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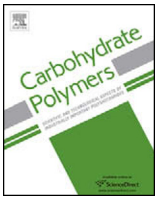

# Characterisation and properties of homo- and heterogenously phosphorylated nanocellulose

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ABSTRACT

Nano-sized cellulose ester derivatives having phosphoryl side groups were synthesised by phosphorylation of nanofibrilated cellulose (NFC) and nanocrystaline cellulose (NCC), using different heterogeneous (in water) and homogeneous (in molten urea) processes with phosphoric acid as phosphoryl donor. The phosphorylation mechanism, efficacy, stability, as well as its influence on the NC crystallinity and thermal properties, were evaluated using ATR-FTIR and $^{13}$C NMR spectroscopies, potentiometric titration, capillary electrophoresis, X-ray diffraction, colorimetry, thermogravimmetry and SEM. Phosphorylation under both processes created dibasic phosphate and monobasic tautomeric phosphite groups at C6 and C3 positioned hydroxyls of cellulose, yielded 60-fold (~1173 mmol/kg) and 2-fold (~1038 mmol/kg) higher surface charge density for p-NFC and p-NCC, respectively, under homogenous conditions. None of the phosphorylations affected neither the NC crystallinity degree nor the structure, and noticeably preventing the derivatives from weight loss during the pyrolysis process. The p-NC showed high hydrolytic stability to water at all pH mediums. Reusing of the treatment bath was examined after the heterogeneous process.

## 1. Introduction

Nano-sized cellulose derived from bio-sources such as wood, straw and cotton by acid hydrolysis or mechanical treatments have gained increasing attention due to their high strength and stiffness combined with low weight, biodegradability and renewability, thus offering numerous applicative opportunities (Camarero Espinosa, Kuhnt, Foster, & Weder, 2013; Habibi, 2014). Using mechanical treatments cellulose fibres are delaminated by high pressure homogenisation, microfluidisers or sonication in order to produce microfibrillated (MFC) or nanofibrillated (NFC) cellulose, depending on the preparation method and source. Weak negative surface-charge can be present as a result of those carboxylic groups ($-\text{COO}^-$) within the residual hemicelluloses that were not removable during purification. On the other hand, by using acid hydrolysis the amorphous domain of the native cellulose structure is hydrolysed with hydrochloric, sulphuric acid or less frequently with phosphoric acid, thus leaving the crystalline domain as nanocrystalline (NCC) cellulose. The hydrolysed nanocelluloses (NC) were reported to have the same crystalline structures as their original fibres (cellulose I), but negative surface potential related to the end-located terminal groups ($-\text{COO}^-$, $-\text{SO}_3{}^2$, $-\text{PO}_3{}^{2-}$) depending on the isolation process.

However, there are only a few reports about the isolation of NC using phosphoric acid hydrolysis (Lemke, Dong, Michal, & Hamad, 2012; Okano, Kuga, Wada, Araki, & Inata, 1999), and only one being recently published (Camarero Espinosa et al., 2013), where the isolated NCC (size of 31 ± 14 nm × 316 ± 127 nm) possessed small numbers of phosphoric groups on the surface with a surface-charge density of 10.8 ± 2.7 mmol/kg cellulose compared to the corresponding sulphuric acid hydrolysis isolation process, which leads to the formation of anionic cellulose sulphate with almost 10-fold higher surface charge density (98.5 ± 8.0 mmol/kg cellulose) of sulphuric groups.

The creating of negatively charged phosphoric groups (such as cellulose phosphate or cellulose phosphite) on cellulose is a well-known strategy when producing materials applicable in orthopaedics (Petreus et al., 2014), biomedical (Granja et al., 2001a, 2001b; Li, Wang, Liu, Xiong, & Liu, 2012; Mucalo, Kato, & Yokogawa, 2009), textile (Aoki & Nishio, 2010; Tzanov, Stamenova, & Cavaco-Paulo, 2002), plastics (as flame-retardants' fillers) (Gérard, Fontaine, & Bourbigot, 2010), fuel cell (Ma & Sahai, 2013),

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bio-chemical separation (as cation exchangers) (Suflet, Chitanu, & Popa, 2006), and other applications (such as protein adsorbents) (Oshima, Taguchi, Ohe, & Baba, 2011).

Given the importance of the phosphoric functional groups, many methods for cellulose phosphorylation have been developed either by the reaction on the cellulose free hydroxyl groups at C2, C3, and C6 positions or via displacement of an ester or ether group already present in the cellulose. The latter are usually applied as homogeneous chemical reactions using mainly toxic reagents and organic solvents that result into partial or total disruption of the original fibrous integrity. The synthesis of phosphorus cellulose derivatives has thus been performed in various solvents [dimethyl sulphoxide (DMSO)–methylamine, DMAc–LiCl, $SO_3$–triethylamine, formic acid, trifluoroacetic acid, N,N-dimethylformamide (DMF)–$N_2O_4$, paraformaldehyde, trimethylchlorosilane–DMF, urea in melt or aqueous–NaOH], with many variants of phosphorous compounds (phosphoric and phosphinic acids, phosphorus oxoacids, phosphorus pentoxide, amidophosphates, alkyl or aryl derivatives of phosphorous acids) (Granja et al., 2001a, 2001b; Isogai & Atalla, 1998; McCormick, Callais, & Hutchinson, 1985; Nifant'ev, 1965; Oshima et al., 2011; Petreus, Bubulac, Petreus, & Cazacu, 2003; Ramos, Assaf, El Seoud, & Frollini, 2005). The highest substitution degree (DS between 1 and 2.8) of water soluble phosphorylated cellulose was obtained from the reaction of MCC with phosphorous acid/urea, either in melt (Inagaki, Nakamura, Asai, & Katsuura, 1976; Suflet et al., 2006) or subsequently to microwave activation (Gospodinova et al., 2002). Phosphorylation of different cellulose derivates (i.e. acetate, propionate, etc.) was also reported using combinations of different solvents as well as phosphoryl donors (i.e. phosphoric acid, ethanol, hexanol, and phosphorus pentoxide or diethyl chlorophosphate, diethyl chlorothiophosphate, dimethyl chlorophosphate with pyridine, etc.) (Aoki & Nishio, 2010; Li et al., 2012).

However, such a functionalisation may not be adequately applied if the integrity of the cellulose polymer chain is to be preserved without a side-reaction such as intermolecular crosslinking (Garcia-Ubasart, Vidal, Torres, & Rojas, 2013). Thus, the phosphorylation of cotton (Tzanov et al., 2002) or NFC (Božič, Liu, Mathew, & Kokol, 2014) using site-specific hexokinase-mediated reactions in the presence of phosphoryl donor adenosine-5′-triphosphate was achieved with low DS of up to 0.43 at the C6 hydroxyl group of the cellulose glucopyranose ring.

This paper was thus the first study on chemical phosphorylation of nano-sized fibrillated and crystalline cellulose using heterogeneous (with aqueous phosphoric acid) and homogeneous (with phosphoric acid in molten urea) methods, respectively, and also providing the dimensional, surface chemistry, crystalline and thermal, as well as hydrolytic stability characterisations of the obtained derivatives.

## 2. Experimental

### 2.1. Materials

The cellulose sludge with moisture content of 48 wt% was supplied by Domsjö Fabrikerna AB, Örnsköldsvik from Sweden and used without any pre-treatment as a raw material for the preparation of nanocellulose (NC). The cellulose sludge was reported to be high within the cellulose content (95%) with some hemicellulose and trace amounts of lignin (Jonoobi, Mathew, & Oksman, 2012).

The cellulose bioethanol residue, industrial residue from wood ethanol production, was supplied by SEKAB E-Technology, Örnsköldsvik, Sweden. It was in the form of dark brown powder, consisting of small particles within the range of 2–100 μm. The product obtained had about 50% cellulose, a high amount of lignin (44%) and some extractives.

Phosphoric acid ($H_3PO_4$), urea ($(NH_2)_2CO$), NaOH, $KH_2PO_4$, 2.6 pyridine di-carboxylic acid (PDC), hexadecyl trimethyl ammonium chloride ($CH_3(CH_2)_{15}N(Cl)(CH_3)_3$) and methanol (MeOH) were purchased from Sigma. All other chemicals were of the highest commercially available grades.

### 2.2. Preparation of nanocellulose (NC)

The fibrillated nanocellulose (NFC) was prepared from cellulose sludge according to the procedure reported by Jonoobi et al. (2012). The cellulose sludge was first suspended within distilled water and dispersed in water to a concentration of 3 wt% by using a mechanical blender (Silverson L4RT, England) at 3000 rpm for 10 min. Then, the suspension was passed through an ultra-fine grinder (MKCA 6–3, Masuko, Japan) at 1440 rpm until a gel was formed. The processing time was 30 min. The AFM image analysis (measured by Nanoscope V software) showed that the produced NFC were chain-like structures of diameters within the range 10–70 nm and the length of micrometre scale (Jonoobi et al., 2012).

The nanocrystalline cellulose (NCC) was extracted from the cellulose bioethanol residue, by following a modified version of the procedure described by (Oksman, Etang, Mathew, & Jonoobi, 2011). Lignin and the extractives were removed after de-waxing, bleaching and cleaning. The purified cellulose was made into 2 wt% suspensions, mixed by shear mixture and passed through the homogeniser 10-times in order to obtain a thick gel of NCC. The produced NCC showed a typical cellulose nanocrystalline structure with a measured diameter of 5–10 nm (Peng, 2014).

### 2.3. Phosphorylation of NC with phosphoric acid in aqueous media (heterogeneously)

Phosphoric acid (85%, v/v) was slowly added to 3.0 wt% NC dispersion, respectively (keeping the temperature below 30 °C) via a dropping funnel until phosphoric acid concentrations of defined molarity (10.7 M, 13 M and 14.8 M) were reached. After acid addition, the reaction vessel was placed in an oil bath that was preheated to defined temperatures (100 °C, 120 °C and 140 °C) and the mixture was stirred over different periods of time (10, 30 and 60 min). The slightly yellow reactive mixture was subsequently cooled in an ice bath to room temperature. The phosphorylated NCs (p-NC) were separated from the liquid by centrifugation at 9000 rpm for 15 min. The supernatant was decanted (and stored for CE and elemental analysis), replaced by an equal amount of fresh ultrapure water, and the mixture was centrifuged again. This procedure was repeated at least ten times until the supernatant was colourless. The p-NC dispersions were further dialysed against ultrapure water for five days by exchanging the water every day until neutral pH (pH ≈ 7). The final dispersions were subsequently sonicated for 2 min at 25% amplitude and stored in a refrigerator or frozen and lyophilised, depending on the further analysis.

### 2.4. Phosphorylation of NC with phosphoric acid in molten urea (homogeneously)

The reaction was performed in a 250 ml, four-necked flask equipped with a condenser, a thermometer and a stirrer. 6.4 g of urea was added to the flask and heated to 140 °C in order to melt the urea. Then 1 g of suspended NC in 1.4 g of water and 5.14 g of phosphoric acid (final 10.7 M phosphoric acid) were added alternatively portion-wise to the molten urea in order to reduce the foaming. The reaction was allowed to proceed at 150 °C for 30 min. The reactive mixture was dissolved in 1 M NaOH and then precipitated

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