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# Cellulose fibers modification through metal-free click chemistry for the elaboration of versatile functional surfaces.

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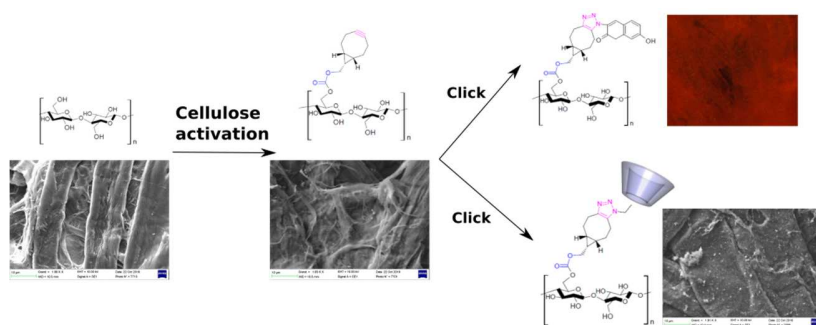
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**KEYWORDS:** cellulose functionalization,  $\beta$ -cyclodextrin, metal-free click chemistry

**ABSTRACT:** In the present work, a novel and versatile way was developed to functionalize cellulose fibers from wipes under heterogeneous conditions. The technic was based on metal-free click chemistry to avoid the persistence of any metal traces on cellulose. First, cellulose fibers were covalently pre-functionalized with bicyclononyne (BCN) to promoted azide-alkyne addition with molecules of interest, like coumarin and  $\beta$ -cyclodextrin derivatives. X-ray photoelectron microscopy (XPS) shows that one BCN group per cellulose unit was modified at the surface of the wipe. 3-azido-7-hydroxycoumarin that can be used as fluorophore for biosensing applications was efficiently bound to the pre-functionalized cellulose with a yield of reaction estimated at 60%. The addition of 6-azido-per-6-deoxy- $\beta$ -cyclodextrin ( $\beta$ -CD derivative) was investigated in order to target future organophosphorus decontamination properties to cellulose wipes. With the best reaction conditions, 60% of triazole groups corresponding to 4 reactive azido groups per  $\beta$ -CD derivative were detected by XPS proving the efficiency of the click reaction. The highest DS values were obtained after 96 h for  $\beta$ -CD 20 Eq (DS = 1.7 % by elementary analysis and DS = 2.5 % by weight measurements). Scanning electron microscopy observations highlighted a globally well distributed  $\beta$ -CD with some aggregates on the surface of the fibers. Thus, the pre-functionalized BCN-cellulose fibers could be used for many applications after an easy clicking of target compounds.



## 1. Introduction

Cellulose is the most abundant organic compound derived from biomass with a worldwide production in nature of many billions of tons per year [1] and so is of a great interest for the research of petroleum-based products replacement. It is used for various applications, especially for biomedical applications. For instance, cellulose is often used as support for biosensing applications. Thus, cellulose-based biosensor was used as chromatographic absorbents for virus purification [2], for the detection of DNA hybridization [3], of the protease activity [4], of the elastase activity [5,6], for the immobilization of enzymes [7]. Moreover, the immobilization of fluorescent molecules on cellulose enables to access to

useful tools for the cellular uptake study on support [8]. In addition, cellulose was used to immobilize proteins. Especially, Mulchandani *et al.* have immobilized organophosphorus hydrolase (OPH) [9] on cellulose by genetically modifying *Escherichia coli* cell to express both cellulose binding domains and OPH before immobilization on the surface of textile hollow fibers [9–11]. Due to the large use of organophosphorus agents as pesticides and as chemical weapons and causing roughly 3 million cases of severe poisonings and 220 000 deaths worldwide annually [10], this strategy is of particular interest to access to efficient decontamination means.

Numerous articles described the chemical modifications of cellulose fibers to bring various properties such as, hydrophobicity[12,13], thermoresponsivity[14], fluorescence[15], photo-responsivity[16], biological properties[17,18], optical properties[19]. All those studies show the interest to have an easy and versatile way to functionalize cellulosic textile.

Anyway, cellulose shows lack of reactivity due to its specific structure, which is its main drawback faced with synthetic polymers. The main described modifications were carried out mostly in homogeneous phase, and in this case, the solubilization of the cellulose leads to a destruction of fibers and consecutively to the loss of its specific properties (mechanical, specific surface). Moreover, for industrial processes, the modification of cellulose in solid phase directly on the fibers is more suitable.

Different methods were described in order to easily functionalize cellulose such as Diels-Alder reactions[20] or thiol-ene “click” chemistry [13,21,22] Recently, “click” chemistry with functional molecules showed its efficiency for the rapid, easy and more environmentally friendly surface functionalization [23,24]. Several groups developed a new cellulosic material thanks to copper azide-alkyne cycloaddition (CuAAC) reaction. To do so, different strategies are applied. The cellulose can be first tosylated before reaction with sodium azide to introduce the azido group. Finally, a “click” reaction is performed in presence of copper with a molecule containing an alkyne function[12,25–28]. An other way is also to adsorb on the cellulose surface carbomethoxycellulose bearing azido group[29].

Surprisingly, they are no report of cellulose modification through strain-promoted azide-alkyne cycloaddition (SPAAC). At the opposite of CuAAC, this reaction is metal free, which is huge asset for biological applications.

We report here the first study of the elaboration of a ready-to-use and biocompatible non-woven cellulose wipe. Our original and efficient strategy is based on the preparation of a pre-functionalized material by covalently attaching bicyclononyne (BCN) at the surface of cellulosic fibers. This modified cellulose wipe is then ready to engaged in a free-metal cycloaddition and it allows to tune on-demand the surface properties of the cellulose with various molecules of interest depending on the further application. Here, we chose to work on the one hand with a coumarin derivative as fluorophore model for bio-sensing applications, and the other hand with  $\beta$ -cyclodextrin ( $\beta$ -CD) to target OP decontamination systems. Indeed, Hatton *et al.* had proved the efficient degradation of chlorpyrifos by an iodosobenzoic acid (IBA) modified cellulose [30]. In another hand, various studies had shown the efficiency of enzyme mimics based on CDs substituted by a prevalent iodine or any  $\alpha$ -nucleophile [31]. Consequently, immobilization of CD derivatives on cellulose could lead to an interesting solution in order to obtain a material having decontamination properties, *i.e.* sponges, protective clothes or even hemodialysis membranes for the treatment of poisoned people. To highlight the modified cellulose, we combined different analyses technics like X-ray photoelectron microscopy (XPS), scanning electron microscopy (SEM) and elementary analyses. To the best of our knowledge, this is the first study that describes the elaboration of highly modular cellulose *via* metal free click chemistry and with a full characterization of the performed modification.

## 2. Materials and Methods

### 2.1 Materials

4-dimethylaminopyridine (**DMAP**), anhydrous dimethylformamide (**DMF**) at 99.8%, anhydrous dichloromethane (**DCM**) at 99.8%, anhydrous methanol (**MeOH**) at 99.99% and White M2R (**calcofluor**) were purchased from Sigma-Aldrich. 3-azido-7-hydroxycoumarin was obtained from

Carbosynth Limited. Non-woven cellulosic wipes were obtained from Kimberley-clark® (Kimwipes® Lite).

#### 2.2 Synthesis of [(1R,8S,9S)-bicyclo[6.1.0]non-4-yn-9-yl] methyl 4-nitrophenyl carbonate (nitrophenylcarbonate-BCN).

(1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate was synthesized according to a known synthetic route [32].

#### 2.3 Synthesis of per-6-azido-β-cyclodextrin

Per-6-azido-β-cyclodextrin (**β-cyclodextrine-N<sub>3</sub>**) was prepared according to literature procedures [33]. The purification method was adapted as followed: after reaction, the raw product was obtained by precipitating the reaction mixture into 800 mL MeOH. After filtration, the product was washed several times with methanol (200 mL x10) until the filtrate becomes colorless. Finally, the product was dried at 60 °C under high vacuum. The compound was collected as a white powder with a yield of 80%.

#### 2.4 Synthesis of bicyclononyne cellulose (cellulose-BCN).

Cellulose-BCN was prepared by reacting nitrophenylcarbonate-BCN (0.411 g, 1.3 mmol, 18 Eq) with a piece of dried Kimwipe® paper (0.01 g, 0.074 mmol, 1 Eq) in presence of DMAP (0.0188 g, 0.15 mmol, 2 Eq) in 2 mL of DMF solvent at 80 °C and under N<sub>2</sub> inert atmosphere. The reaction mixture was stirred during 3 days before to be thoroughly cleaning, one time in DMF and twice with DCM. Finally, the sample was dried under vacuum. This reaction was realized twice. The substitution degree was determined at 16 % by elemental analysis.

#### 2.5 Synthesis of cellulose-β-cyclodextrin (cellulose- β-CD)

Dried cellulose-BCN (0.0038 g, 0.02 mmol, 1 Eq) was engaged in a “click” reaction with β-cyclodextrin-N<sub>3</sub> (0.567 g, 0.43 mmol, 22 Eq) in 2 mL of DMF under inert atmosphere at 80 °C during 5 days before to be thoroughly cleaning one in DMF and twice in DCM. Finally, the sample was dried under vacuum. The substitution degree was determined at 1.7 % by elemental analysis.

#### 2.6 Synthesis of cellulose-fluo

Dried cellulose-BCN (0.0012 g, 0.006 mmol, 1 Eq) was engaged in a “click” reaction with 3-azido-7-hydroxycoumarin (0.0196 g, 0.097 mmol, 15 Eq) in 2 mL of DMF under N<sub>2</sub> inert atmosphere at 80 °C during 5 days before to be thoroughly cleaning once in DMF and twice in DCM. Finally, the sample was dried under vacuum.

#### 2.7 Fourier Transform Infra-Red (FTIR) spectroscopy

FTIR spectra were recorded on an AVATAR 360 FTIR spectrometer (Thermo Fisher) using the ATR mode (equipped with a diamond crystal). 200 scans were collected for each measurement over the spectral range 4000–650 cm<sup>-1</sup>.

#### 2.8 Scanning Electron Microscopy (SEM)

SEM pictures were recorded on a Neoscope JCM 6000. The surfaces were fixed on a carbon tape and subsequently cold-coated using a JFC 1300 Autofine coater (metallization step).

#### 2.9 X-ray photoelectron microscopy (XPS) measurements

XPS analyses were performed using an Omicron Argus spectrometer (Taunusstein, Germany) equipped with a monochromated Al K $\alpha$  radiation source (h $\nu$  = 1486, 6 eV) working at an electron beam power of 300 W. Photoelectrons emission was analyzed at a takeoff angle of 90°; the analyses were carried out under ultrahigh vacuum conditions ( $\leq 10^{-10}$  Torr) after introduction via a load-lock into the main chamber. Spectra were obtained by setting up a 100 eV pass energy for the survey spectrum, and a pass energy of 20 eV was chosen for the high-resolution regions. Binding energies were calibrated against the C 1s binding energy of aliphatic carbon atoms at 284.3 eV. Element peak

intensities were corrected by Scofield factors [34]. Casa XPS v.2.3.15 software (Casa Software Ltd, U.K.) was utilized to fit the spectra, and the Gaussian/Lorentzian ratio was applied (G/L ration = 70/30).

## 2.10 Elemental analysis

Elemental analysis were performed on FLASH 2000 apparatus from Thermofisher with EAGER 300 as software. Found values for carbon, hydrogen, and nitrogen (if present) were within 0.4% of the calculated values for the proposed formula.

## 2.11 Optical microscopy

### 2.11.1 Calco-fluor analysis

The samples were placed in calcofluor white M2R fluorescent probe (0.01% w/v) that reacts with the OH group of cellulose. After rinsing in MilliQ® water, the samples were dried. Finally, the samples were observed by optical microscopy in fluorescence mode using a Leica DMLM device. The filter used was purchased from Zeiss and has the following characteristics: filter cube L5; excitation range: blue; excitation filter: BP 480/40; dichromatic mirror: 505; suppression filter: BP 527/30. All the pictures were taken with  $\times 10$  lens with 600 ms of exposure time.

### 2.11.2 Fluorescence imaging of fluorescent dye covalently bound to cellulose

Optical microscopy in fluorescence mode using a Leica DMLM device was carried out to check the effective “click” reaction between the cellulose-BCN and 3-azido-7-hydroxycoumarin. The filter previously described was used. All the pictures were taken with  $\times 10$  lens with 600 ms of exposure time.

## 2.12 Determination of the degree of substitution (DS)

### 2.12.1 By weight measurements

$$M_{cellulose-BCN} = M_{cellulose} + \%DS \times M_{add} \quad (1)$$

With  $M_{add}$  the molecular weight of the added group or added molecule on the cellulose unit.

From this formulae, we can deduce DS:

$$DS = 100 \times \frac{M_{cellulose-BCN} - M_{cellulose}}{M_{add}} \quad (2)$$

if  $n_1$ ,  $m_1$  and  $m_2$  referred to the number of mole of the native cellulose and of the weight of, respectively, the native cellulose sample before the reaction and the obtained cellulose-BCN after the reaction, we can deduce from equation (2):

$$DS = 100 \times \frac{\frac{m_2}{n_1} - M_{cellulose}}{M_{add}} \quad (3)$$

$$DS = 100 \times \frac{M_{cellulose}(m_2 - m_1)}{m_1 \times M_{add}} \quad (4)$$

With  $M_{cellulose} = 162.14 \text{ g.mol}^{-1}$  and  $M_{add} = 176.21 \text{ g.mol}^{-1}$

### 2.12.2 By elementary analysis of cellulose-BCN

The rate of carbon atom in cellulose-BCN was calculated as follows:

$$\%C = \frac{(n_C \times M_C)_{cell} + \%DS \times (n_C \times M_C)_{cell-BCN}}{M_{cell} + \%DS \times M_{cell-BCN}} \quad (7)$$

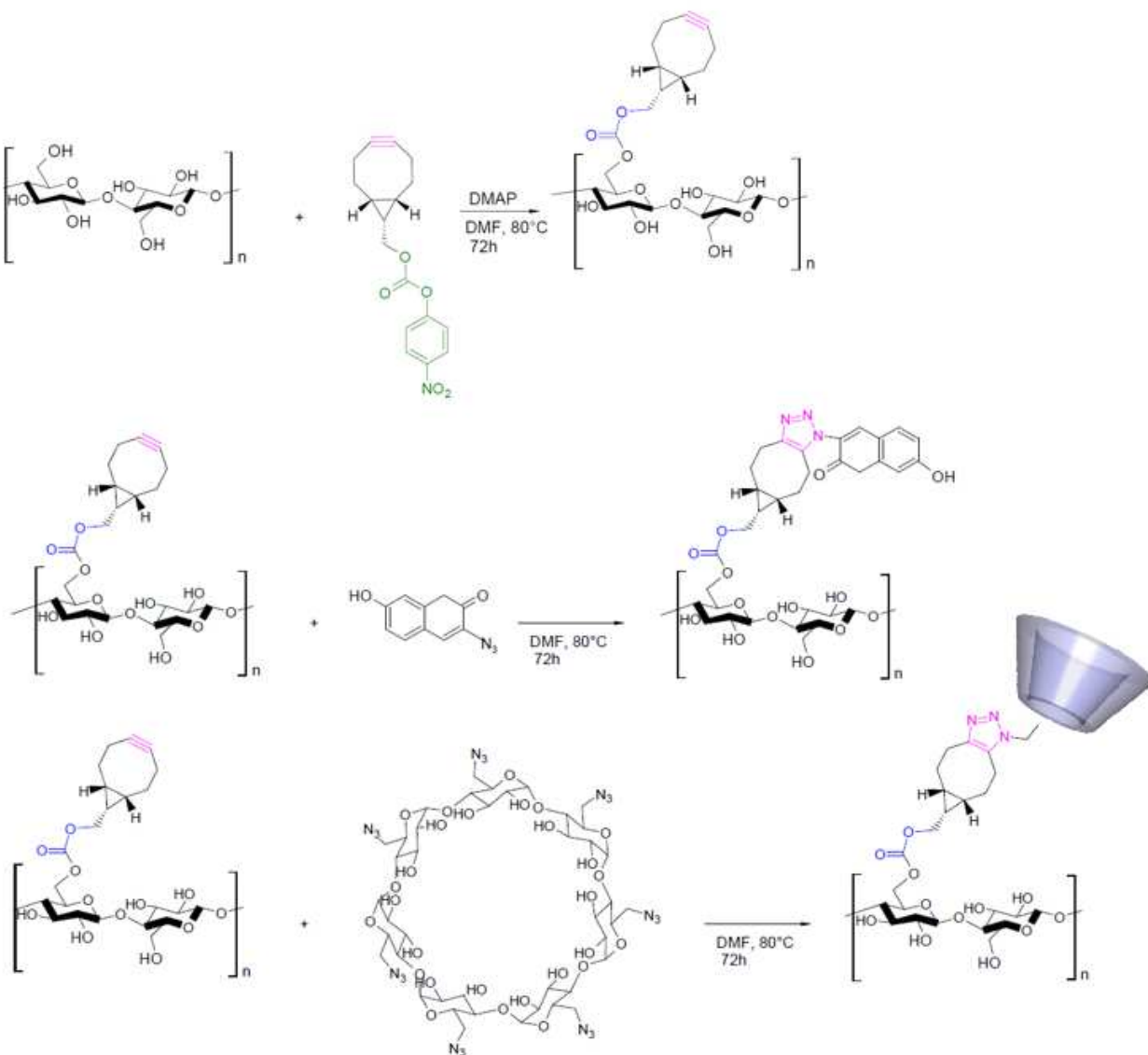
with  $n_C$  = the number of carbon atoms in the structure,  $M_C$ ,  $M_{cell}$  and  $M_{cell-BCN}$  the molecular mass of respectively, carbon atom, one cellulose unit and one cellulose-BCN unit. From this formulae, we deduce DS as following:

$$DS = 100 \times \frac{(n_C \times M_C)_{cell} - \%C \times M_{cell}}{M_{cell-BCN} \times \%C - (n_C \times M_C)_{cell-BCN}} \quad (8)$$

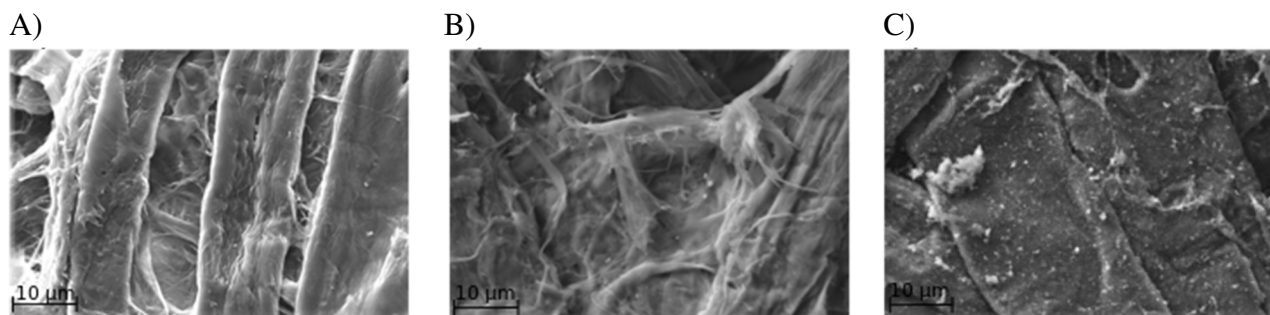
### 3. Results and discussion

#### 3.1. Synthesis and characterization of the bicyclononyne cellulose (cellulose-BCN).

Cellulose-BCN was prepared by reacting nitrophenylcarbonate-BCN with a piece of dried wipe (Scheme 1). Due to the nitrophenyl group, the wipe turns yellowish after reaction and thoroughly rinsing. The influence of the reaction on the aspect of the cellulose fibers was checked by SEM. According to SEM pictures, no morphological change of the fibers was detected after the reaction with nitrophenylcarbonate-BCN (Figure 1A and Figure 1B). The fluorescent optical analysis proves the effective reaction of nitrophenylcarbonate-BCN with cellulose. Calco-fluor has the particularity to react with polysaccharides especially with that contain  $\beta$ -1,4 linkages like cellulose, this affinity depending of the structural polysaccharide microfibril formation [35]. Indeed, we can observe in Figure 2 that the fluorescence of cellulose-BCN is less than native cellulose. Besides, the -OH IR band of cellulose-BCN is smaller, compared to the -OH band of cellulose (Figure 3). The IR spectra shows also the apparition of two bands respectively at  $1750\text{ cm}^{-1}$  and  $1270\text{ cm}^{-1}$  proving the presence of carbonate linkers. Moreover, we can notice the absence of the two characteristics bands of the  $\text{NO}_2$  function at  $1510\text{ cm}^{-1}$  and  $1360\text{ cm}^{-1}$ , indicating the release of the leaving group and the formation of a covalent bond between BCN and the cellulose. This result highlights that nitrophenylcarbonate-BCN is not absorbed on the cellulose surface, and this is confirmed by elementary analysis wherein no trace of nitrogen was found.

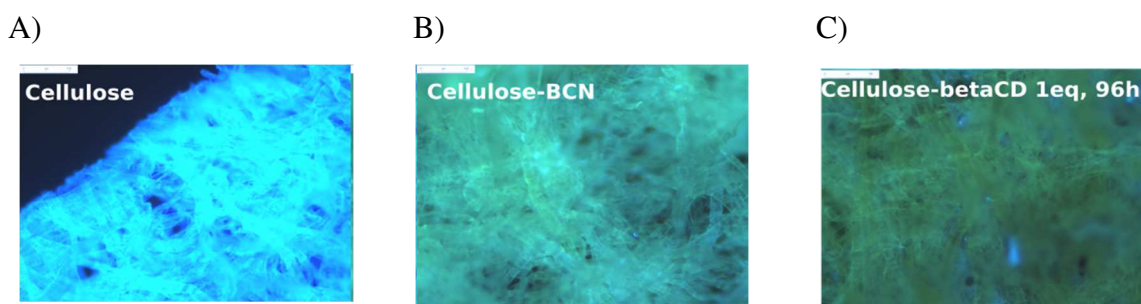


**Scheme 1:** Strategy of modification of cellulose.

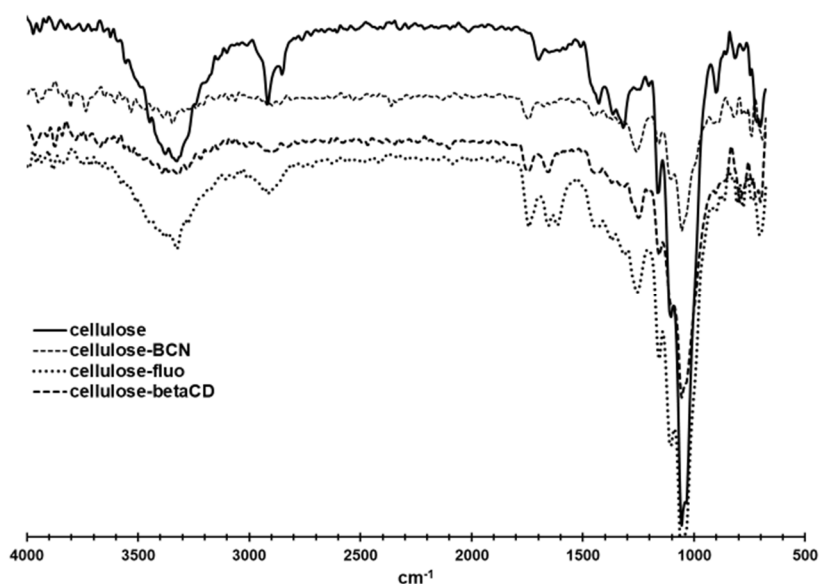


**Figure 1:** SEM pictures: A) Native cellulose wipens, B) cellulose-BCN and C) cellulose-β-CD after click reaction. Magnitude:  $\times 1800$ .





**Figure 2:** Calco-fluor analysis A) native cellulose, B) cellulose-BCN and C) cellulose- $\beta$ -CD



**Figure 3:** IR spectra of cellulose, cellulose-BCN, cellulose-fluo and cellulose- $\beta$ -CD

The degree of substitution (DS) determined on 2 synthesis by weight measurements, were respectively 19% and 18%. We next calculated DS from elementary analysis (Table 1) that is obviously a more accurate technique. The cellulose from wipes contains 41.8% of carbon elements. For both samples, the DS is around 16% high means that approximately 1 in 6 hydroxyl groups reacted considering all the sample.

**Table 1:** Elementary analyses and corresponding DS of cellulose, cellulose-BCN and cellulose- $\beta$ -CD wipes.

Sample Name	C (EA) (%)	N (%)	(EA) DS (%)	(EA) DS (w) (%)
Cellulose	41.8	0.0	/	/
Cellulose-BCN 1	46.7	0.0	15.9	19
Cellulose-BCN 2	46.7	0.0	15.8	18
Cellulose-fluo	/	/	/	11
Cellulose- $\beta$ -CD (1 Eq, 24 h)	44.4	0.7	1.6	1.0
Cellulose- $\beta$ -CD (1 Eq, 96 h)	45.9	0.8	1.7	1.0
Cellulose- $\beta$ -CD (20 Eq, 96 h)	46.2	1.4	1.7	2.5

EA: results obtained by elementary analysis. w: results obtained by weight measurements. ND: not determined

To go further, we determined the elemental composition of the upper layer (*ca.* 10 nm) of the cellulose fwipes by X-ray photoelectron microscopy (XPS) analysis. In the C1s spectrum of native cellulose (Figure 4A), the peaks characterizing contributions from carbon atoms can be deconvoluted into three components: one peak at 284.3 eV corresponding to C-C contributions, one peak at 286.1 eV from C-OH contributions and 287.6 eV from C-O-C contributions. The determined ratio between each contribution (1:3.7:1.3) is in good agreement to the theory (1:4:1) (Table 2). Those peaks are also present into the C1s spectrum of cellulose-BCN (Figure 4B), but the intensity of the peak at 284.3 eV is significantly higher. This can be explained by the addition of C-C and C $\equiv$ C contributions of the bicyclononyne group. Moreover, the apparition, on the C1s region, of a contribution at high binding energy (289.9 eV) specific to C=O moiety together with a new contribution at 531.0 eV on the O1s region also assigned to C=O groups, proves the presence of the carbonate link. The percentage of nitrogen is below 1% (Table 2) for both samples, cellulose and cellulose-BCN. This is in agreement with the IR and elementary analyses which demonstrate the absence of the NO<sub>2</sub> group. In the O1s spectrum of native cellulose (Figure 4B), the spectrum from oxygen atoms can be deconvoluted into two main components: one peak at 532.1 eV corresponding to C-O-C contributions and one peak at 533.1 eV from C-OH contributions. In the O1s spectrum of cellulose BCN, these two peaks are presents but one other contribution at 531.0 eV should be added to fit perfectly to the signal. This corresponds to the addition of C=O from the carbonate link. The contribution of each was used to calculate the different ratios: C=O/C-OH, C=O/C-O-C and C-OH/C-O-C which are respectively of 0.485, 0.21 and 0.435 (Table 2). If we consider that only one hydroxyl group per cellulose unit will react with one nitrophenylcarbonate-BCN, those ratios should be in theory, respectively 0.5, 0.25 and 0.5 which are very closed to the experimental results. Therefore, there is one BCN group per unit of cellulose at the surface of the wipe.

A) C1s

B) O1s

C) N1s

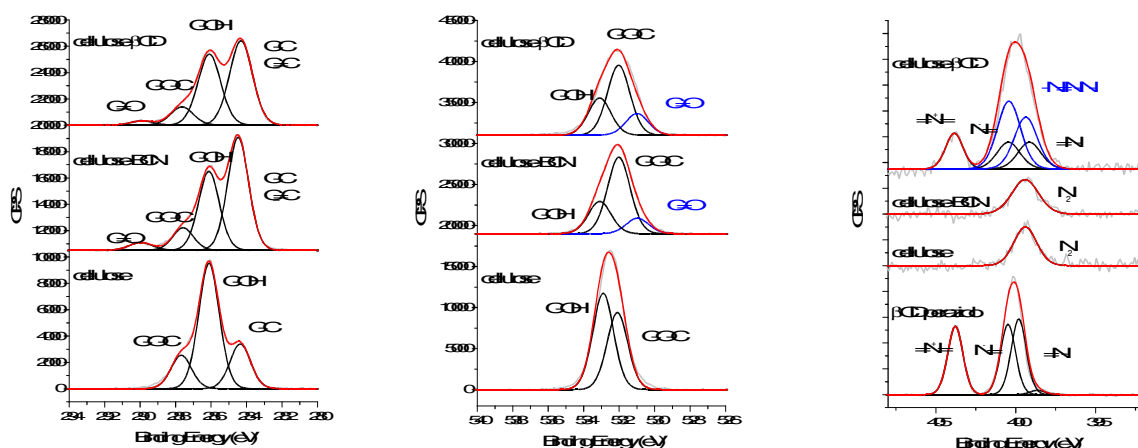


Figure 4: XPS spectra: A) C1s , B) O1s, C) N1s

Table 2: XPS results for native cellulose, cellulose-BCN and cellulose-β-CD wipes.

Meye Biyogo *et al.* : Table 2

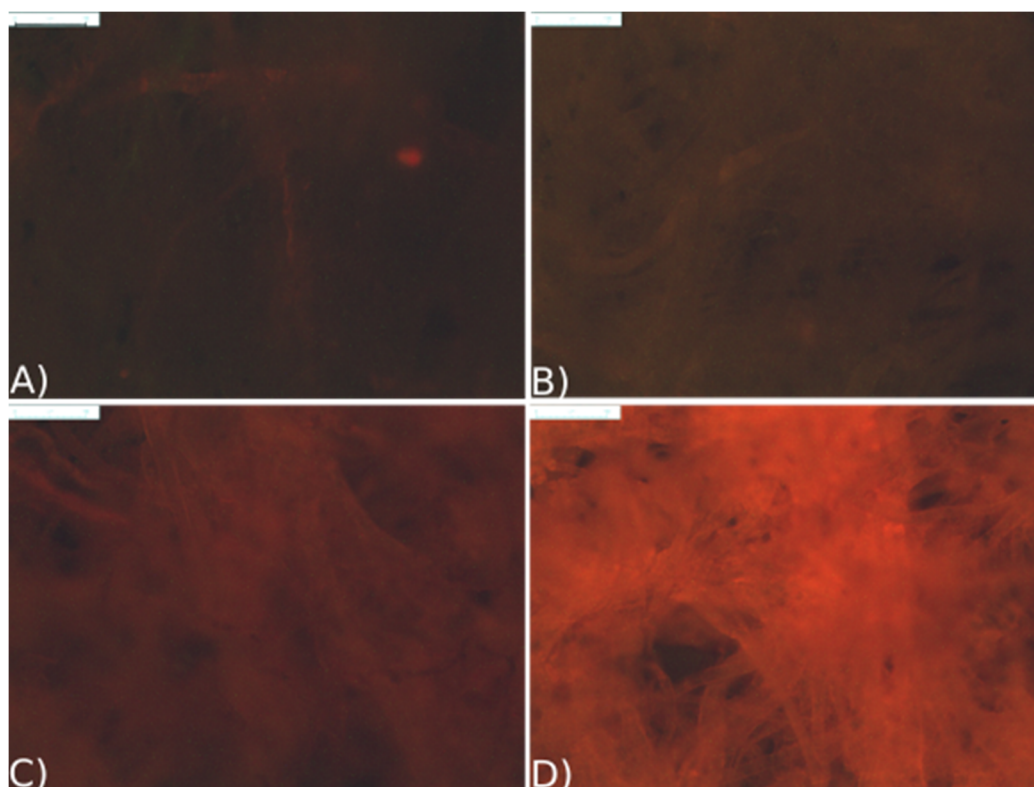
BE (eV)	C1s				O1s						N1s			
	284.3 C-C C=C	286.1 C-O	287.6 C-O-C	289.9 C=O	531.0 C=O	532.1 C-O-C	533.1 C-OH	RATIO determination			399.4 N <sup>-</sup> NH	400.5 N	403.9 N <sup>+</sup>	Unreacted azido*
Native Cellulose	65.4 %				33.7%						0.9 %			
	14.3 %	40.3 %	10.8 %	-	-	15.0%	18.7%				1.25	0.9 %		
Cellulose-BCN	72.8 %				26.2%						0.9%			
	36.8%	25.7%	7.4%	2.9%	3.3%	16.1%	6.8%	0.48	0.21	0.43	0.9%			
Click β-CD 1eq 24 h	65.7%				28.2%						6.1%			
	30.0%	27.8%	6.9%	1.0%	3.2%	15.3%	9.4%	0.34	0.21	0.61	1.2%	1.2%	1.2%	3.6 %
											1.3%	1.2%	-	
Click β-CD 1eq 96 h	66.6%				28.6%						4.9%			
	31.5%	26.5%	6.9%	1.7%	4.8%	15.4%	8.2%	0.59	0.31	0.53	0.65%	0.65%	0.65%	1.95 %
											1.3%	1.7%	-	
Click β-CD 20eq 96 h	59.7%				33.5%						6.8%			
	29.5%	23.4%	6.1%	0.7%	4.0%	18.7%	10.7%	0.37	0.21	0.57	1.4%	1.4%	1.4%	4.2 %
											1.2%	1.4%	-	

\*Sum of the percentages of N<sup>-</sup>, N<sup>+</sup> and N

### 3.2 Synthesis and characterization of the cellulose-hydroxycoumarin (cellulose-fluo).

Cellulose-BCN was then engaged in « click » reaction with a pro-fluorogenic compound, 3-azido-7-hydroxycoumarin (Scheme 1). First, FTIR analysis (Figure 3) still shows the peak of the carbonate group. The intensity of the band at  $3310\text{ cm}^{-1}$ , characteristics to the presence of OH groups from the fluorescent dye, increases. Moreover, peaks of aromatic rings from the coumarin derivatives are respectively detected at  $3000\text{ cm}^{-1}$ ,  $1660\text{ cm}^{-1}$  and  $1610\text{ cm}^{-1}$ . DS was determined by weight measurements method and was equal to 11%. So, the yield of the reaction can be estimated at 60%. 3-azido-7-hydroxycoumarin has the particularity to show an intense fluorescent answer in the presence of a triazole group. Thus, we compared the fluorescent intensity of native cellulose, cellulose-BCN, cellulose-fluo and a blank. In the case of the blank, cellulose and 3-azido-7-hydroxycoumarin were mixed together in the same conditions than for the cellulose-fluo synthesis. We detected no fluorescence for cellulose and cellulose-BCN (Figure 5 A) and B)). Figure 5 C) shows a weak fluorescence of the blank, probably due to some absorbed fluorescent dye on the

surface. Finally, the intense fluorescence observed with cellulose-fluo (Figure 5 D)) proves the presence of the triazole ring formed during the click process.

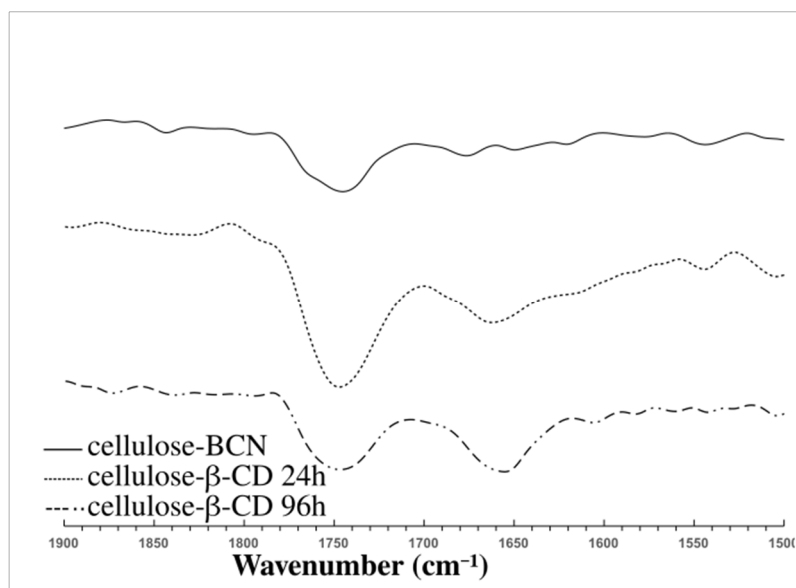


**Figure 5:** Fluorescent OM pictures: A) Native cellulose wipers, B) cellulose-BCN and C) cellulose-β-CD (1 Eq, 96 h)

### 3.3 Synthesis and characterization of the cellulose-β-cyclodextrin (cellulose-β-CD) (scheme 1)

We decided to use per-6-azido-per-6-deoxy-β-CD as model, because it can be concurrently monosubstituted at secondary face [36]. Thus, this strategy will be useful in the future to immobilize cyclodextrin-based enzyme mimics such as OP scavengers. We tested three different conditions of reaction: a) 1 Eq/cellulose unit – reaction time: 24 h; b) 1 Eq/cellulose unit – reaction time: 96 h; b) 20 Eq/cellulose unit - reaction time: 96 h.

No significant change was observed in the case of cellulose-β-CD IR analysis compared to IR spectra of both native β-CD and cellulose, except the increase of the band intensity at  $1650\text{ cm}^{-1}$  on the IR spectrum (see Figure 3). The higher the duration reaction, the stronger the increase of the signal intensity was, according to the progressive chemical environment change of the carbonate function during the “click” reaction (Figure 6). Calco-fluor analysis of cellulose-β-CD shows no significant change compared to cellulose-BCN (Figure 2) because it mainly reacts with cellulose.



**Figure 6:** Zoom of IR spectra of cellulose-BCN, cellulose- $\beta$ -CD 24h and cellulose- $\beta$ -CD 96h

SEM images of the samples (Figure 1) show small dots on the surface of cellulose while nothing appeared for cellulose-BCN. However, when  $\beta$ -CD are globally well distributed on the surface, some areas present aggregation of  $\beta$ -CD. The C1s XPS spectrum cellulose- $\beta$ -CD shows the same deconvolution than for cellulose-BCN (Figure 4 A)). Thus, all the BCN moieties did not react. However, we also note the reduction of the contribution of C-C peak due to the  $\beta$ -CD immobilization on the surface. The O1s spectrum obtained after “click” reaction is difficult to use because cellulose and  $\beta$ -CD have similar signal. Moreover  $\beta$ -CD being a large molecule, it can shade off the signal of cellulose. Nevertheless, it can be noted that the ratio of hydroxyl groups increases. The evolution of N1s contribution was followed by XPS analysis before and after the “click” reaction (Figure 4C) revealing an important change. At the bottom, the N1s signal for powder of perazido  $\beta$ -CD can be deconvoluted in 3 main components with a ratio of 0.96:1:1.06. These components respectively correspond to  $N^+$  at high binding energy (403.9 eV), N (400.5 eV) and  $N^-$  at low binding energy (399.4 eV). They are perfectly consistent with the presence of azido group. After the click reaction, we observe an increase of the signal intensity at 399.4 eV which corresponds to  $N^-/NH$  and at 400.5 eV which corresponds to N. This is characteristic of the triazole rings, and proves the efficiency of the “click” reaction performed without metal. We don’t expect that all the azido groups present in the  $\beta$ -CD react which means that azido group will still remain after the “click” reaction. From N1s spectrum, considering that each nitrogen from azido group will be present at the same ratio, the percentage of triazole groups can be deduced (Table 2). Under the conditions a) and b), 41% and 60% respectively of triazole groups are formed at the end of the reaction (that corresponds to 4 reactive azido groups on 7 per CD unit for the condition b)). Surprisingly, the increase of the amount of CD reactant (conditions c) did not lead to an increase of the triazole groups. Indeed, 38% of triazole groups (*i.e.* 3 reactive azido groups on 7 per CD unit) are present after reaction while the DS determined by elemental analysis (Table 1) is similar with that calculated under conditions b). For each condition, the  $\beta$ -CD is immobilized on the surface through several triazole rings which means that the primary face is always directed to the top. This result is promising because  $\beta$ -CD is generally used to form inclusion complex with another

molecule and the control of the direction of the two faces is in this case important to assure the complex formation.

### **Conclusion:**

In conclusion, we developed a new and versatile way to functionalize cellulose fibers. This innovative strategy is based on a strain-promoted azide-alkyne cycloaddition under heterogeneous conditions. The metal-free click chemistry is a very attractive approach to immobilize a large diversity of molecules on a textile support, and it avoids the persistence of any metal traces on cellulose after rinsing. Thus, we can extend the use of the functionalized textile to biological applications without any risk. Moreover, we deduced the substitution degree of the cellulose-BCN from a complete characterization, especially involving a XPS analysis to accurately determine the efficiency of the cellulose substitution. Thus, this pre-functionalized support opens the way to a wide number of uses after clicking the target compounds. Indeed, covalent links between the cellulose material and the immobilized derivatives ensures the strength of bonds, preserving the modified cellulose from any degradation regardless the further applications. In the future, additional investigations could be performed in order to prepare new cellulose based supports as decontamination means against organophosphorus nerve agents.

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### **Data availability**

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

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