Allyl, glycidyl methacrylate and cyclodextrin-modified nanocelluloses. Preparation, characterisation and adsorption-release specific properties.

E. Vismara[*], M. Zarattini[*], A. Bernardi[*], D. Nanni[**], S. Bertini[***] and C. Freire[****]

[*]Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, 20131 Milano, Italy, elena.vismara@polimi.it
[**]Department "Toso Montanari" Università di Bologna, 40136 Bologna, Italy, daniele.nanni@unibo.it
[***]"Giuliana Ronzoni" Institute, 20133 Milano, Italy, bertini@ronzoni.it
[****]CICECO/University of Aveiro Campus de Santiago, 3810-193, Aveiro, Portugal, cfreire@ua.pt

ABSTRACT

Oxidized (ONC) and hydrolyzed (HNC) nanocelluloses were prepared from high-grade pure cotton fibers (CFT), too short to be spun. They maintained the CFT crystallinity (IC=0.59). ONC and HNC were fully O-allylated affording ONC-ALL and HNC-ALL, respectively. ONC-ALL and HNC-ALL became completely amorphous. Bacterial nanocellulose (BNC) and ONC were graphted with glycidyl methacrylate (GMA) affording BNC-GMA and ONC-GMA, respectively. Substitution degree (DS= GMA residue *versus* glucose unit) range in BNC-GMA and ONC-GMA is 0.4-0.6. As GMA grafting forms a new C-C bond between cellulose and GMA and keeps OH cellulose groups unchanged, GMA nanocellulose maintains the cristallinity of the starting nanocellulose. The non polar allyl and GMA appendages decrease the hydrophilicity of cellulose. ONC-ALL and HNC-ALL were further functionalized by mono 6-[(mercaptotetramethylene) thiol]-β-cyclodextrin (CySH). GMA epoxide group was opened by water and by β-cyclodextrin (Cy) affording BNC-GMA-OH and BNC-GMA-Cy. GMA and allyl appendages induce nanocellulose capability to adsorb 2-naphthol (2N) and amoxicillin (A).

Keywords: nanocellulose, allyl chloride, glycidyl methacrylate, cyclodextrin, amoxicillin.

1 RESULTS AND DISCUSSION

Advances in the chemical modification of nanocelluloses have been recently reviewed by Youssef Habibi [1].

Our work aims to enlarge the number of nanocellulose chemical modifications and broaden the horizon of nanocellulose applications, see Table 1.

Different nanocelluloses were considered as starting materials, oxidized (ONC), hydrolyzed (HNC) nanocellulose and bacterial (BNC) nanocellulose in order to prepare different modified nanocelluloses suitable for more or less sophisticated applications. Allylation and GMA grafting on one hand provide nanocelluloses with new hydrophilic–hydrophobic balance. On the other allylation and GMA grafting are suitable to further several modifications, by addition on the double bond and by opening the epoxide group, respectively. Finally, allylation affords amorphous nanocellulose while GMA grafting affords crystalline nanocellulose.

ONC and HNC were prepared from short high-grade pure cotton fibers (CFT), too short to be spun. CFT is a waste provided by an Italian company (CFT Masserini), but what waste! Figure 1 reports the CFT solid-state ^{13}C NMR spectrum (CP MAS), where the cellulose profile confirms the purity. The two C4 signals around 90 ppm integration gives the crystallinity index (IC=0.59), left C4 crystalline part, right amorphous part. ONC was prepared by TEMPO mediated oxidation [2]. HNC was prepared according to the well-known procedure [3]. The ONC (0.59) and HNC (0,68) crystallinity was measured by their CP MAS spectra, not reported. ONC-ALL and HNC-ALL preparation took inspiration from an efficient method of preparing allyl cellulose [4]. CP MAS spectra showed that ONC and HNC were fully allylated and amorphous. For convenience only one spectrum is reported, Figure 2 shows the CP MAS spectrum of ONC-ALL. ONC-ALL and HNC-ALL were further functionalized with CySH by free-radical thiol–ene coupling, according to click chemistry methodology, using visible and UV light [5]. CySH prepared according to the methodology described by Wenz was used to achieve ONC-ALL-CySH and HNC-ALL-CySH that merge nano-properties with cyclodextrin inclusion property [6]. Cyclodextrins are used in food, pharmaceutical, drug delivery, and chemical industries, as well as agriculture and environmental engineering. ^{13}C CP MAS spectra of CySH visible light modified ONC-ALL and HNC-ALL were investigated. It is very difficult to distinguish ciclodextrin signals from cellulose signals due to the commun carbohydrate structure. Allyl and tetramethylene groups can be distinguished. In the CP MAS spectrum profile of ONC-ALL-Cy reported in Figure 3 the allyl group is partly preserved and partly modified and aliphatic signals (tetramethylene group) are significantly increased, compared to ONC-ALL spectrum. Furthermore the integration of the allyl group between 115-150 ppm decreases compared to the integration of anomeric carbohydrate signals between 100-110 ppm.

Table 1. Preparation and adsorption on HNC, ONC and BNC				
modifications	allylation	HNC-ALL	ONC-ALL	-
	GMA grafting	HNC-GMA	ONC-GMA	BNC-GMA
	GMA's epoxide opening	HNC-GMAOH	ONC-GMAOH	BNC-GMAOH
	Cy linking	-	-	BNC-GMA-Cy
	CySH linking	HNC-ALL-Cy	ONC-ALL-Cy	-
absorptions	β-naphtol	HNC-ALL	ONC-ALL	-
			ONC-GMA	-
	amoxicillin	HNC-ALL	ONC-ALL	BNC-GMA
			ONC-GMAOH	BNC-GMAOH

Table 2. Adsorption of amoxicillin (A) and 2-naphtol (2N)			
Nanocell.	**time (h)**	**A/2N**	**mg /mg nano**
BNC-GMA	0	A	0
	24		3,72
	48		4,27
	52		2,53
BNC-GMAOH	0	A	0
	24		1,32
	48		0,46
HNC-ALL	0	A	0
	24		1,12
ONC-ALL	0	A	0
	24		0,97
ONC-GMAOH	0	A	0
	16		0,06
HNC-ALL	0	2N	0
	24		0,05
ONC-ALL	0	2N	0
	24		0,04
ONC-GMA	0	2N	0
	24		0,02
	42		0,025
BNC-GMA	0	2N	0
	48		0,1

These evidences support the occurrence of the coupling between CySH and ONC-ALL/HNC-ALL, even though the structures are not totally clarified ONC-ALL and HNC-ALL coupling with CySH mediated by UV light afforded ^{13}C CP MAS spectra difficult to explain.

ONC and BNC [7] were functionalized by surface radical glycidyl methacrylate (GMA) grafting, transferring the approach used for cellulose textiles to nanocellulose. [8] BNC is highly crystalline, see CP MAS spectrum in Figure 4. The GMA grafting occurred as confirmed by the IR BNC-GMA and ONC-GMA spectra where GMA structure was identified by the glycidyl ester and epoxide group signals. For convenience only BNC-GMA spectrum is reported in Figure 5. Glycidyl group was hydrolized to glycerol to afford ONC-GMA-OH and BNC-GMA-OH. BNC-GMA-OH was put in reaction in strong base with β-cyclodextrin to afford BNC-GMA-Cy. Several modified nanocelluloses were tested to adsorb amoxicillin (A) and 2-naphtol (2N), see Table 2. The best results were obtained with BNC-GMA. Its A adsorbtion is an equilibrium process as evinced by the trend *versus* time. Preliminary results show its capability to A controlled release in water. It can be studied for manufacturing innovative nanocellulose for drug delivery. BNC-GMA could be also further investigated for manufacturing innovative adsorbent nanocellulose for the removal of aromatic compounds from contaminated waters. Amoxicillin (A) shows a good affinity with the allyl group. This result suggests that also HNC-ALL and ONC-ALL absorbtion capability can be further investigated.

2 EXPERIMENTAL PART

2.1 HNC and ONC preparation

HNC-Grind CFT (2.4 g) was treated with H_2SO_4 64% w/w (130 mL) at 45°C for 6 hrs

Reaction mixture was poured in 700 mL cold H_2O and left to precipitate 1 day. The supernatant was centrifuged at 4000 rpm for 15 min. The recovered solid was washed exaustively with distilled water under centrifugation at 8000 rpm for 15 min, then suspended in water and lyofilized.

ONC- Grind CFT (1.0 g) was suspended and stirred in distilled H_2O (100 mL) for 30 min. 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO), (40 mg) and NaBr (172,7 mg) dissolved in H_2O (50 mL) were added. NaClO 10% (3 mL) followed by NaOH 0,1M added dropwize to reach pH=10 induced the oxidation process. The process run 5h under stirring (500 rpm) at room temperature maintaining the pH=10. EtOH (2 mL) and HCl 0,1M added dropwize till pH=7 quenched the reaction. The precipitate was recovered, exaustively washed with water under centrigation at 15000 rpm, suspended in water and lyofilized.

2.2 Allylation

Nanocellulose (348 mg) was suspended and stirred in DMSO (30 mL) under Ar flux. After 10 min tetra-n-butylammonium fluoride (TBAF) (4 g) was added, temperature was raised at 60°C and maintained for 1h. Colour changed to yellow. At 50°C, NaOH in powder (7,3 g) was added followed by DMSO (50 mL). The reaction was kept in the dark for 30 min. Allyl chloride (18 mL) was added. The reaction run at 50°C for 48 hrs. Colour changed to brown. Methanol (100 mL) was added and the precipitate was recovered and washed with methanol under centrifugation at 6000 rpm for 15 min. The reaction product was extracted in Soxlet with n-hexane for 24 hrs.

2.3 Free-radical thiol–ene coupling

βCD-SH (130,2 mg) was dissolved in H_2O (5 mL) and sonicated for 10 min. Allyl nanocellulose (122,7 mg) was then added under stirring. (2,2-dimethoxy-2-phenylacetophenone (DPAP), (15,2 mg) was added. The reaction was lightened with Tungsten lamp 150W, 220-230V at room temperature for 1 hr. The recovered solid was filtered, exaustively washed with water and lyofilized.

2.4 GMA grafting

In a 500 mL two neck round bottom flask 200 mL of water and 966.1 mg of ONC were added, then stirred for 30 minutes in a thermostatic bath at 80°C. Then 56.7 mg $FeSO_4$ (0.17 mmol) and 6 mL of H_2O_2 30% (58.74 mmol) were introduced. The mixture was stirred for 25 min (100 rpm) at 80°C. At the end of the activation time 4.8 mL of GMA (35.16 mmol) was added to the mixture, under vigorous stirring to bring the monomer in solution. After 15 min (quenching time) the solid material was extracted from the reacting mixture using a Gooch funnel, and washed exhaustively with hot water in order to remove the non-grafted GMA polymer. The extracted solid was centrifuged 3 times (9000 rpm, 15 min) with water, and then filtered again using a Gooch funnel, and exaustively washed with water and diethyl ether.

2.5 Adsorption experiment

Aqueous solutions of the tested compounds (amoxicillin trihydrate and 2-naphthol) at 2.0x10-3 M concentrations were prepared in 25 mL flasks. Different types of nanocellulose were tested (BNC-GMA, BNC-GMAOH, HNC-AL, ONC-AL, ONC-GMAOH). All the flasks were shaken at 100 rpm in a thermostatic bath (Julabo SW22) for the time reported in Table 2.

The concentration of the solutions was determined at different times by UV-spectrophotometer (Jasco V-650), see quantitative results reported in Table 2.

HNC-AL = 100 mg for each experiment (2-Naphthol and Amoxicillin)

ONC-AL = 100 mg for each experiment (2-Naphthol and Amoxicillin)

2.6 Beta-cyclodextrin linking

An amount of 150 mg (0.135 mmol) of β-cyclodextrin was put in a three necked round-bottomed flask equipped with reflux condenser and bubbler. The flask was filled with Ar and then 40 mL of DMF (previously dried over 4 Å molecular sieves and degassed) were added. The flask was then put in an ice bath to start the slow addition of 25 mg of NaH (60% dispersion in mineral oil, 0.625 mmol). When the evolution of H_2 was finished, BNC-GMAOH (23.1 mg) was added, and the reaction was left stirring 30 hours under argon flow at room temperature. A solid was recovered and washed three times with methanol and two times with water. Gravimetric analysis shows a weight increase of the 2.2 % that suggests a very low reactivity of BNC-GMAOH toward β-cyclodextrin.

2.7 IR and NMR characterization

The following figures 1-5 report for convenience some examples of the characterization experiments made for the starting nanocelluloses and for the modified nanocelluloses reported in Table 1.

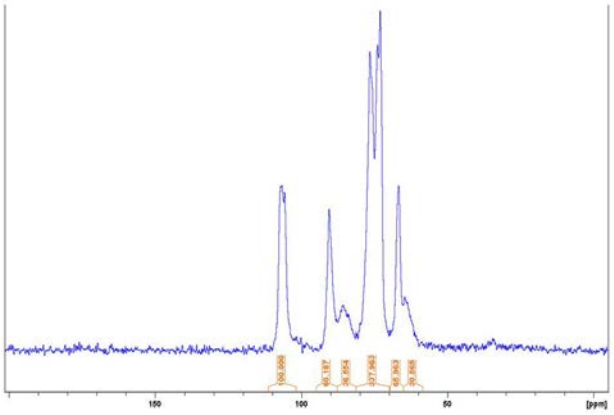

Figure 1. CP MAS of CFT

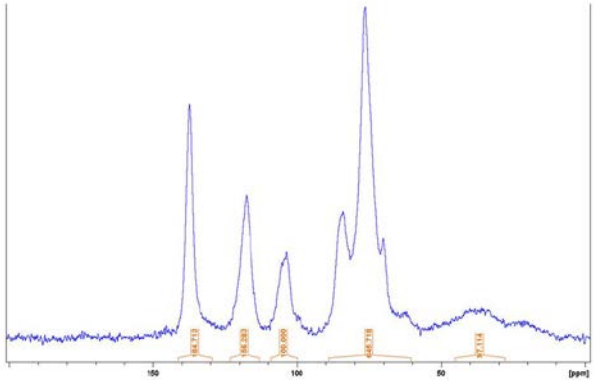

Figure 2. CP MAS of ONC-ALL

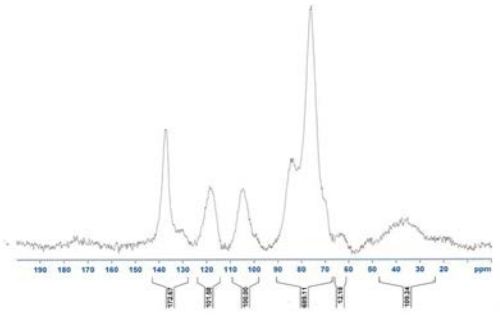

Figure 3. CP MAS of ONC-ALL-Cy

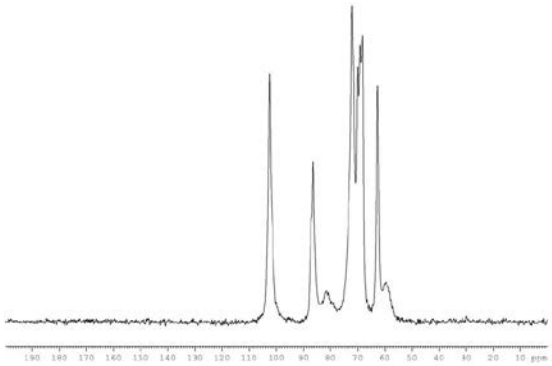

Figure 4. CP MAS of BNC

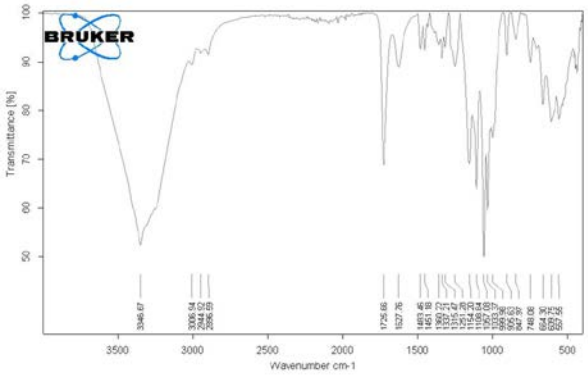

Figure 5. IR of BNA-GMA

REFERENCES

[1] Y. Habibi Chem. Soc. Rev. 43, 1519-1542, 2014.

[2] Y. Okita, T. Saito, A. Isogai, Biomacromolecules 11, 1696–1700, 2010.

[3] G. Siqueira, J. Bras, A. Dufresne Biomacromolecules 10, 425–432, 2009.

[4] T. Heinze, T. Lincke, D. Fenn, A. Koschella Polymer Bulletin 61, 1–9, 2008.

[5] Dondoni, A. Marra Chem. Soc. Rev . 41, 573–586, 2012. A. Massi, D. Nanni Org Biomol Chem. 10, 3791-807, 2012.

[6] G. Nelles, M. Weisser, R. Back, P. Wohlfart, G. Wenz, S. Mittler-Neher J. Am. Chem. Soc. 118, 5039-5046, 1996.

[7] L. C. Tome´, S. C. M. Fernandes, D. S. Perez, P. Sadocco, A. J. D. Silvestre, C. P. Neto, I. M. Marrucho, C. S. R. Freire Cellulose 20, 1807–1818, 2013.

[8] E. Vismara, L. Melone, G. Gastaldi, C. Cosentino, G. Torri Journal of Hazardous Materials 170, 798-808, 2009.

Acknowledgments: COST FP1205; partial financial support from MIUR, Rome (PRIN 2010-2011-010PFLRJR, “PROxi” project); C.F.T. PIETRO MASSERINI S.R.L. Company- Gazzaniga – BG, Italy for providing cotton fibers.