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Phosphorylation of Cellulose in the Presence of Urea. Mechanism of Reaction and Reagent Impact.

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Abstract

A mechanism of cellulose phosphorylation in presence of urea is proposed. This model is correlated with the thermal stability of phosphorylating reactive mixture and the yield of phosphorylation for two different reagents: phosphoric acid and lauryl phosphate monoester. Three main successive reactions are involved in the formation of cellulose phosphate: (1) generation of ammonia by urea decomposition, (2) formation of phosphoramidate as intermediate reactive due to the reaction of ammonia with phosphate species, and (3) grafting the phosphate moiety to substrate due to the transfer of phosphate from phosphoramidate to cellulose. The proposed in vitro mechanism is supported by similar phosphorylation processes observed in some biochemical reactions. The limiting factor in the phosphorylation of cellulose is the formation of phosphoramidate intermediate.

KEYWORDS: *cellulosic fibers; phosphorylation reaction; reagents; urea; phosphoramidate; mechanism.*

Graphical abstract

INTRODUCTION

During the last few decades, biomaterials have gained an increasing interest as alternatives to decrease the dependency on petroleum-based products. Among these biomaterials, cellulose is the most abundant organic raw material that is a renewable and biodegradable resource and finds applications in different areas. However, to impart additional and desirable features, which are not normally found in the pristine cellulose, physical or chemical modification of its surface is an essential step. Consequently, there is a rising interest in cellulose derivatives due to the perpetuity of this biomaterial. One of these cellulose derivatives is cellulose phosphate, more precisely named cellulose-*O*-phosphates, which can be obtained by chemical reaction to graft phosphate groups onto cellulose fibers. These cellulose phosphates have displayed several properties that promote their use in many fields, such as flame retardant materials (Inagaki et al. 1976; Jain et al. 1985; Zhang et al. 2020; Shi et al. 2021), water purification and ion exchange capacity for some transition metals (Nada and Hassan 2003; Ubaidullaev et al. 2004; Oshima et al. 2008; Luneva and Ezovitova 2014), superabsorbent materials (Leo J Bernardin 1972; Saito et al. 1991; Shi et al. 2015), osteogenic materials (Mucalo et al. 1995; Wan et al. 2007; Leone et al. 2008), energy storage/supercapacitor (Jiang et al. 2012; Belosinschi and Tofanica 2018; Rana et al. 2020), agriculture (Harada et al. 2021), antimicrobial materials (Blanchette et al. 2020; Wu et al. 2021) and other fields (Kim et al. 1998; Wu et al. 2021).

Various methods of phosphorylating cellulosic materials have been investigated. (Tzanov et al. 2002) described the first enzymatic phosphorylation using hexokinases to transfer one phosphate moiety from adenosine triphosphate (ATP) to the C6-hydroxyl groups in cellulose. This method has been adopted by (Božič et al. 2014; Liu et al. 2015). The highest degree of substitution (DS) obtained (i.e., average number of phosphate moiety grafted to the anhydroglucose unit (AGU) of cellulose) was about 0.4. This value is supposed to be significant using enzymes. However, the enzymatic catalysis remains a phosphorylation method exclusively for fundamental research, having very little chances to be scaled-up in the near future. The major shortcomings for this method are the high price of enzymes such as kinases, the relative ease of inhibition of biocatalysts, and the limitation of enzymes to one specific substrate. Phosphorylation with trivalent phosphorus derivatives (Gospodinova et al. 2002) (e.g., hydrogenphosphite HPO_3^{2-}) is another common reaction used to phosphorylate cellulose. However, the product obtained with these phosphite species is unstable redox and brings two times less charges for each grafted moiety on the cellulose surface given the diprotic nature of phosphorous acid. This situation significantly limits the application potential of phosphorylated cellulose with phosphite reagents; therefore, this case will not be considered herein. Phosphorylation with condensed phosphoric species in organic solvents is another pathway

that has been followed to phosphorylate cellulosic substrates. In this case, phosphorus pentoxide (P_2O_5) and polyphosphoric acid (PPA) alone or in mixture with phosphoric acid (H_3PO_4) are used as phosphorylating reagents. To obtain a high degree of substitution ($DS \sim 2.5$), aprotic organic solvents, such as alcohols like hexanol, propanol, butanol (Touey 1956; Granja et al. 2001b, a) and dimethylformamide (Kim et al. 1998; Kowalik et al. 2000) are required to dissolve these condensed phosphoric reagents without solvolysis. The obtained phosphorylated fibers are completely soluble in water, depending on the reaction conditions and the size of the cellulosic substrate. Nevertheless, this phosphorylation procedure is impossible to scale-up due to the high environmental hazard posed by the usage of these toxic solvents. Phosphorylation with acid chlorides derivatives is another route to phosphorylate cellulose. Phosphorous oxychloride ($POCl_3$), alkyl, aryl, and polyol phosphorochloridates ($ClP(O)(OR)_2$) are some phosphorylating reagents that fall into this category (Nifant'ev 1965; Richard Horrocks and Zhang 2001; Aoki and Nishio 2010). The presence of chlorine in the chemical structure of the reagents generates hydrochloric acid as a by-product. An acidic environment causes the degradation of cellulosic substrates and, therefore, a basic scavenger such as ammonia, sodium hydroxide, or pyridine must be used to neutralize the acid produced during this reaction. In some specific cases, organic solvents such as dimethylformamide or chloroform are used to dissolve the phosphorylation reagents. All these aspects, along with the high price of the phosphoric acid chlorides derivatives, limit this phosphorylation approach to a narrow application field of fire retardants, where small quantities of grafted phosphate are needed.

Phosphoric acid (H_3PO_4) is one of the main pentavalent phosphorus precursors used in cellulose phosphating reactions. However, when it is used alone, this reagent shows very little reactivity toward cellulose. Moreover, its acidic character promotes the hydrolysis of the amorphous domain of the native cellulose structure and shows lower esterification reactivity, which results in a significant degradation of the cellulosic substrate (Niu et al. 2020). This drawback is partially overcome if urea is added in the phosphorylation system. The introduction of urea among the phosphorylation reagents, has been widely investigated and remarkably increases the reaction yield (Kokol et al. 2015; Ghanadpour et al. 2015; Noguchi et al. 2017; Rol et al. 2019, 2020; Ait Benhamou et al. 2021).

Despite the importance of urea in the phosphorylation reaction, the degradation of cellulose substrate is an inescapable issue. Derivatives of phosphoric acid, catalysts, and special reaction conditions must be present to increase the reaction rate, to avoid the substrate degradation, and to obtain a reasonable phosphorylation yield. So far, no study has been able to develop a process that meets all the criteria listed above. As a result, cellulose phosphate is the only ordinary cellulose derivative that is not commercially available despite its tremendous potential of applicability. In a previous work, we developed an effective process using phosphate esters instead of phosphoric acid as phosphorylating reagent in the presence of urea (Belosinschi et al. 2017), and the obtained phosphorylated cellulosic substrates have remarkable ionic charge in water, up to 7000 mmol/kg without noticeable signs of degradation.

Among the procedures mentioned above, phosphorylation of cellulose in presence of urea is believed to be the most effective procedure with real chances to be successfully implemented at industrial scale. The latter continues to be delayed because so far, and to the best of our knowledge, no study has proposed a viable reaction mechanism of phosphorylation in presence of urea. The most advanced study on this topic dates from 1956 and was published by (Nuessle et al. 1956). The authors concluded that the urea serves as solvent medium for the phosphorylation reaction. Two pieces of evidence contradict their conclusion: 1) the urea and the phosphoric acid form a deep eutectic solvent at around $100^\circ C$ but 2) the phosphorylation yield is significant only when the temperature exceeds $140^\circ C$ (i.e., after the urea starts to decompose). The present paper is thus the first study to propose a viable mechanism of cellulose phosphorylation that considers the main changes and all possible interactions between reagents at different temperatures in the reaction pathway. Two different routes were adopted to produce cellulose phosphate: (i) phosphorylation with H_3PO_4 -urea, and (ii) phosphorylation with lauryl phosphate monoester-urea. A comparison based on the proposed mechanism will be discussed.

Materials and Methods

Materials

The cellulosic substrate used in this study was bleached softwood kraft fibers (BSKF) supplied by Kruger Wayagamack (Trois- Rivières, Canada). Lauryl alcohol ($C_{12}H_{26}O$; CAS Registry Number: 112-53-8), polyphosphoric acid (115%, $(H_{(n+2)}P_{(n)}O_{(3n+1)})$; CAS Registry Number: 8017-16-1), phosphoric anhydride (P_4O_{10} ; CAS Registry Number: 1314-56-3), phosphoric acid (85%, H_3PO_4 ; CAS Registry Number: 7664-38-2), urea (99% NH_2CONH_2 ; CAS Registry Number: 57-13-6), Analytical grade chemicals and solvents were always used as received without further purification.

Methods

Synthesis of lauryl phosphate monoester (LPM)

The phosphate ester used in the study is laboratory synthesized according to the method elaborated by Tracy and Reiersen (2002) as shown in Fig. 1. This treatment yields mainly monoalkyl phosphates. The preparation process is as follows: 200 g (1.073 mol) of lauryl alcohol were added into an 800 ml capacity beaker and slightly heated on a plate at 40 °C until completely melted. The beaker was then immersed in a coolant (ice/water slush mixture) prior to adding the other reactants because the reaction is highly exothermic and requires a cooling system. Then, 57 g of 115% polyphosphoric acid and 28 g (0.2 mol) of phosphoric anhydride were progressively added to lauryl alcohol under vigorous agitation to maintain the temperature of the reaction under 60 °C. The esterification of lauryl alcohol with phosphate reagents was performed at 85 °C for 11 hours. The crude product was used without further purification and, according to this method, the mixture contains 80% of lauryl phosphate monoester on average.

Fig. 1 Synthesis of LPM

Phosphorylation of bleached softwood kraft fibers BSKF

Phosphorylation of BSKF was carried out by two different routes, using lauryl phosphate monoester (LPM) and phosphoric acid (H_3PO_4) as phosphoryl donors (Fig. 2).

Fig. 2 Phosphorylation of BSKF

Phosphorylation of BSKF with LPM

Phosphorylated BSKF were synthesized by the esterification reaction in molten urea between LPM and BSKF following the method described by (Belosinschi et al. 2017). In the first step, 25 g of LPM and 11.5 g of urea were added in a crystallizing dish (170 mm by outer diameter). The dish was placed in a preheated oven at 105 °C and atmospheric pressure for 20 minutes to allow urea to melt and form a homogenous liquid. In the second step, 15 g of disk-shaped dried BSKF (150 mm in diameter) were impregnated over the molten reaction mixture (LPM and urea) for 5 minutes, and the cellulose substrate was then overturned on the other side and the impregnation was continued for 5 additional minutes. In the third step, the mixture was cured at 160 °C for 30 minutes. In the fourth step, the reaction was stopped by adding 400 ml of DI water to the cellulose cake at room temperature, and

the mixture was left for 15 minutes. The reaction mixture was then poured into a 1L beaker, and the fibers were mixed well with a hand mixer. The modified BSKF thus obtained were separated from the liquid phase by a filter assembly consisting of a Buchner funnel and a paper machine wire. The final consistency of the solid was about 25%. This fourth step was repeated 3 more times with water and two times with denatured ethanol to ensure a thorough wash of fibers and a complete elimination of side products.

Phosphorylation of BSKF with H₃PO₄

Phosphorylation of BSKF with H₃PO₄ (11g) was carried out with the same procedure, described above. The reaction parameters such as pressure (atmospheric pressure), temperature (160 °C), time (30 minutes), and stoichiometry (AGU of cellulose/ phosphorylation reagent/urea of 1/1/2) were kept the same.

Thermogravimetric analysis (TGA)

Thermal stability analysis (TGA and DTGA) of the samples was carried out in a Perkin-Elmer (Pyris Diamond) Thermoanalyzer. Samples of pure urea and two different phosphorylation mixtures (phosphoric acid/urea and lauryl phosphate monoester/urea) were heated in open platinum pans from room temperature to 250 °C, under a nitrogen atmosphere, at a heating rate of 5 °C/min. The latter was chosen at this low value to increase chances of capturing the most important chemical structural changes of the phosphorylation reaction mixture with the temperature.

Determination of total charge of fibers

The total charge density of the phosphorylated fibers was determined by conductimetric titration using a Thermo (USA) Orion conductometer (Model 150) and a Metrohm Brinkmann (USA) automated titrator (Dosimat 765) under nitrogen. In short, an appropriate amount of acid-washed fibers was dispersed in a 1 mM aqueous sodium chloride solution and titrated with 0.1 N NaOH. More details of the procedure was described in our previous work (Shi et al. 2015).

Dimensional characteristics of fibers

The mean arithmetic fiber length, length weighted percentage of fine and fiber width were analyzed with an OpTest Fiber Quality Analyzer (FQA) device. The data collected allow to highlight the impact of phosphorylation procedure on fiber morphology.

Scanning electron microscopy (SEM)

The SEM images of typical samples were obtained with a JEOL JSM-5500 Scanning Electron Microscope. Samples were gold coated using an Instrumental Scientific Instrument PS-2 coating unit. The SEM operating voltage was at 15.0 kV.

RESULTS AND DISCUSSIONS

It is worth noting that the purpose of TGA was to reveal the most important chemical structural changes of the phosphorylation reaction mixture while maintaining the same conditions of the reagents during the phosphorylation reaction. Thus, a heating pre-treatment prior to thermal analysis for both reaction mixtures (phosphoric acid/urea and lauryl phosphate monoester/urea) was necessary. This pre-treatment was conducted as follows: a small amount of H₃PO₄ 85% and urea 99% with a molar ratio of 1/2, respectively, were added to a beaker and heated in an oven up to 105 °C, for no longer than the time needed to melt the urea and to turn the mixture into a homogeneous liquid. The mixture was then removed from the oven and left several minutes to cool down to the room temperature before thermal analysis. A similar procedure was conducted for the mixture lauryl phosphate monoester/urea. Here, two remarks must be taken into consideration: 1) the lauryl phosphate monoester is synthesized in laboratory as crude product, and 2) the molar ratio of lauryl phosphate monoester/urea is 1/2 respectively and was calculated considering the crude product as composed of 100% lauryl phosphate monoester.

Mechanism of cellulose phosphorylation with H_3PO_4 -urea

All the phosphorylation reactions that we performed at temperatures below 140°C under atmospheric pressure yielded a very low or even no additional charges on cellulose fibers. It appears from this fact that the phosphorylation reaction is conditioned by the onset temperature of urea decomposition in the reaction medium. This assumption is confirmed by evaluating the thermal stability of urea. As shown in Fig. 3, the thermogravimetric analysis indicates that neat urea decomposes at a significant rate beyond 150°C (point A on the DTGA curve of urea).

Fig.3 a) TGA and b) DTGA of urea

As mentioned above, the production of cellulose phosphate via phosphorylation reaction must be carried out at temperatures exceeding 140°C . Despite this, thermal behavior information below this critical temperature is crucial to investigate the final phosphorylation yield. Thus, a second thermal analysis of urea in the presence of phosphorylating reagent was performed. Fig. 4 shows the thermal behavior of phosphoric acid-urea mixture. As it can be observed, the water contained in H_3PO_4 85% begins to evaporate significantly from the reaction mixture at a temperature of around 120°C , as marked by point A on the DTGA curve of urea-phosphoric acid mixture. The evaporation rate increases as the temperature of the mixture increases as well. When the free water was completely evaporated, the condensation of phosphoric acid took place to form pyrophosphate structures as shown in Eq.1. In addition, urea starts to decompose into ammonia and isocyanic acid, as shown in Eq.2. Here, it should be noted that the onset temperature of urea decomposition shifted to low temperature (140°C point B on the DTGA curve of phosphoric acid-urea mixture) because H_3PO_4 forms eutectic mixture with urea. At this point, the weight loss was supposed to be instantaneous since both products resulting from decomposition of urea are gases. However, the DTGA curve records a reverse trend (i.e., a reduction of the weight loss rate). This evolution suggests that at least one of the gases resulted from the urea decomposition is retained in the condensed phase. The first reaction that is most likely to happen is the neutralization between the acidic species of phosphoric acid and ammonia as a base as shown in Eq.3 and Eq.4.

Fig.4 a) TGA and b) DTGA of H3PO4-urea

Eq.1. Pyrophosphate formation through thermal dehydration of phosphoric acid

Eq.2. Decomposition of urea with a temperature exceeding 140 °C

Eq.3. Partial neutralization of pyrophosphoric acid with ammonia

Eq.4. Partial neutralization of phosphoric acid with ammonia

The neutralization process is partial at elevated temperatures (i.e., only one molecule of ammonia will bond for each phosphate moiety present in the mixture). At room temperature, the ammonia is capable to neutralize two of the three protons of phosphoric acid with the formation of diammonium phosphate. Meanwhile, the neutralization reaction is limited to a single proton beyond 140° C. This situation can be confirmed by comparing thermal stability of diammonium phosphate with that of monoammonium phosphate. Diammonium phosphate begins to lose one molecule of ammonia at temperatures below 100° C (<https://wcms.agro-schuth.de/sicherheitsdb/DiammoniumPhosphate.pdf> 2003), while the monoammonium phosphate is stable up to 190° C.

Based on work by (Nuessle et al. 1956), It appears that the diammonium pyrophosphate is the active species of phosphorylation process. However, this hypothesis is not sustainable because this species can be obtained by direct condensation of monoammonium, as shown in Eq.5, or diammonium phosphate compounds. Thus, the phosphorylation of cellulose with monoammonium or diammonium phosphate reagents could be theoretically performed in the absence of urea. However, from a practical point of view, the presence of urea in the reaction mixture is always mandatory regardless of the phosphorylation reagent used.

Eq.5. Further condensation of monoammonium phosphate to diammonium pyrophosphate

In addition, the DTGA curve of phosphoric acid-urea mixture shows a significant reduction in weight loss between points B and C. It suggests that additional amounts of ammonia are kept in the condensed phase. A second reaction between ammonia and phosphate that may increase the retention rate of ammonia is likely to happen. In these circumstances, the only way a second molecule of ammonia could bind to phosphate is as an amine function rather than a regular ammonium counterion. The product of this reaction is an intermediate phosphoramidate as shown in Eq.6. The reaction equilibrium is pushed to the right (i.e., toward phosphoramidate formation) due to the anhydrous medium, the high temperatures and the availability of ammonia in large quantities. The formation of the phosphoramidate intermediate is the key to an efficient phosphorylation of cellulose. Evidence of phosphoramidate formation can be found in some biochemical reactions where the ammonia kinase transfers one phosphate from adenosine triphosphate (ATP) to a nitrogenous group like NH₄⁺ as an acceptor (Dowler MJ 1968).

Eq.6. Formation of phosphoramidate intermediate

The amine bond in phosphoramidate is unstable in the presence of alcoholic OH groups of cellulose. A transfer of phosphate from phosphoramidate to cellulose via OH occurs and ammonia is released at high temperatures, which results in the formation of cellulose phosphate (Eq.7). A similar process can be found in some biochemical reactions where the hexose phosphotransferase transfers the phosphate from donors like phosphoramidate to an alcohol group of hexoses like glucose (Smith and Theisen 1966).

Eq.7. Formation of cellulose phosphate

Theoretically, the ammonia is quantitatively returned in the reaction medium. Thus, it can be considered as the real catalyst for the phosphorylation reaction. At this point, it can be concluded that the phosphorylation of cellulose could be performed with ammonia instead of urea, according to the proposed mechanism. However, the attempts in this direction did not give the expected results. In fact, the gaseous state of the ammonia makes its manipulation as catalyst in the phosphorylation of cellulose almost impossible. By comparison, urea exhibits some undeniable advantages such as: a high melting point that allows a safe handling, a large amount of ammonia enclosed in the molecule, formation of a deep eutectic solvent with acidic phosphate species, and, most importantly, generation of ammonia in situ during the phosphorylation process. Moreover, the decomposition of urea occurs at elevated temperatures that force a second ammonia (NH_3) molecule to bond to phosphate moiety as an amine ($-\text{NH}_2$) rather than as an ammonium ion (NH_4^+). The major drawback of using urea is that its decomposition is incomplete and generates as much isocyanic acid as ammonia. The isocyanic acid is very reactive at high temperature, being involved in a series of side reactions during phosphorylation such as: carbamylation of cellulose by grafting carbamate as a secondary moiety in addition to phosphate (Eq.8); decomposition with water to liberate ammonia and carbon dioxide (Eq.9); condensation with another molecule of urea to form biuret as by-product in the final reaction medium (Eq.10).

Eq.8. Formation of cellulose carbamate

Eq.9. Decomposition of isocyanic acid with water

Eq.10. Formation of biuret

Mechanism of cellulose phosphorylation with (LPM-urea)

Phosphorylation of cellulose with lauryl phosphate monoester follows a similar pattern as the one proposed for phosphoric acid, with some small particularities. The thermal decomposition of LPM-urea mixture was tested using thermogravimetric analysis, as shown in Fig. 5.

First, the water and other volatile organic compounds contained in the crude lauryl phosphate monoester were evaporated by heating the reaction mixture (the gap between the point A and B on the DTGA curve of LPM-urea mixture). Starting from 140° C, the ammonia emerged in the system because of urea decomposition. Simultaneously, one of the two protons of the LPM was neutralised by ammonia base as shown in Eq.11. This partial neutralization was expected to occur because the pKa values of LPM were determined in a previous paper (Shi et al. 2014) and they were found close to first and second pKa of phosphoric acid.

Fig.5 a) TGA and b) DTGA of LPM-urea

Eq.11. Partial neutralization of lauric phosphate monoester with ammonia

A second molecule of ammonia can attack and cleave the ester bond in the phosphate ester at such high temperatures. The result of this reaction is the formation of intermediate phosphoramidate, as shown in Eq.12. The intensity and the area of the peak between point B and C on DTGA curves of the reaction mixtures are proportional to the ability of the phosphorylating reagent to absorb ammonia by partial neutralization and

amination reactions. This peak is clearly more intense and larger when phosphoric acid is used as phosphorylating reagent than the lauryl phosphate monoester. This proves that ammonia is much more efficient in the cleavage of the P-O-P bonds from pyrophosphates than C-O-P bonds from phosphate esters. Consequently, it is expected that the rate of phosphorylation is higher in the case of using phosphoric acid rather than lauryl phosphate monoester as phosphorylating reagent.

Eq.12. Formation of phosphoramidate intermediate from lauryl phosphate monoester

The last reaction of the phosphorylation is the transfer of phosphate from phosphoramidate to cellulose via hydroxyl groups with the formation of cellulose phosphate as stated before. This transfer raises an apparent issue in this case because two different OH—one from cellulose and the other from lauryl fatty alcohol—are present simultaneously in the system. The phosphate moiety goes with predilection to cellulose due to higher availability, lack of steric hindrance and greater polarity of hydroxyl in cellulose than in lauryl fatty alcohol.

Impact of the phosphorylating reagent

The charge content of phosphorylated cellulose samples was determined by the electric conductivity titration method (Shi et al. 2015). The results of this titration are shown in Fig. 6.

Fig.6 Conductimetric titration of a) phosphorylated BSKF with H₃PO₄-urea mixture and b) phosphorylated BSKF with LPM-urea mixture

The total charge content is given by the following equation:

$$CC(\text{mmolkg}^{-1}) = \frac{C_{\text{NaOH}} \times V_{\text{NaOH}}}{W_{\text{phosphorylated BSKF}}} \times 1000$$

Where V_{NaOH} is the added volume (L) corresponding to the plateau length, C_{NaOH} is the employed concentration of NaOH (M), and W_{phosphorylated BSKF} is the used weight of phosphorylated samples (g).

Pristine cellulose fibers contain a small amount of carboxyl and sulfonate charges most probably generated by kraft cooking and bleaching treatments. The phosphorylation grafts a large quantity of phosphate moiety on the fiber surface and, consequently, the charge density increases substantially as shown in Table 1.

Table 1: Charge density and dimensional characteristics of cellulose fibers before and after phosphorylation

Sample	Charge content (mmol/kg)	Average length (mm)	Average diameter (μm)	Fines (%)
BSKF	80 $\pm$ 10	1.52 $\pm$ 0.02	25.3 $\pm$ 0.1	0.6 $\pm$ 0.05
Phosphorylated BSKF with H_3PO_4	5500 $\pm$ 60	1.54 $\pm$ 0.03	29.55 $\pm$ 0.12	0.5 $\pm$ 0.1
Phosphorylated BSKF with LMP	5350 $\pm$ 50	1.53 $\pm$ 0.03	29.03 $\pm$ 0.15	0.5 $\pm$ 0.1

Although a higher phosphorylation yield was expected using phosphoric acid due to a more efficient formation of the phosphoramidate, phosphoric acid and lauryl phosphate monoester gave almost the same phosphorylation yield. This is due to the high molecular weight of LPM ($M_w = 267 \text{ g/mol}$), compared to that of phosphoric acid (98 g/mol), which allows for better impregnation and high distribution of the mixture when the phosphate ester is used as phosphating agent. Moreover, the aliphatic chains of LPM behave as a lubricant medium that prevents the cellulosic substrate from over-drying because of high temperatures used during the curing process. In addition, as it can be noticed in the Table 1, results of fiber dimensions (average length and fines %) clearly show that the phosphorylation method adopted in this work prevents the cleavage of the fibers and preserves their initial sizes. In fact, in all types of phosphorylation processes, fibre degradation is considered an inevitable issue, which directly affects the length of pristine fibers. For instance, (Ablouh et al. 2021) phosphorylated cellulose substrate by using the same phosphorylating reagents with a large amount of urea. The latter is considered an essential element to obtain high phosphorylation yield. However, the average length of the fibers was reduced by more than half after phosphorylation. This decrease in fibers length results in a significant increase of the fines percentage, which clearly confirms the importance of the method used for fiber phosphorylation regardless of the amount of urea. Scanning electron microscope (SEM) was employed to understand more about the surface morphology of fibers. SEM images of BSKF and phosphorylated BSKF are shown in Fig. 7. The surface of non-phosphorylated BSKF in Fig 7 (a-b) is relatively rough and consists of small amounts of microfibrils located at the surface of BSKF. These fibrils disappeared after phosphorylation, regardless of the phosphorylating reagent used (Fig. 7 c-d-e-f), and the fibers became smooth compared to these of BSKF.

Fig.7 Scanning electron microscopy micrographs at different magnifications of BSKF (a-b), phosphorylated BSKF with LPM-urea mixture (c-d) and phosphorylated BSKF with H_3PO_4 -urea mixture (e-f)

Conclusions

The present work is the first scientific paper that proposes a viable mechanism of cellulose phosphorylation. The mechanism is consistent with the results from thermogravimetric analyses and is supported by similar processes found in some specific biochemical reactions. These fundamental advancements pave the way for the scale-up of the phosphorylation process in a near future.

Ammonia is the real catalyst of the phosphorylation process of cellulose in presence of molten urea. The gaseous state of ammonia makes the retention of this catalyst very difficult in the phosphorylating mixture. Hence, a source of ammonia such as urea, capable to generate the catalyst in-situ, is preferred.

The phosphorylation is entirely conditioned by the formation of phosphoramidate as reaction intermediate. This species is formed at a higher rate when phosphoric acid rather than lauryl phosphate monoester is used as

phosphorylating reagent. The transfer of phosphate moiety from phosphoramidate to cellulose is the final step of the phosphorylation process. The phosphorylation method proposed in this study allows for the production of phosphorylated fibers with good yield, low energy consumption, low addition of chemical reagents, and most importantly with minor signs of fiber degradation.

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Figure Legends

Fig. 1 Synthesis of LPM

Fig. 2 Phosphorylation of cellulose

Fig.3 a) TGA and b) DTGA of urea

Fig.4 a) TGA and b) DTGA of H₃PO₄-urea

Fig.5 a) TGA and b) DTGA of LPM-urea

Fig.6 Conductimetric titration of a) phosphorylated BSKF with H₃PO₄-urea mixture and b) phosphorylated BSKF with LPM-urea mixture

Fig.7 Scanning electron microscopy micrographs at different magnifications of BSKF (a-b), phosphorylated BSKF with LPM/urea mixture (c-d) and phosphorylated BSKF with H₃PO₄/urea mixture (e-f)

Declarations

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Conflicts of Interest / Competing Interests

The authors declare no conflicts of interests

Availability of data and material (data transparency)

The authors confirm that all the data supporting the findings of this study are available within the article.

Code availability (software application or custom code)

Not applicable

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The mechanism of reaction and experimental part were proposed by Dan Belosinschi. The authors share equal contributions on all other aspects.

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