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# The role of deep eutectic solvents in the production of cellulose nanomaterials from biomass

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**Abstract:** In recent years, the demand for environment-friendly products has been on an increasing trend among researchers and industry for sustainable development. Deep eutectic solvents are green solvents which, due to their properties (biodegradability, recyclability, low cost, availability, easy preparation, low toxicity, chemical and thermal stability), can be used in various fields such as polymer chemistry, which includes nanocellulose isolation and polysaccharides processing. Several studies have illustrated the effectiveness of using deep eutectic solvents instead of the conventional reaction system to produce and disperse nanomaterials. This work summarizes the use of deep eutectic solvents in the isolation of cellulosic nanomaterials from different types of biomass. Deep eutectic solvents demonstrate high effectiveness in swelling lignocellulosic biomass and producing cellulose nanomaterials. Overall, deep eutectics solvents represent an innovative and effective pretreatment process for the fractionation of raw cellulose-containing fibres to promote subsequent isolation of nanomaterials made from cellulose.

**Keywords**: deep eutectic solvents, isolation, nanocellulose, nanocrystals, nanofibers, polymers

## Introduction

Recently, cellulose has resurfaced with renewed attention due to its recoverability, biocompatibility, and natural abundance (Jiang et al., 2021). Cellulose is one of the most inexhaustible renewable biopolymer in the world. It is virtually an unlimited source of raw materials saturating the growing demand for the production of ecological materials (Gan et al., 2019). Cellulose is a major component of plant cell walls, and can therefore be isolated and extracted from plant sources and agricultural waste. In order for cellulose to be a beneficial substance, it must be extracted (Asyraf et al., 2020). Cellulose can be extracted in different sizes depending on the intended application. The extraction can be performed by several methods such as mechanical, chemical, and bacterial cellulose production (Omran et al., 2021).

Cellulose nanomaterials represent a new class of cellulose particles, with various properties and functions, from molecular cellulose and wood pulp. Nanocellulose can be extracted from microorganisms, grasses, aquatic plants and animals and it can be classified into three categories based on its morphology and functional characteristics depending on the cellulose source and method of isolation (Dhali et al., 2021).

Nanocellulose is divided into cellulose nanofibrils (CNFs), cellulose nanocrystals (CNCs), and bacterial nanocellulose. Cellulose nanoparticles are cellulose elements with at least one distance below 100 nm. CNFs have lengths in the micron scale and widths from 10 nm to a few hundred nanometers. CNCs are rod-like particles ranging in length from 100 to 300 nm and in width from 5 to 15 nm (Omran et al., 2021). CNCs typically refer to short and rigid nanocrystals with deformed amorphous region completely degraded along the elementary fibrils while CNFs commonly refer to long and flexible nanofibrils (Jiang et al., 2021).

New sources of nanocellulose, new modifications, and treatment methods are developed to decrease the high energy required during its production, and to design a more suitable industrial-scale production process. Dimensions of cellulose nanomaterials depend on the original cellulose source as well as on the processing method, reaction time, and temperature (Gan et al., 2019). Nanocellulose has attractive properties (large specific surface area, modifiability, biodegradability), and a wide range of applications. Surface properties and interfacial compatibility of nanocellulose are suitable for further development (Chu et al., 2020). Nanocellulose materials (bionanomaterials) are recyclable and sustainable; (NFs and CNCs have found applications in the automotive, medical, packaging, construction, and paper industries) (Omran et al., 2021).

### *Production of cellulose nanofibrils by deep eutectic solvents*

Nanomaterials derived from cellulose, CNCs, and CNFs have been researched due to their many fascinating properties (high strength, large specific

surface area). These nanocellulosic materials are isolated from treated, delignified pulp, originating from various lignocellulosic materials (biomasses). The pre-processing includes processes to remove lignin, hemicelluloses, and other compounds (extractives and inorganics) to obtain pulp with high cellulose content (Hong et al., 2020). In several studies (Shen et al., 2019; Guo et al., 2019), the authors have found that lignin can be dissolved in deep eutectic solvents (DESs) which can, in addition, be used for efficient extraction and fractionation of biomass to separate its main components.

DESs are prepared by mixing two or more substances at a proper ratio, normally with moderate heating. Typically, quaternary ammonium salts, which act as hydrogen bond acceptors (HBAs), are mixed with hydrogen bond donors (HBDs) to form DESs (Abad-Gil et al., 2021). The simple preparation method, eco-friendly characteristics, recyclability, and biodegradability are the major advantages of using DESs. Moreover, they possess various unique properties such as non-flammability, high thermal stability, relatively wide liquid range, and low vapor pressure (Ma et al., 2019). Depending on whether the nanocellulose surface is modified, DESs systems for nanocellulose isolation can be classified into two types: nonderivatization and derivatization DES (Jiang et al., 2021).

CNFs can be isolated from a variety of cellulose sources, including bleached kraft pulp, bleached sulfite pulp, wheat straw, bagasse, and corn cob (Liu et al., 2016). Although wood is the main raw material for CNF production, industrial and agricultural wastes have attracted great attention in recent years and are used as an alternative fibre source for CNF preparation due to their abundance, low cost, and renewablility (Chávez-Guerrero et al., 2016; Nechyporchuk et al., 2016).

Yan et al. (2021) applied a natural DES system as a swelling solvent. This treatment was followed by screw extrusion and permeation to obtain unmodified CNFs with good thermal stability and high yield. Needle bleached kraft pulp was used as the main raw material in this experiment. The DES system was prepared by mixing choline chloride and ethylene glycol in the molar ratio of 1:1 (100 °C for 1h). Hydrated DESs were mixed with water in the mass ratio of 80:20, 60:40, 40:60, and 20:80. Raw biomass samples were pretreated with DES in the mass ratio of 1:8 (120 °C for 120 min). Mechanical agitation was used for sufficient swelling. Crushed cellulose was obtained using a screw machine in three 3 cycles at 100 °C. $H_2SO_4$ was used as catalyst in 0, 0.2, 0.4, 0.6 and 0.8 wt % added dropwise to crushed cellulose and the obtained samples were diluted to 0.8 % with DES and distilled water. At the end of this experiment, CNF samples were dialyzed and placed in a colloid mill for further purification and preparation of CNF suspensions. All diameters of the obtained CNFs were below 100 nm. Permeation and swelling of the cellulose fibers by DES were in favor of increasing reactivity. Water-mixed DES was used to minimize cost and to reduce the DESs' viscosity showing that DES is significant in cellulose swelling. In addition, ethylene glycol showed good compatibility with cellulose. It was concluded that DES can promote cellulose fibrillation (high temperatures) due to strong interactions with cellulose chains. This work showed that the properties of solvents are related to CNFs preparation.

A green and ecological method of CNFs preparation, in terms of cost, was implemented by Li et al. (2021). Different distributions of CNF diameters were obtained using DES and mechanical methods. The authors investigated the use of a DES system for the pretreatment of cellulose from okara. DES was synthesized by choline chloride and oxalic acid in the molar ratio of 1:1. Biomass (okara) and DES were mixed in the ratio of 1:20. The reaction was performed at 100 °C for 30—100 min. Distilled water was used at the end of the reaction to precipitate cellulose. Initially, the 2 % cellulose suspension was homogenized 15 times under the pressure of 60 MPa, and then the 2 % heterogeneous cellulose mixture was poured into a mixer and processed (3 min, 6000 rpm). At the end, the 2 % cellulose suspension was dissociated for 5 min using an ultrasonic grinder. CNF diameters from 5 nm to 45 nm with the mean diameter of 30 ±14 nm were obtained by high pressure homogenization. Ultrasound treatment revealed that the CNFs diameters were between 6—50 nm and showed a more even distribution. CNF prepared by high speed mixing had the mean diameter of 27 ±2 nm. The authors carried out rotary evaporation to recover DES. The DES system could be recycled up to three times with the recovery rate of more than 90 %. The yield of cellulose from okara was between 20.07—24.86 %.

DES systems were used to pretreat raw ramie fibers. Yu et al. (2020) treated ramie fibers directly to produce CNFs. Three different DESs (choline chloride/urea (1:2); choline chloride/oxalic acid dihydrate (1:1); and choline chloride /glycerol (1:2)) were applied. Short ramie fibers (2 g) were mixed with 200 g of DES, then the mixture was stirred for 2—10 h at a set temperature (140 °C for choline chloride/urea, 100 °C for choline chloride/oxalic acid dihydrate, and 180 °C for choline chloride/glycerol). Effectiveness of DES was evaluated based on chemical composition analyzes and changes in morphology and structure; reaction time also proved to significantly influence the efficiency of

DES pretreatment. Cellulose hydrolysis, cellulose removal, and reduced hemicellulose removal can be achieved by DES based on choline chloride and oxalic acid dihydrate. After DES pretreatment, cellulose content in the fibers of the processed biomass increased due to the elimination of lignin, pectin, and hemicelluloses. Furthermore, weakening of the cell wall structure and partial fiber damage were observed. AFM microscopy revealed that most cellulosic fractions in the ramie fibers were eliminated after DES treatment.

Based on previous studies (Yu et al., 2020), acidic DES (choline chloride/oxalic acid dihydrate; 1:1) was applied to treat biomass (ramie) and subsequently to separate the cellulose-rich fraction. Then, this fraction was pulped into certain CNFs in a ball mill. Characteristics of thus prepared CNFs were evaluated and compared with CNFs isolated directly from raw fibers and degummed fibers. In a round bottom flask, 2 g of ramie and 200 g of DES (4 h, 100 °C) were mixed. The results showed that DESs can fractionate lignocellulosic biomass with high cellulose selectivity. The obtained CNFs showed the same morphology with the length of 523.38 nm, and the width of 14.29 nm. Thermal stability was around 308 °C and crystallinity was 79.17 %. DES treatment has proven to be a reliable and effective way to isolate nanocellulose without the need for a bleaching step.

Hydrogels derived from natural polymers are currently gaining great interest. Deng et al. (2021) CNFs used ball milling and DES as a crosslinking agent to strengthen hydrogels. DES was used to pretreat waste cotton fabric. DES were prepared by mixing oxalic acid and choline chloride in the molar ratio of 1:2 under gentle heating. Cotton fiber powder (1:20) was added to the prepared DES solution and the reaction was continued at 110 °C (3 h). Next, the obtained cellulose was washed with distilled water and processed in a ball mill for 16 h. The treated cotton had disturbed and irregular structure due to the DES treatment. The CNF yield was 49.7 %. The water retention value (WRV) was 436.2 % and the degree of polymerization (DP) was 426. Zeta potentials of CNFs ranged in negative numbers (–25.63 mV) and the crystallinity index (CrI) of CNF obtained by DES treatment was 57.9 %. Results of the experiments showed that DES disrupts the intramolecular hydrogen bonds of cellulose, thereby reducing its crystallinity. The obtained CNFs showed fibrous structure of native cellulose (width 50—100 nm; length 1—2 μm). CNFs isolated after DES pretreatment had negatively charged surface, which is a good precondition for forming a good dispersed suspension. These experiments have shown that CNFs can improve hydrogels by improving mechanical and biological properties, and delaying the development of various hydrogels for drug delivery.

Li et al. (2021) used DES as a pretreatment medium, and for the production of sulfated CNFs by supermass colloid. The DES system was prepared in the mass ratio of 1:3, and 2 g of raw fibers (bleached sulfate poplar pulp) were added to the DES at a constant temperature. DES treated and untreated pulp was nanofibrillated using a shredder. Mass ratios of sulfamic acid and cellulose fraction were 1:6, 1:9, and 1:12 at 100 °C (1 or 1.5 h). Yields of DES-treated cellulosic pulp ranged from 80.8 % to 95.2 %, and decreased with the increasing sulfamic acid content and reaction time. Fiber width increased after DES application (15.5—17.4 nm) due to an increase in the degree of swelling of the fibers (DES weakened internal hydrogen bonds by penetrating the fiber structure). The results confirmed that longer reaction time leads to more pronounced swelling and higher degree of substitution (DS) (replacement of hydroxyl groups by sulfate groups). The increase in WRV was caused by defibrillation, and shorter fibrils were visible on AMF images compared to CNF from the original pulp (10—25 nm). Due to the presence of sulfamic acid, the zeta potential value was measured in the range between –18.3 mV to –35.2 mV.

Ji et al. (2021) investigated the cleavage of hydrogen bonds in biomass, and the subsequent preparation of CNFs using ternary DES, microwave deconstruction, and ultrasonic separation. DESs were prepared by mixing choline chloride with lactic acid or oxalic acid, and $AlCl_3 \cdot 6H_2O$ (1:1:0.2), and 10 g of DES and 0.1 g of sugarcane bagasse were mixed in a microwave tank. The reaction temperature was about 80—120 °C. Ternary DES was applied to the biomass under microwave irradiation (2400 W, 20 min) to isolate cellulose-containing fractions. Such obtained residues were subjected to ultrasonic crushing to be nanofibrillated, thereby preparing CNFs. After adjustment of DES and microwave radiation, the content of cellulose increased, while the content of lignin and hemicelluloses showed a decreasing trend. The values of surface charge density and zeta potential after DES pretreatment based on choline chloride/lactic acid/$AlCl_3 \cdot 6H_2O$ were: 0.84 mmol/g, and –35.82 mV. Results of the experiments showed the width of CNFs ranging between 15—17 nm, length between 400—600 nm, and height between 6—7 nm. CNFs had good thermal stability and high crystallinity (58.05—60.71 %).

Further mechanical disturbance of the pretreated samples resulted in nanofibers with the width of 20—80 nm. Liu et al. (2021) obtained the yield of 84.19 % (CNFs from bleached softwood kraft pulp)

using DES formed by mixing choline chloride and anhydrous citric acid (1:1) at 90 °C for the pretreatment. This treatment was followed by high pressure homogenization, and the resulting CNFs showed high crystallinity (81.36 %) and diameters between 21 ±3 nm.

Green solvents (DES) show capability for extraction of biomass and its components, and foe cellulose nanofibrillation. In Liu et al. (2021), carboxylic acid-based DESs and choline chloride DESs were used to prepare CNFs from bleached hardwood kraft pulp. Mechanical extrusion was used along with the DES treatment to esterify and swell cellulose, and the CNFs were subsequently processed by colloidal milling. Carboxylic acids and choline chloride were mixed in the molar ratio of 1:9 (lactic acid), 1:2 (formic acid, and acetic acid), and 1:1 (malonic acid, oxalic acid and citric acid). The raw material was mixed with DES in the weight ratio of 1:8, and the mixtures were stirred for 3 h (50—100 °C). After treatment in a colloid mill, the CNFs mixtures were dialyzed in distilled water. SEM analysis showed that with increasing DES pretreatment temperature, diameters and lengths of CNFs decreased significantly. At 85 °C, the CNFs obtained had an average width of 80 nm and a length of more than 1 μm (6—10 μm). The results suggest that CNFs with different morphologies can be obtained by controlling the pretreatment temperature of cellulose. As the temperature increased to 100 °C, CNFs averages decreased to 20 nm, as did the lengths. DES treatment led to esterification modification and efficient swelling of cellulose (high yield of CNFs); yields of 72—88 % were obtained at DPs of 300—500. In addition, esterified CNFs were used as reinforcement to prepare polylactic acid composites (to improve mechanical properties of composites).

The aim of this work (Liu et al., 2021) was to use DES based on choline chloride and urea as a system for the synthesis of composite films (CNF/reduced graphene oxide). A part of the work was to investigate the effect of DES content, DES component mass ratio, processing temperature, and DES recycling time on electrical properties of the composite film. *Phyllostachys pubescen* (Moso bamboo) was chosen as the CNF source. Lactic acid/choline chloride (9:1) mixture was mixed with bamboo powder at 120 °C (3 h). Subsequently, water and ethanol were added to the mixture to obtain cellulose. The product was refined using a homogenizer, and ultrasound was used to obtain CNF. DES used in the CNF/reduced graphene oxide composite synthesis process was prepared by mixing choline chloride with urea (1:1) at 80 °C until a homogeneous solution was formed. CNF/graphene oxide suspensions weighing 30 mg (weight ratios: 2:1, 1:1, 1:2) and mixed with 20 g DES (15 min) were used to make the composite films. The reaction system consisted of 15 mg of CNF, 15 mg of graphene oxide sheets, DES (25 %, 50 % and 75 % of total weight), and water. The effect of temperature was monitored in an aqueous system with 25 % DES in the temperature range from 50 to 100 °C. Morphological analyzes of the films revealed that graphene sheets coated the surface of CNF, which is in favor of tensile stress. The produced composite electrode with the weight ratio (CNF/reduced graphene oxide) of 1:2 had the highest electrical conductivity of 26.47 $S \cdot cm^{-1}$, and the maximum area specific capacity could reach up to 226.83 $mF \cdot cm^{-2}$ (promising substrate for supercapacitors and sensors based on cellulose). Hong et al. (2020) investigated betaine/glycerol hydrochloride-based DES as a pretreatment medium for cellulose (bleached birch pulp) from birch (*Betula pendula*), and subsequent production of CNFs by microfluidization. Various conditions of DES application (temperature, molar ratio of DES components, and time) were analyzed, and at the same time, physical, chemical, and mechanical properties of the prepared CNFs and cellulose nanofilms were investigated. DES was synthesized by mixing using various molar ratios from 1:6, 1:3, 1:2, to 2:3 (betaine hydrochloride/glycerol). The weight ratio of cellulose to DES was 1:25, the reaction was run at 130, 140 and 150 °C, and the required time was 30 min or 1 h. The solid fraction was collected and diluted for further mechanical disintegration using a microfluidizer. Nanocellulosic films were prepared by vacuum filtration from a suspension of CNFs. The results showed that the maximum weight yield was 72.5 %, and the molar ratio between the DES components had a decisive influence on cellulose modification. DES served as a swelling agent and weakened the hydrogen bonds in cellulose. Isolated CNFs diameter of 17—20 nm with CrI in the range of 67.7—74.4 % were obtained.

The aim Suopajärvi et al. (2020) was to use six different DESs (5 acidic, and 1 alkaline DES), for delignification and nanofibrillation of cellulose from agricultural by-products. DES treatment was performed at 100 °C for 8 h, at 100 °C for 16 h, and at 80 °C for 24 h. Dried, ground samples of wheat straw, corn stalks, and rapessed stem were used as feedstock. Composition of the DES solutions obtained with lactic acid/choline chloride was (2:1 and 5:1); with levulinic acid/choline chloride it was (2:1); with malic acid and glutaric acid with choline chloride it was (1:1), and finally with glycerol/$K_2CO_3$ it was (5:1). To the suspension with 5 g of water, 3 g of raw material were added and the mixture was stirred, followed by an addition of ethanol to stop the reaction. DES-treated

samples were mixed and subsequently nanofibrillated using a microfluidizer to obtain nanocellulosic suspensions. Nanofibers were produced from nanofibrillated samples by filtration and tested for mechanical properties. The average width of the prepared individual nanofibrils was 4-6 nm with all raw materials. Lactic acid/choline chloride and alkaline glycerol/$K_2CO_3$ provided the highest delignification yields (11.8—5.8 wt %), nanocellulose viscosity, and CrI (54—38 %).

***Production of lignin-containing cellulose nanofibrils/cellulose nanocrystals/nanocellulose by deep eutectic solvents***

In Hong et al. (2020), lignin and hemicelluloses were imperfectly separated from biomass of luffa sponge using DES composed of choline chloride/oxalic acid dihydrate. DES was used to delignify luffa biomass and separate a cellulose-rich solid fraction. The residue after DES treatment underwent a two-step ultrasonication treatment to produce lignin-containing CNFs and CNCs fractions. DES of choline chloride/oxalic acid dihydrate with the molar ratio of 1:1 was prepared. Luffa samples were fractionated in DES with the mass ratio of 1:20 at 70 °C, 80 °C, 90 °C and 100 °C for 1 h, 1.5 h, 2 h, and 2.5 h. After the DES treatment, the residue was washed and separated by centrifugation (turbid supernatant and solid residue). The following ultrasonication treatment led to CNC (turbid supernatant) and CNF (solid residue). The lignin-containing CNC fraction consisted of a mixture of short needle-like crystals and elongated nanofibers with diameters ranging from 11 to 28 nm and lengths between 188 and 3125 nm. CNFs from the DES-treated luffa had diameters ranging between 11 and 134 nm with the average diameter of 28 nm. Under the optimal reaction condition (90 °C, 150 min), a solid fraction with the cellulose content of 76.4 wt % and the residual lignin amount of 10.7 wt % was produced. A two-step ultrasonication process resulted in lignin-containing CNF and CNC fractions with the yield of 59.1 wt %.

Mongolian oak was used by Kwon et al. (2021) as a starting material for the production of lignocellulosic nanofibrils (LCNFs) applying DES pretreatment and mechanical defibrillation. Lactic acid (carboxyl HBD), glycerol (hydroxyl HBD), and urea (amide HBD) were selected as HBDs, and choline chloride was used as HBA. DESs were prepared in the molar ratios of 1:2, and 1:6 (100 °C, 10 min). The reaction was run with 1 g of biomass and 20 ml of DES solution at 100 °C and 140 °C (2, 6, 12 h). The fraction containing cellulose was washed with distilled water and acetone, and used to produce LCNFs. The results showed that, after the DESs treatment, the cellulose content decreased with increasing molar ratio, temperature and time. Defibrillation caused a decrease in crystallinity (from 71.2 to 68.5 %) when choline chloride/lactic acid based DES was used. At higher temperatures, this decrease was more significant. Choline chloride/urea led to an increase in crystallinity (31.3—62.2 %) after defibrillation, which was more pronounced at higher temperatures. All obtained LCNFs showed a network structure of nanofibrils, and individual nanofiber widths varying according to the DES type (1.56 to 2.41 nm).

In their experiment, Li et al. (2021) isolated lignin-containing CNFs from corncob by combined DES pretreatment, enzymatic hydrolysis, and finally, high pressure homogenization. Choline chloride was applied as HBA for all solvent systems. Formic, lactic and acetic acids were used as HBDs. Choline chloride DES was prepared as follows: formic acid/choline chloride (2:1); lactic acid/choline chloride (5:1); acetic acid/choline chloride (2:1); oxalic acid/choline chloride (1:1); urea/choline chloride (2:1); glycerol/choline chloride (2:1). Corncob and DES were mixed in the mass ratio of 1:25 (water bath 90 °C, 9 h). Lignin-containing CNFs showed good fluorescence, thermal stability (353 °C), hydrophobicity, and diameters between 60—90 nm.

The development of environmentally friendly materials and reagents is of great importance for the production of lignin-containing nanocellulose (LNC). Shu et al. (2022) used DESs to prepare LNC from poplar applying microfluidization. Ternary DESs consisting of choline chloride (HBA) and two HBDs (lactic acid and *p*-toluenesulfonic acid) were used. Various DES pretreatment conditions (molar ratio, and temperature of process) were investigated. DESs were mixed in the molar ratios of 2:6:1, 2:8:1, 2:9:1, 2:10:1, and 2:12:1 (choline chloride/lactic acid/*p*-toluenesulfonic acid). Then, 2 g of poplar samples were added to 100 g of DES at temperatures of 80 °C, 90 °C, 100 °C, 110 °C and 120 °C, and stirred evenly for 3 h. Water was added to the mixture to complete the reaction, and the solid fractions were separated by centrifugation, dialyzed, and disintegrated using a microfluidizer. LNC with the width of approximately 23 nm and thickness of 1.16 nm were obtained at the molar ratio of 2:10:1 and 100 °C. The yield and lignin content in LNC reached 64.65 % and 27.65 %, respectively. LNC showed high thermal stability (370 °C), high viscosity and gel behavior (valuable for thermoplastic composites applications).

The acid DESs treatment process was applied to thermomechanical softwood pulp, and subsequently to the production of lignin-containing CNCs. In Jiang et al. (2020), ternary and binary DESs based

on choline chloride/oxalic acid (1:1), and choline chloride/oxalic acid/*p*-toluenesulfonic acid (2:1:1) were used. The application of DESs was followed by a mechanical process to produce CNCs. Here, 1 g of pulp was added to DES, and the mixture was stirred at 80 °C for 3 h or 6 h. The obtained suspension was centrifuged, and the supernatant was represented by CNCs. Regarding binary DES hydrolysis, due to the increase in time, the cellulose content increased from 54.8 % to 56.9 %, and it decreased to 46.0 % and 40.6 % due to hydrolysis with the application of ternary DES. These results suggest that higher cellulose content is degraded in the presence of a strong acid. The obtained CNCs showed morphology with the thickness of 3.3 nm obtained by atomic force microscopy (AFM), and width of 5.9 nm as obtained by transmission electron microscopy (TEM).

DES-treated lignin-containing CNCs contribute to the improvement of nanomaterial production. Zargar et al. (2022) pointed to the environmental impact of the production of lignin-containing CNCs from thermomechanical pulp after the application of acidic DES based on several analyses. Furthermore, the effect on the production of lignin-containing CNCs depending on the DES chemical composition, dose, and reuse was studied selecting three factors: DES components (choline chloride, oxalic acid dihydrate, *p*-toluenesulfonic acid), temperature (80—90 °C), and hydrolysis time (3—6 h). Subsequently, the mixture was dialyzed, mixed with a high speed stirrer, and centrifuged to obtain lignin-containing CNCs. The assessment was carried out through a life cycle analysis in terms of global warming potential (GWP), acidification potential (AP), and cumulative energy consumption (MJ). The main goal of lignin-containing CNCs isolation was to achieve higher yield than in the production of CNCs. The results showed that higher yields were achieved at the expense of higher environmental impact. The most significant reduction in GWP, AP, and energy consumption was achieved by reducing the DES input volume to 20 % of the default value.

In Jiang et al. (2020), thermomechanical softwood pulp was analyzed as a raw material for LNC isolation after DES application. The effect of DES choline chloride/oxalic acid dihydrate treatment at molar ratios of 1:2, 1:1, 2:1, and 4:1, and reaction temperatures of 80 °C and 90 °C was monitored. The treatment comprised mixing and heating of 1 g of the sample in 50 g of DES at a given temperature for 6 h. The mixture was dialyzed, mixed, and centrifuged at the end to obtain LNC. With the increasing pretreatment temperature and oxalic acid concentration, the yield increased up to 57 % obtained at the DES ratio of 1:1 at 90 °C and 6 h. Under these conditions, banded (≈ 7.1 nm, thickness ≈ 3.7 nm) LNCs with 60 % cellulose crystallinity and 32.6 % lignin content were isolated. Lignin nanoparticles diameter of 20—50 nm was reached and they showed high thermal stability.

### *Production of cellulose nanocrystals by deep eutectic solvents*

Processing of biomass with DES to produce nanocellulose has attracted considerable attention. However, after the application of DESs, a mechanical or a chemical process is still required to isolate and produce nanocellulose (Li et al., 2021).

By removing the amorphous phase from cellulose-containing fibers, CNCs is obtained in liquid, gel, or solid form. Natural fibers are chemically hydrolyzed to form nanocrystalline cellulose, which has nanoscale sizes, excellent strength and optical properties (Ilyas et al., 2020). Due to their properties (low density, large surface area, recoverability, high crystallinity), CNCs have versatile applications. CNCs production using DESs has been reported in several recent studies.

In Wu et al. (2021), the preparation of CNFs and CNCs using DES followed by ball milling was designed. Microcrystalline cellulose was used as the starting biomass. DES was prepared by mixing choline chloride/formic acid in the molar ratio of 1:2. Biomass with the fiber consistency of 5 wt % was added to the DES solution. The reaction was run for some time in an oil bath at 90 °C. Centrifugation followed to separate the solid residue and the supernatant. The treated cellulose was then placed in a ball mill. Grinding time has been shown to be a significant factor influencing the yield and properties of nanocellulose. The results revealed that the use of DES in biomass pretreatment reduced fiber aggregation dimensions and thus helped defibrillate to cellulose products in nanometers. With the increasing grinding time, the zeta potential values decreased and the yield of nanocellulose production was 95 %.

In another experiment (Fan et al., 2020), CNCs were isolated and extracted from grape pomace using a green solvent (choline chloride/lactic acid; 1:2). CNCs were then used to produce self-curing hydrogels. Grape pomace was crushed, extracted and dried. The flask containing biomass, distilled water and sodium hydroxide was placed in an oil bath at 60 °C under mechanical stirring. The biomass was washed and dried to obtain cellulose. Lactic acid and choline chloride were added to the flask at 60 °C to form DES. A mixture of cellulose and DES (1:100, wt %) was heated at 80 °C under mechanical stirring for 6 h. The CNCs produced

**Tab. 1.** Comparison of different CNCs isolation techniques (Raza et al., 2022).

| Isolation method | Conversion path | Advantages | Disadvantages |
|---|---|---|---|
| Mineral acid hydrolysis using $H_2SO_4$ | – mainly delignification and bleaching,<br>– sometimes steam explosion,<br>Conditions:<br>H2SO4 concentration (60—65 wt. %),<br>temperature = 45—90 °C,<br>reaction time = 30—90 min | fast method<br>authentic technology | corrosion (equipment) lower yield degradation of cellulose difficult recovery<br>toxic pollutants |
| Mineral acid hydrolysis using HCl | – always delignification and bleaching,<br>– rarely steam explosion,<br>Conditions:<br>HCl concentration (4—6 mol/l),<br>temperature = 40—80 °C,<br>reaction time = 3—5 h | thermal stability > $H_2SO_4$<br>yield > $H_2SO_4$<br>degradation of CNCs < $H_2SO_4$ | CNCs prone to flocculation corrosion<br>difficult recovery<br>toxic pollutants |
| Organic acid hydrolysis | – formic acid, oxalic acid, maleic acid, p-toluenesulfonic acid,<br>Conditions:<br>acid concentration (30—80 wt. %),<br>temperature = 80—120 °C,<br>reaction time (30—240 min) | higher yield > mineral acids<br>thermal stability > mineral acids<br>better crystallinity index<br>recyclable acid<br>less corrosive<br>ecofriendly | long residence time<br>high temperature |
| Enzymatic hydrolysis | – different enzymes<br>– Cellulase, viz. Cclast<br>– Cellulase (700 EGU/g)<br>– Cellic CTec2<br>– Endoglucanase<br>Conditions:<br>temperature = 40—50 °C | no toxic residues<br>lower energy process<br>better morphology (CNCs)<br>not corrosive to equipment<br>less waste and byproducts<br>higher conversion | long time<br>high cost of enzymes |
| Ionic liquids | – salts with melting point < 100 °C<br>– environmentally friendly solvent<br>– mild conditions | chemically stable<br>thermally stable<br>nontoxic<br>easy recovery<br>biocompatible<br>biodegradable | high price |
| DES | -easily prepared<br>- mixing renewable, cheap, and biodegradable components<br>- conducted under mild conditions | easily available<br>biocompatible<br>nontoxic<br>recyclable<br>better energy efficiency<br>no pollution | yield (investigation) |

had the length of 241.5 ±45.3 nm and diameter of 22.0 ±3.9 nm. Hydrogels prepared from these CNCs showed good mechanical properties and the self-treatment efficiency of about 90 %.

DES pretreatment and bleaching preceded the isolation of CNCs from oil palm by sulfuric acid hydrolysis. The DES system set up by Gan et al. (2020) was prepared by mixing potassium carbonate and glycerol in the molar ratio of 1:7 (80 °C, 2 h). A mixture of 10 g of biomass and 200 ml of DES was heated at 80 °C for 2 h. The hydrolysis with sulfuric acid was terminated by adding cold distilled water to the system. The diameter and length of the CNCs produced were 6.78 ±2.12 nm and 160.06 ±32.58 nm, respectively. Due to the high crystallinity and thermal stability of cellulose, lignin and hemicellulose removal was effective after bleaching and DESs application. CNCs of even higher crystallinity and the particle diameter of less than 10 nm were reached after the hydrolysis.

The aim of Lim et al. (2021) was to use cellulose pulp (after alkaline DES treatment) to isolate CNCs using acidic DESs, and to analyze the application of DES (choline chloride/oxalic acid, 1:1) to produce CNCs from bleached pulp from rice straw. About 1 g of bleached pulp was added to 10 g of DES (preheated to 80 °C) under stirring for 4 h. The diameter and length of the isolated CNCs

were 17.9 ±0.5 nm and 332.1 ±9.6 nm, respectively, and TEM and scanned electron microscopy (SEM) images revealed rod-shaped nanofibers of CNCs after DES application and the CrI value of 76.7 %. The CNCs produced were in the nano-size range with the polydispersity index (PDI) of 0.56. Stable CNC collision dispersion with the zeta potential of 52.1 ±5.2 mV was prepared. DESs were able to

**Tab. 2.** Isolation conditions and properties of nanocellulose after pretreatment with DES (Jiang et al., 2021).

| Solvent (DES) | Molar ratio | Isolation conditions | Sample | Mechanical treatment | Yield (%) | Type of nano-cellulose | Morphology |
|---|---|---|---|---|---|---|---|
| Choline chloride/ Lactic acid | 1:2 | 80 °C 6 h | grape pomace | – | – | CNCs | length: 214.5 nm width: 22.0 nm |
| Choline chloride/ Lactic acid | 1:9 | 50–100 °C 3 h | hardwood bleached kraft pulp | mechanical extrusion (3 times) + colloid mill dispersion (40 min) | 72–88 | CNFs | 85 °C: length > 1 µm width: 80 nm 100 °C: width: 15–30 nm |
| Choline chloride/ Malonic acid | 1:1 | 50–100 °C 3 h | hardwood bleached kraft pulp | mechanical extrusion (3 times) + colloid mill dispersion (40 min) | 72–88 | CNFs | 85 °C: length: 6–10 µm width: 80 nm 100 °C: width: 20–50 nm |
| Choline chloride/ Oxalic acid dihydrate | 1:1 | 80–90 °C 6 h | softwood thermo-mechanical pulp | blending (30 min) | 57 | lignin containing nano cellulose | 90 °C: thickness: 3.7 nm width: 7.1 nm 20–50 nm in diameter |
| Choline chloride/ oxalic acid/ *p*-toluene sulfonic acid | 2:1:1 | 80 °C 3–6 h | softwood thermo-mechanical pulp | blending (30 min) | 66 | lignin containing CNCs | 80 °C, 3h: thickness: 3.3 nm width: 5.9 nm 20–50 nm in diameter |
| Choline chloride/ Levulinic acid<br>Choline chloride/ Malic acid<br>Glycerol/ $K_2CO_3$ | 1:2<br>1:1<br>5:1 | 100 °C 8–16 h,<br>80 °C 24 h | wheat straw, corn stalk, rapeseed stem | microfluidizer (9 passes) | 32–70 | CNFs | width: 4–6 nm |
| Ammonium formate/ Glycolic acid | 2:1 | autoclave 180 °C 4 h | softwood pulp | ultrasonication (30 min) | – | CNCs | length: 100–300 nm width: 10 nm |
| Choline chloride/ Imidazol | 3:7 | 60–100 °C 15–180 min | hardwood kraft pulp | microfluidizer (5 passes) | 90–100 | CNFs | width: 20–50 nm |
| Betaine hydrochloride/ Glycerol | 1:2 | 150 °C 1 h | bleached birch pulp | microfluidizer (6 passes) | 72.5 | CNFs | length: 75–125 nm width: 17–20 nm |

hydrolyze the amorphous region of cellulose and subsequently release CNCs after the DES treatment.

Vorobiov et al. (2022) studied two types of CNCs: CNCs prepared by DES, and CNCs prepared by acid hydrolysis, as well as their influence on 3D printing (rheological behavior of dispersions). Microcrystalline cellulose with an average particle size of 50 μm was used as the raw material. Acid hydrolysis was carried out for 1 h (45 °C) mixing 5.2 g of raw material in 52 ml of 64 % (w/w) sulfuric acid. To obtain transparent DES, 13.92 g of urea and 16.26 g of choline chloride were added to the flask (stirring at 80 °C). Microcrystalline cellulose (0.62 g) was added to DES, and the dispersion was stirred at 110 °C for 4 days in an oil bath. The cloudy upper phase enriched by DES was separated from the lower phase by decantation. CNCs were used to prepare inks with CNC content of 6—15 wt % in polymerizable DES based on choline chloride/acrylic acid. The results showed that all inks prepared were non-Newtonian fluids for shear dilution. Cellulose flakes were formed in CNC-containing inks prepared in DES, while CNC inks (after acid hydrolysis) were stable to agglomeration up to 9 wt %. The highest printing accuracy was achieved for the ink with 15 wt % CNC (after acid hydrolysis). Ionic conductivity decreased from 0.26 $mS \cdot cm^{-1}$ to 0.011 $mS \cdot cm^{-1}$ with the increase in CNC content (after acid hydrolysis) from 6 wt % up to 15 wt %.

## Future and perspectives

There is a growing interest in the development of biodegradable and renewable materials due to the urgent need to protect the environment. Scientists are focused on finding ecological materials from renewable sources, specifically cellulose, which is the most widespread biopolymer on Earth. Cellulose is a ubiquitous polymer from nature that can be isolated from a wide range of biomass. Pretreatment is often required to extract and subsequently fractionate cellulose from various lignocellulosic materials. To obtain cellulosic nanomaterials, the cellulose-containing fraction has to be purified by dissolving non-cellulosic components.

Intensive research into nanocellulose is based on its unique nanostructure and excellent chemical and physical properties (dimensions in nanometers, large specific surface area, high strength, surface chemistry modification, non-toxicity). Due to its many advantages, nanocellulose is widely used for the production of high-performance and environmentally friendly materials used in engineering and biomedical fields. Due to their renewable resources and biodegradable nature, nanocellulose-based materials are being increasingly used in a variety of applications. Whenever the properties of a material can be improved and easily modified, the material will cover multiple applications. The nanoscale range has contributed to a very large surface area, which is suitable for adsorption and modification of pollutants, making nanocomposites an attractive material for energy and environmental applications.

The price of cellulose nanomaterials depends mainly on the choice of raw materials and the method of nanocellulose synthesis. The choice of lignocellulosic material is very important because its cellulose content and local availability will affect the yield and production costs. Optimization of the pretreatment protocol has been constantly sought as it is specific for lignocellulosic biomass and depends on factors such as the nature of biomass, process and environmental parameters, as well as economic viability. Industrial nanocellulose production indicates that the demand for cellulosic nanomaterials constantly increases. The demand for pulp is expected to continue to grow due to its growing and expanding applications.

The lignocellulose-based bond has proven to be green and a substitute for conventional fossil resources in meeting the growing energy demands. The global nanocellulose market is projected to grow at the rate of 20.4 % in value, from $ 247.2 million in 2019 to $ 1.08 billion by 2027, according to an analysis of reports and data.

## Conclusion

To achieve a balance between economicy and ecology, it is necessary to explore a greener and cheaper method of producing cellulosic nanomaterials. This article provides an overview of recent advances in environmentally friendly and sustainable technologies for the isolation of cellulosic nanomaterials using DES. The most recent papers published mainly in 2020—2021 focusing on the development and technological research related to the generation of nanocellulose crystals and fibrils using DES were studied.

Pretreatment processes play a crucial role in removing lignin and other impurities embedded in cellulosic fibers, and in improving the quality of cellulosic nanomaterials (CNF and CNC). The use of DES in biomass processing is promising as they can be used for biomass fractionation as well as for acceleration of cellulose nanofibrillation. This strategy has several potential benefits, such as high efficiency, low energy consumption, and the use of environmentally friendly chemicals. The

versatile DES solvent systém allows for CNF and CNC preparation. DES pretreatment contributes to the disruption of the structure of raw cellulose fibers; DES have the ability to remove amorphous cellulose and non-cellulosic components such as hemicelluloses, water-soluble substances, pectin, and lignin from the cellulose structure.

A summary of the results and conclusions of the studies described in this article shows that DESs are important in cellulose swelling, and they promote high temperature cellulose fibrillation. DES pretreatment seems to be an excellent way to extract nanocellulose from raw cellulose fibers, not requiring any further bleaching steps. Furthermore, DES properties have been found to affect the preparation and isolation of cellulosic nanomaterials, while reaction time and temperature are also important parameters affecting DES efficiency. DES-treated biomass showed irregular and broken morphology, suggesting that DES treatment causes a loss of fibrous structure and facilitates the recovery of the cellulose-rich fraction and the subsequent preparation of cellulose nanomaterials from different biomasses. In general, a mechanical or a chemical process is required after DES pretreatment to convert isolated cellulose to nanocellulose. Regarding the comparison of different methods of nanocellulose isolation, hydrolysis with mineral acids can be considered a reliable and fast method but it is prone to equipment corrosion and difficult acid recovery. Enzymatic hydrolysis is a very expensive process due to the very high cost of enzymes. DES has many advantages in terms of solvent recyclability, low toxicity, biodegradability and low cost.

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