

†Supplementary Data:

General Procedure for synthesis of 6-carboxy cellulose (6CC):

Cellulose (10 gm) was taken in a 2-neck round bottom flask, equipped with overhead teflon stirrer. 140 ml acid mixture in 2:1 ratio (v/v) of 65% HNO₃ and 85% H₃PO₄ was added slowly over a period of 5 minutes. The acid mixture was allowed to get absorbed in the cellulose for 10-15 minutes. To this was then added 1.96 gm of NaNO₂ (1.4 w/v %). As soon as the NaNO₂ was added, reddish fumes of NO₂ gas were evolved. To prevent escaping of NO₂ gas, the side neck of round bottom flask was plugged with a stopper. The reaction was performed at three different high temperatures 40°C for 24 and 48h, 50°C for 12h and at 70 °C for 8h. The reaction was quenched by adding distilled water (five times the volume of reaction mixture) and allowed to stand for half an hour.

The reaction product at 40°C was obtained as 2 crops. The solid residue obtained was the 1st crop. The decanted portion was centrifuged at 12000 rpm to obtain a gel like material; this gel like material was taken as the 2nd crop. Each of the two crops were continuously washed separately with 2:1 ratio of methanol and distilled water, until filtrate had neutral pH. Final washing was done by acetone and the products were dried in a lyophilizer. The reaction product obtained at 50°C and 70°C produced only one crop, which consisted entirely of spherical nanoparticles. The products were characterized for carboxyl groups by the well established Ca-acetate method (USP 1995). The 1st crop was fibrous in nature and had sizes ranging from 2-10µm. Where not mentioned in the table, only one crop is obtained, i.e., it is 1st crop with sizes 2-10µm unless specified as NP (nanoparticles).

Table 1: Percent carboxyl content and yield of 6-carboxy celluloses as a fibre and nanoparticles at different temperatures and time periods. (NP : Nanoparticles)

Time (h)	-COOH Content (%) *				Yield (%)			
	25°C	40°C	50°C	70°C	25°C	40°C	50°C	70°C
1h	1.7	1.7	-	-	84.0	83.0	-	-
3h	3.0	3.0	-	-	73.0	72.0	-	-
6-8 h	8.6	8.6	-	13.9	71.0	68.0	-	16.0 (NP)
12h	14.1	14.1	13.2	-	69.0	60.0	46.0 (NP)	-
24h (I crop) (II crop)	19.7	16.0 18.0	-	-	63.0	25.0 5.0(NP)	-	-
48h (I crop) (II crop)	22.0	17.0 21.5	-	-	45.0	22.0 5.0 NP)	-	-

In the high acid concentration reaction medium, cellulose is known to degrade into several products such as glyoxalic acid, glucuronic acid, oxalic acid, levulinic acid, and so on (see J. H. Arendt, J. P. Carriere, P. Bouchez, J. P. Sachetto, *J. Polym. Sci. Symp.*, 1973, **42**, 1521). Degradation as a function of time and temperature has been reported in several papers and patents, such as *US 6,627,749 B1* dated 30 September 2003 and S.Wei, V. Kumar, G.S.Banker, *Int. J. Pharm.*, 1996, **142**, 175. Such side reactions increase with time and temperature, and reduce the yield.

Dynamic Light Scattering (DLS)

DLS studies were conducted using a Brookhaven Instruments Corp. Instrument using the 90 Plus Particle Sizing Software Ver. 3.94

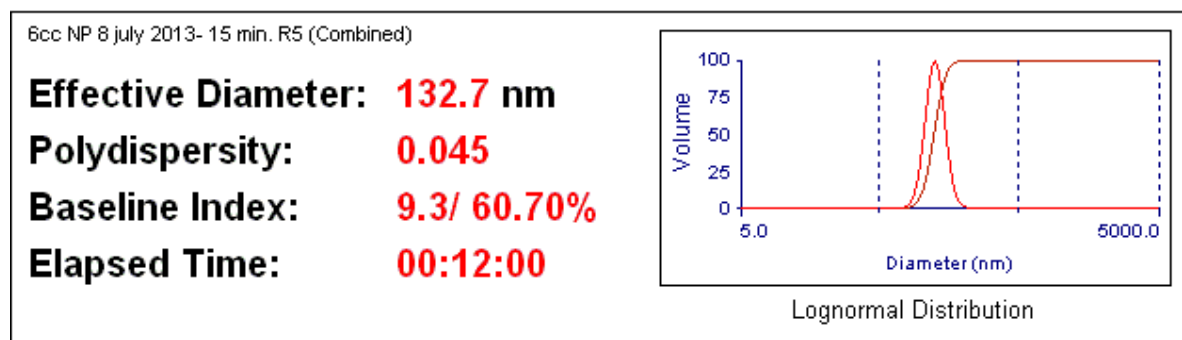


Fig. 4 : DLS of 6CC

Scanning electron microscopy (SEM)

Surface morphology of oxidized cellulose samples were studied using scanning electron microscope (SEM). The scanning electron micrograph were obtained using dual beam scanning electron microscope (FEI company, model Quanta 200 3D) operating at 30 kV. The samples were loaded on stubs and sputtered with thin gold film to prevent surface charging and also to protect them from thermal damage due to electron beam.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) studies of cellulose nanoparticles were carried out by using FEI –Technai G²-20 instrument. A 10 μ L aliquot sample of 1mg of oxidized cellulose in 10 ml distilled water was mounted on freshly glow discharged carbon coated Cu grids (200 mesh, ICON Analytical, India)

Atomic Force microscopy (AFM)

Atomic force microscopy (AFM) studies were done by using atomic phase microscopy (MAK-VECCO MMAFM-LN) in non contact mode. An aliquot of 10 μ l sample drop of 0.01% concentration was cast on silicon wafer.

Fourier transform infrared spectrometry (FTIR)

A Perkin Elmer Spectrum One instrument was used to record FTIR in transmission mode, between 450 to 4000 cm^{-1} . A total of 6 scans were taken per sample with a resolution of 4 cm^{-1} .

Solid-State NMR Spectra

The NMR experiments for various samples were performed on Bruker AV-300 spectrometer operating at a ¹³C frequency of 8 KHz. The instrument was equipped with 7.05 T superconducting magnet, having 4mm Bruker probe. The samples were packed in 4 mm zirconia rotor and spun at 10 kHz.

WAXRD

Wide Angle X-ray Diffraction (WAXRD) was analysed by using powder XRD Xpert -1217 diffractometer. The scanning speed was 4°/min, with radiation obtained from CuK- α . The samples were scanned from 2 θ values of 5° to 60°.

Dispersion Studies with single walled (SWCNT) and multi walled carbon nano tube (MWCNT)

50 mg of CNT equally mixed with 50 mg of oxidised cellulose sample in 10 ml distilled water and sonicate for 10 minutes and observed the dispersion.

Degree of Polymerization (DP) : The particles were not soluble in water due to low degrees of substitution (13.2% for 6CC nanoparticles). Therefore, it was convenient to evaluate the degree of polymerization (DP) of the celluloses by viscosity studies in cupriethylenediamine solution according to TAPPI T 254 cm-10. The assumption made in this case is that the TAPPI method will hold for celluloses with low degrees of substitution.

In our case the graph of viscosity (cP) versus DP was plotted with data taken from pg.99 of the standard book “Wood and Cellulose Science” by A.J. Stamm (Ronald Press Co., N.Y., 1964). More details can be found in the link below :

http://www.ipst.gatech.edu/faculty/ragauskas_art/technical_reviews/Laboratory%20Procedures.pdf

Pulp (cellulose) viscosity values were determined in accordance with TAPPI standard T230 om-94 “Viscosity of Pulp (capillary viscometer method).” The moisture content was determined for air-dried and was used to weigh 0.2500g o.d. of

cellulose. The weighed cellulose was solvated with cupriethylenediamine and passed through SCHOTT (kapillar-Viskosimeter) at 25°C. The viscometer were carefully cleaned with nitric acid water and acetone and dried between measurements. Two separate viscometer readings were performed for each sample and each sample was run twice (total of four viscometer readings). The viscosity was converted to the degree of polymerization (see Morton, J.H.; Viscosity /DP Relationships for Cellulose Dissolved in Cuprammonium and Cupriethylene Diamine Solvents. In *Proceedings of the Chemistry and Processing of Wood and Plant Fibrous Materials*, Cellucon 1994. Bangor, U.K. p. 151-158.(1996)) as follows :

$$D.P = -449.6 + 598.4 \ln [\eta] + 118.02 (\ln [\eta])^2$$

Where D.P is the degree of polymerisation and η is the viscosity in cP measured according to TAPPI T 230 om-89.

Anti-microbial study with E.Coli

The percent inhibition of cellulose, 6CC, 6CC-NP was determined for E.coli- DH5 alpha isolates from National Collection of Industrial Microorganisms (NCIM), Pune, India. E.coli strain were streaked onto the appropriate media and incubated overnight at 37°C. Single colonies were selected and inoculated into 10 ml of sterile broth. Inoculated broth was incubated at 37 °C overnight under constant agitation. Overnight cultures were diluted 1:100 and 1:1000 in broth, resulting in 1×10^6 and 1×10^7 cfu, as confirmed by plating serial dilutions.

Samples serially diluted at 1×10^6 and 1×10^7 cfu and inoculated in the LB agar plates for CFU. Plates were incubated in incubator at 37°C for 6h. The viable count of microorganisms after 1 day in presence of cellulose and in oxidized cellulose (6CC, 6CC-NP) at 4.0 % (w/v) is shown by the photograph in Fig. 3 in the manuscript text.