

SUPPLEMENTARY INFORMATION

Nanocellulose with Dual Carboxy and Aldehyde Functionality: a Modular Platform for Hydrogel Formation and Sustained Drug Release

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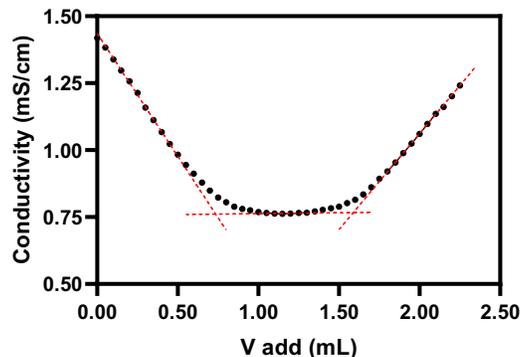


Fig. S1 Conductometric titration to quantify carboxylated groups on CNCs-COOH.

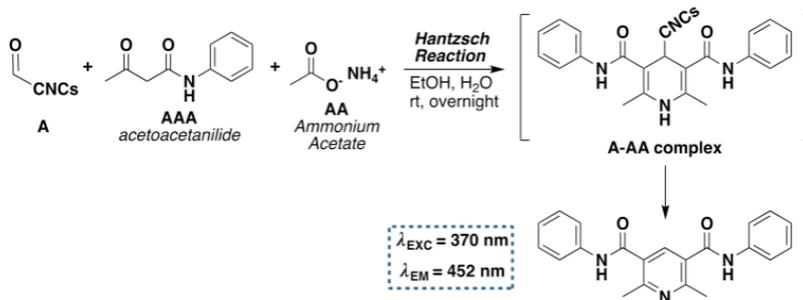


Fig. S2 Hantzsch reaction for aldehyde quantification.

Aldehydes Quantification Test: The amount of aldehyde content in CNCs-COOH-CHO was determined by a fluorometric method. Five-standard solution for the calibration curve and a series of oxidised CNCs samples in a 10 mL volumetric flask were prepared as follows: 5 mL of 4M Ammonium Acetate (AA), 2 mL of 0.2M Acetoacetanilide (AAA) in EtOH, 2 mL of ethanol and a series of 4-chlorobenzaldehyde 5mM in EtOH or oxidised CNCs samples (A). To reach 10 mL of final volume, the required quantities of H₂O were added and the mixture left under stirring overnight at room temperature. Fluorescence intensities of the reagent blank, 4-chlorobenzaldehyde standards, and sample solutions were recorded at 470 nm with excitation at 370 nm. Aldehyde concentrations were then calculated using the corresponding calibration curve. ($\lambda_{\text{exc}} = 370 \text{ nm}$; $\lambda_{\text{em}} = 452 \text{ nm}$; filter of 420 nm; $\Delta\lambda = 2$).

Table S1. Calibration line data

Standard	[A] μM
Std 1	5
Std 2	10
Std 3	20
Std 4	50

Calibration Line	
Slope	1.139E+02
Intercept	1.779E+03
R ²	0.989
Sample	mmolCHO/gCNCs
CNCs - CHO	0.277996

Table S1. Left: Concentrations of 4-chlorobenzaldehyde standards prepared to build the calibration curve for the aldehydes' quantification. **Right:** Information of the calibration line used to determine the concentration of aldehydes in unknown samples of oxidised nanocellulose (CNCs-CHO)

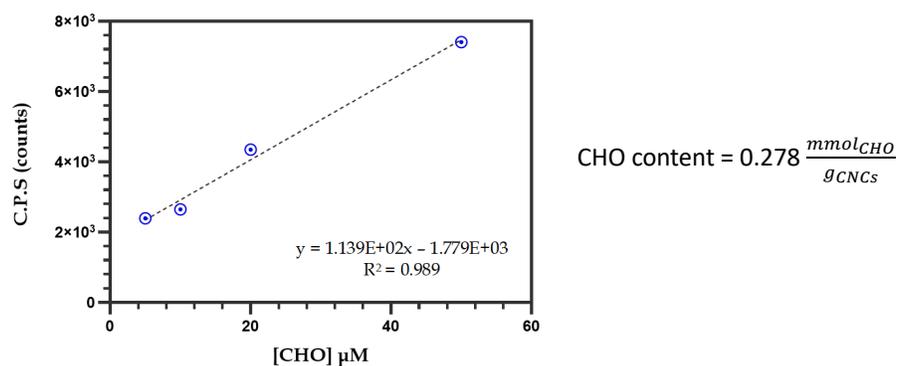


Fig. S3 Calibration curve obtained by standard solutions presented in Table S1.

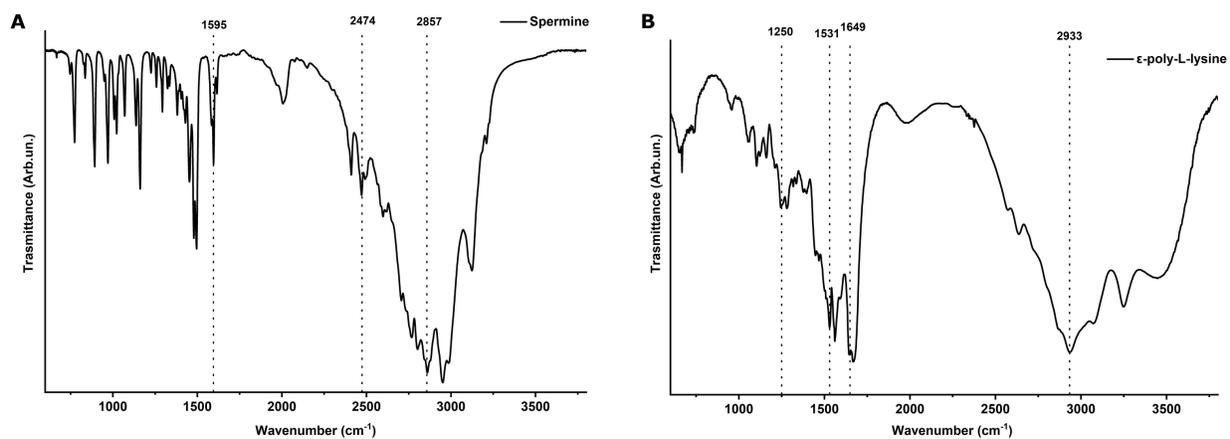


Fig. S4 The ATR-FTIR spectra of the starting materials: (A) Spermine, (B) ϵ -poly-L-lysine.

	P1	P2	P3	P4	C1	C2	C3	C4
Spermine (mg/ml)	25	25	-	-	2	2	-	-
ϵ -Poly-L-lysine (mg/ml)	-	-	1.3	1.3	-	-	4	4
EDC/NHS (mg)	-	-	-	-	39/23	39/23	39/23	39/23
CaCl ₂ (mg/ml)	0.3	0.3	-	-	0.09	0.09	-	-

Table S2 Hydrogels' composition. Amount of reagents used for hydrogels' formulation.

A

Standard	[Doxo] μM/mL	Peak Area (mAu·s)
Std 1	0.10	0.400
Std 2	0.20	0.791
Std 3	0.40	1.696
Std 4	0.80	3.727
Std 5	1.50	6.632
Std 6	3.00	13.195

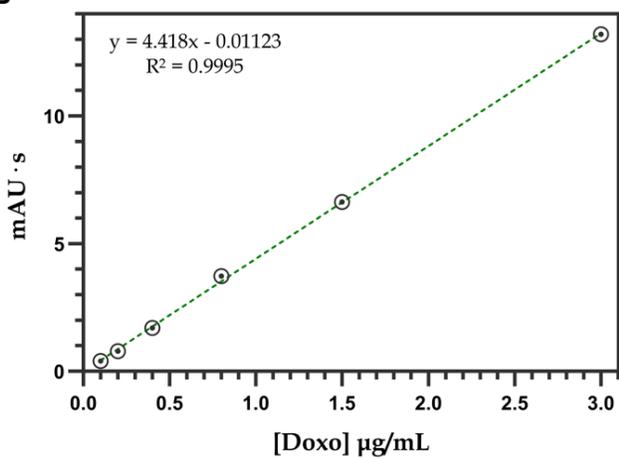
B

Fig. S5 (A) Doxorubicin standard solutions' concentration and the corresponding HPLC peak area. (B) Calibration line used for doxorubicin release quantification through HPLC-MS with the corresponding curve equation and R^2 .

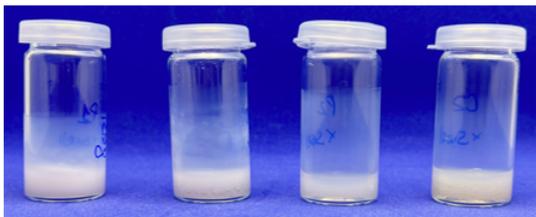
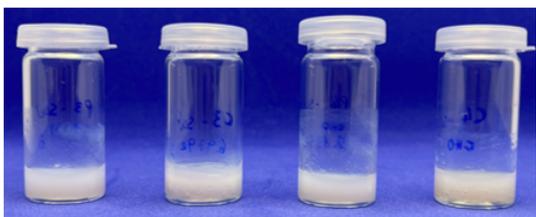
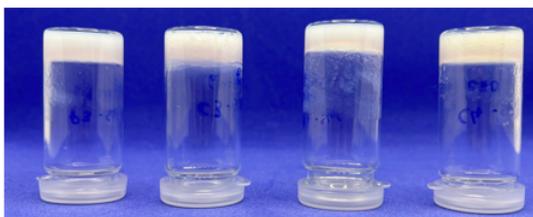
A**B****C****D**

Fig. S6 Tilt test evaluation. (A) Spermine hydrogels: from left to right P1, C1, P2, and C2. (B) Tilt-test of spermine hydrogels. (C) ε-poly-L-lysine hydrogels: from left to right P3, C3, P4, and C4. (D) Tilt-test of ε-poly-L-lysine hydrogels.