Supporting Information

Biodegradable Cellulose Nanocrystals Composites Doped with Carbon Dots for Packaging and Anticounterfeiting Applications

Shiva Singh,[1] Keshav Dev,[2] Shakshi Bhardwaj,[1] Dakuri Ramakanth,[1] Khushboo Rani Singh,[3] Krishna Mohan Poluri,[3] Kaushik Ghosh,[2] Pradip K. Maji[1]*

[1]*Department of Polymer and Process Engineering, Indian Institute of Technology Roorkee, Saharanpur Campus, Saharanpur- 247001, India*
[2]*Department of Chemistry, Indian Institute of Technology, Roorkee 247667, India*
[3]*Department of Biosciences and Bioengineering, Indian Institute of Technology Roorkee, Roorkee-247667, Uttarakhand, India*
Corresponding author:* ***Pradip K. Maji,* *Email: pradip@pe.iitr.ac.in*
Contact No.: +91-7895965010

1. Supporting Information Section S1:

S1.1. Moisture content:

The determination of the equilibrium moisture content was conducted in accordance with the methodology specified in "TAPPI T 550 om-08".[1] In a tared dry weighing vial, two samples of pulverised Black Lentils, each weighting 2 g, were analysed. The weighed substance is introduced into a preheated 105 ℃ hot air oven. The substance was removed from the oven after one hour and placed in a desiccator to prevent it from absorbing moisture. It was necessary to repeatedly weigh the sample until a constant weight was observed.

S1.2. Solvent extractives:

The quantity of solvent-soluble organic extractives in the source material was determined using the "TAPPI T 204 cm-97" method.[2] An 8 g test sample was placed within an extraction pipette. The complete grommet was secured using a Soxhlet assembly. In the round-bottom flask, a 1:75 solvent-to-fiber ratio was vaporised using a thermal mantle set to 40% power. In order to reduce the loss of solvent, ice-cold water was circulated via the condenser. The examination occupied six hours of time. Following completion, the extracted substance was extracted from the receptacle and subjected to a 30-minute heating process at 40 ℃ in order to dehydrate the solvent that had been absorbed. The extractives underwent evaporation separation using a rotary evaporator unit, while the purified solvent was systematically collected. After placing the residual solvent extractives in a Petri dish and subjecting them to a heated air oven at 45 ℃ for an entire night, the extractive content was determined by weighing the mixture the following day.

S1.3. Acid insoluble lignin:

For this assessment, the methodology outlined in "TAPPI T 222 om-02" was implemented.[3] Two 1.0-g test samples of the raw material were thoroughly combined with a glass rod at a temperature of 2 ℃ for 15 minutes in a container containing 15 ml of 72% (v/v) H_2SO_4. Following thorough blending, the beakers were immersed in a constant-temperature water reservoir set at 20 ℃ for a duration of three hours. Following dilution with 575 ml of double-distilled water, the Soxhlet unit was utilised to heat the acid-fiber mixture. The remaining substance was collected onto tared filter paper. Prior to being weighed, the acid-insoluble lignin residue-containing filter paper was desiccated overnight in a heated air oven.

S1.4. Holocellulose and alpha Cellulose:

In order to determine the quantity of holocellulose, samples containing two extractives, each weighed 2.5 g, were decomposed in 80 ml of double-distilled water. Following the addition of one gramme of sodium chlorite ($NaClO_2$) and several droplets of acetic acid, the suspension was transferred to a heated oil bath. The previously mentioned reaction was programmed to operate at 75 ℃ continuously for one hour. Following an hour, the procedure was replicated four times utilising 1 g of sodium chlorite ($NaClO_2$) and a few droplets of acetic acid. The specimen was filtered, washed multiple times with distilled water, and dried at 50 °C for an entire night. By conducting a weighting of the dehydrated sample, the concentration of hollocellulose in the sample could be ascertained.[4]

"TAPPI T 429 cm10" was applied to the alpha-cellulose estimation.[5] The procedure comprised two distinct components. In the initial stage, two 1 g samples of holocellulose were extracted and subjected to digestion in 50 ml of 1N NaOH at 75 ℃ for a duration of two hours. Double-distilled water was employed for the rinsing and filtration of the sample. The remaining substance was dissolved in 50 ml of distilled water for the second phase, followed by the addition of 17.5% NaOH to the suspension at 75 ℃ for 30 minutes. After filter paper was used to rinse the sample with distilled water, the residual substance was desiccated in an oven overnight prior to being weighed.

S1.5. Ash content:

Inorganic ash in the fibers was quantified using the "TAPPI T 211 om-02".[6] A 1 g sample was transferred into two tared crucibles. The crucibles were heated in a muffle furnace at a 25% heating rate and up to a constant temperature of 525 °C. The muffle contained the sample for a duration of 105 minutes, of which 45 minutes were devoted to achieving the desired temperature and 60 minutes were spent at 525 ℃. The collected samples were deposited in a desiccator to prevent the ash from absorbing moisture. At last, the ash content of the samples was determined through a process of weighting.

Supporting Information Section S2:

S2.1 Extraction of cellulose nanocrystals (CNC_{BL})

Black lentil shell waste was washed twice with hot and cold water, dried in a hot air oven at 60 °C for a day, and grounded into powder. The dry powder was dewaxed with 2:1 (v/v) toluene/ethanol in a Soxhlet extractor for 6–7 h. Delignification was performed on the dewaxed product using 5 wt% $NaClO_2$ and 2 ml glacial CH_3COOH. After 6 h of agitation at 75 °C, the solution was washed with DW and vacuum-filtered. Two chlorite bleachings were repeated. To dissolve chlorine complexes from chlorite bleaching, the vacuum-filtered product was

treated with 2 wt% sodium sulfite. The reaction was stirred for 2 h at 75 °C. The product was vacuum-washed with distilled water after sulfite bleaching. Sulphite bleached product was treated with 7 wt% potassium hydroxide (35 g pellets diluted with 500 ml DW) for 5 h at 80 °C. The product was vacuum-filtered and washed with lukewarm water till pH = 7. Chemically Pure Cellulose (CPC_{BL}) was produced by heating the filtered product at 60 °C. A 64% (w/v) sulfuric acid solution was used to treat cellulose at a 1:20 fiber-to-acid ratio. The reaction lasted 45 min at 50 °C. The hydrolysis was stopped by discharging the reaction mixture into icy water. For a day, the suspension was covered and isolated to separate the water and CNC_{BL} mixture by gravity. The mixture was decanted to remove as much water as feasible after CNC_{BL} sedimentation. The residual suspension was centrifuged for 15 min at 8000–10,000 RPM using the REMI; R-24, India. Separating the supernatant from the stock. The process was repeated 4–5 times with a few ml of distilled water. The dialysis was done using Dialysis Membrane-110 with a capacity of 3.63 ml/cm. The prepared CNCs were packed in the dialysis membranes and subjected to the DW container at room temperature; the water of the container was kept on changing in every 6 hr to maintain the concentrating gradient, and removal of acid was done till the achieved residues was neutralized (pH: 7).

S2.2 Extraction and preparation of different CDs

The waste shells were washed thoroughly and dried in a hot air oven at 50 °C and grounded in smaller sized powder like form. Then 2 g material was stirred in a beaker in 150ml double distilled water. After the solution became uniformly dispersed it was then poured into a sealed reactor, which can be a stainless-steel autoclave or a Teflon-lined vessel. The reactor is placed in an oven or a heating mantle and heated to the desired temperature, typically ranging from 150 to 220 °C. The reaction mixture is maintained at the elevated temperature for a specified time, typically ranging from 4 to 6 hours. After the reaction time, the reactor is allowed to cool down slowly to room temperature. The CD_{BL} are then collected from the reaction mixture by filtration trough Whatman filter paper and are further purified to remove any impurities or

unreacted precursors filtered using 20-micron PTFE Ram filter. Then dialysed overnight in the DI water for overnight.

One common method for adjusting or enhancing the PL characteristics of photoluminescent materials is by doping. There have been reports of using dozens of elements, including N, S, and P in different doping techniques to adjust the characteristics of CDs. Here, we used Ammonium persulfate (1.5 g) for Sulfur (S) doping and EDA (Ethylene diamine) (7.5) as Nitrogen (N_2) doping and Raw TG leaves as the source for Carbon. Hydrothermal process was used for the synthesis of both N (NCD_{BL}) and S (SCD_{BL}) doped CDs using double distilled water as the solvent. The reaction temperature was kept 200°C and for 4 hours in a hot air oven.

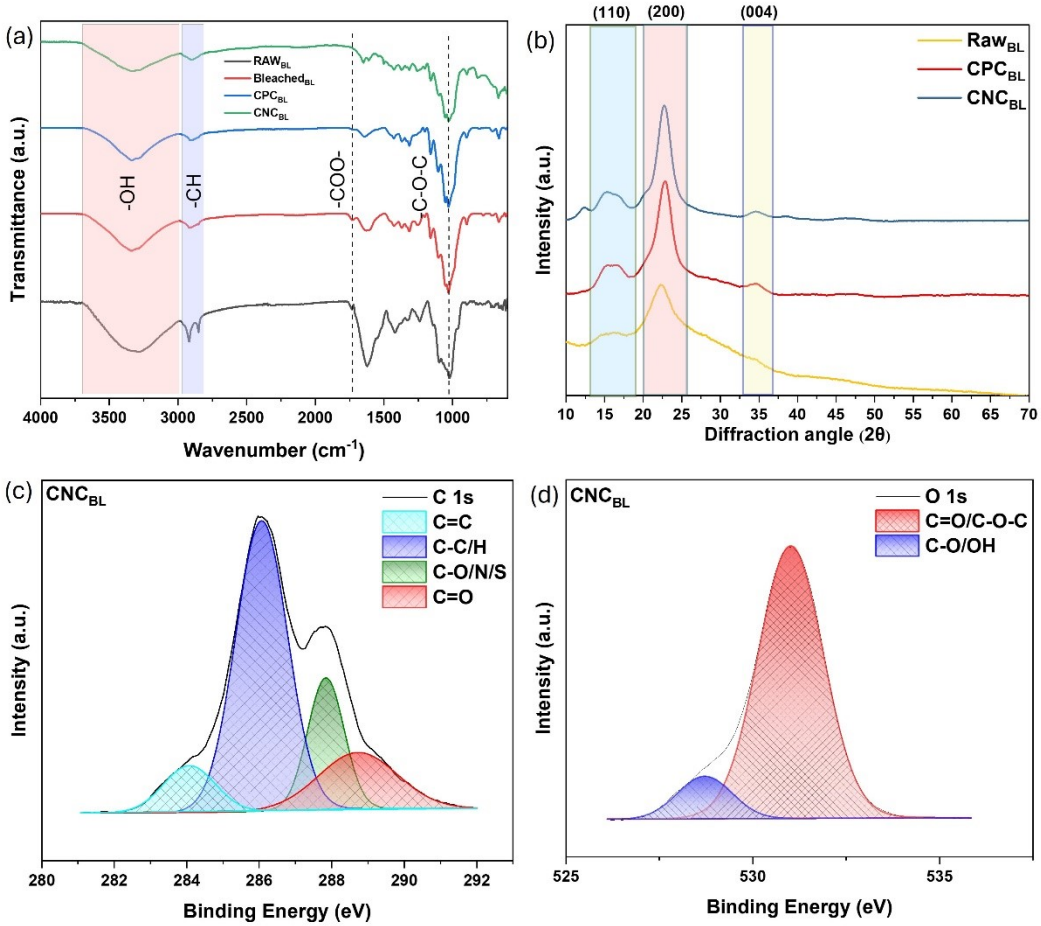

Figure S1 *(a) FTIR analysis of the extracted CNC_{BL} from RAW_{BL}, (b) powder XRD spectra of RAW_{BL}, $CPC_{BL,}$ and CNC_{BL}, (c) C1s XPS spectra of CNC_{BL}, and (d) O1s XPS spectra of CNC_{BL}*

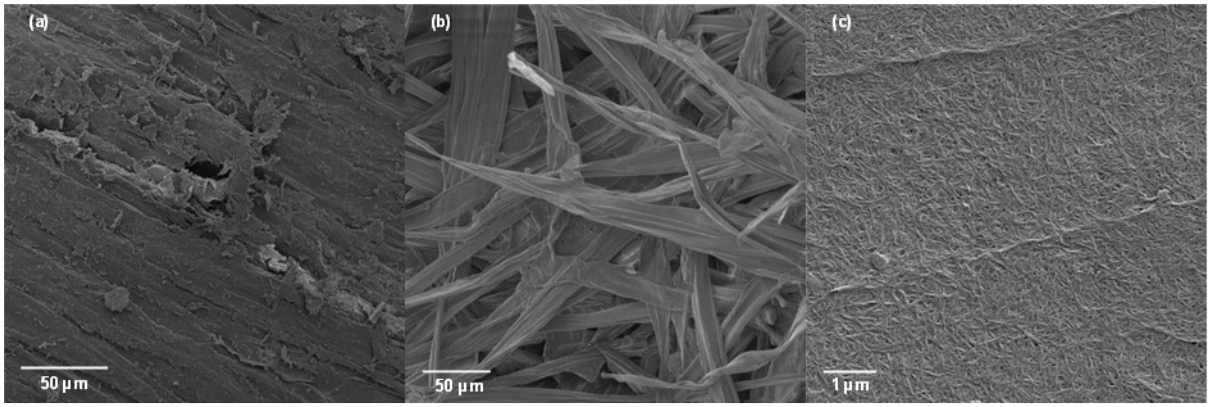

Figure S2 *(a) FESEM of RAW_{BL}, (b) FESEM of CPC_{BL}, and (c) FESEM of prepared CNC_{BL}*

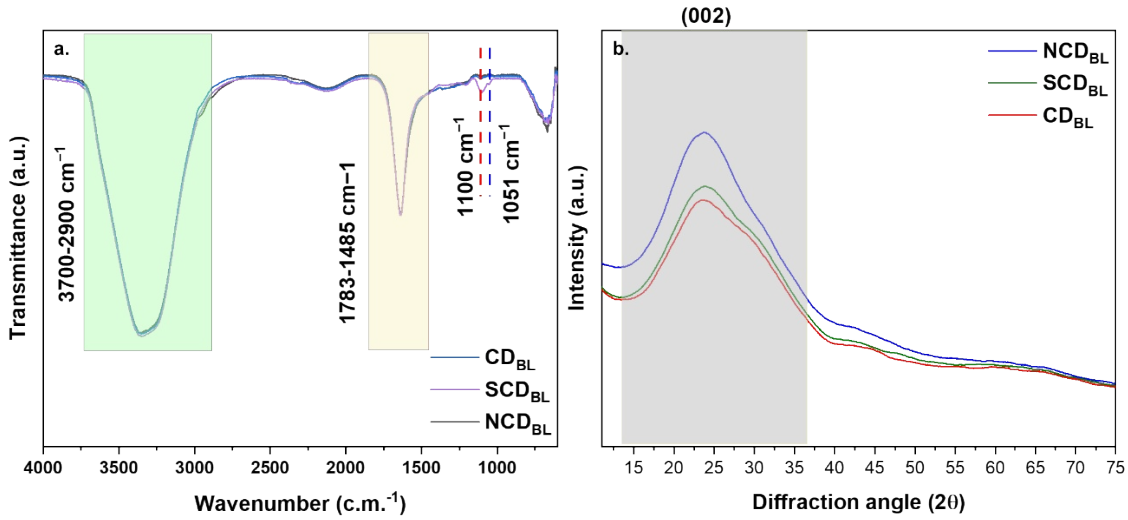

Figure S3 *(a) FTIR analysis of the prepared $CD_{BL,}$ $SCD_{BL,}$ and NCD_{BL} from RAW_{BL} and (b) XRD analysis of the prepared $CD_{BL,}$ $SCD_{BL,}$ and NCD_{BL} from RAW_{BL}*

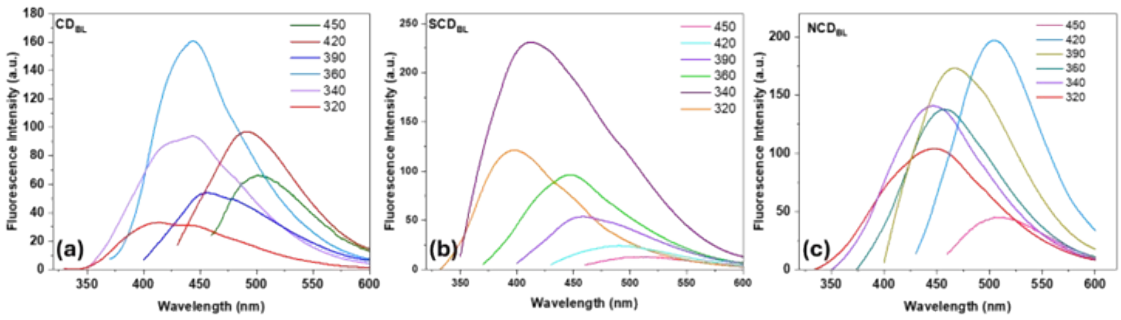

Figure S4 *Fluorescence emission spectra of (a) CD_{BL} at 360 nm, (b) SCD_{BL} at 340 nm (c) NCD_{BL} at 420 nm and (d) NCD_{BL} solutions at different pHs from 2 to 12*

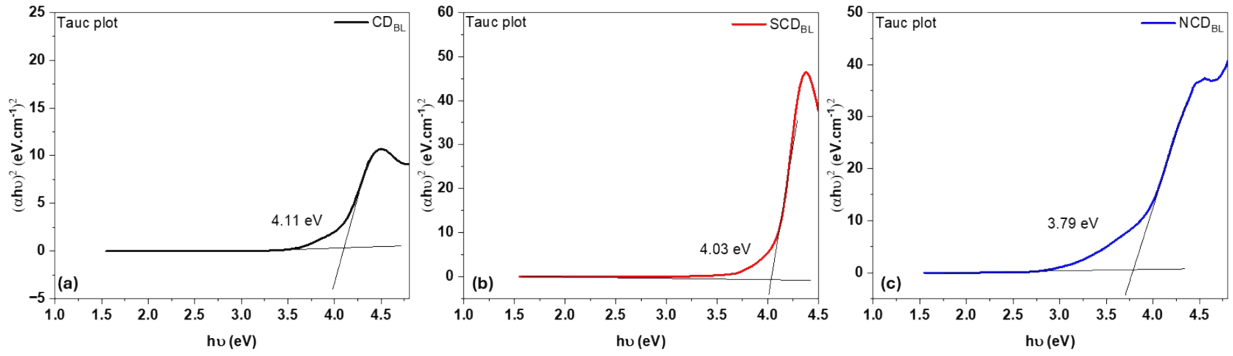

Figure S5 *Tauc plots for band gap calculation of (a) CD_{BL}, (b) SCD_{BL}, and (c) NCD_{BL}*

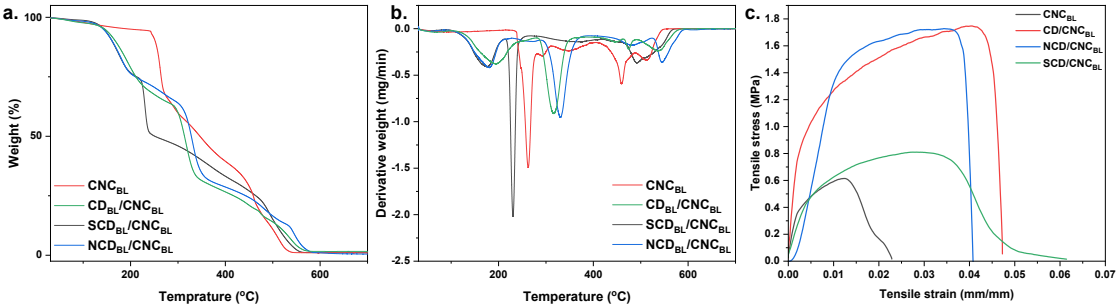

Figure S6 *(a) TGA, (b) DTG and (c) mechanical properties of the prepared sensors*

Table S1. *Summary of proximate, and ultimate chemical analysis*

Proximate Analysis			
Sr. No.	**Analysis**	**Content (%)**	**Deviation (±)**
1	Moisture	1.71	0.07
2	Ash	1.6	0.04
3	Wax	8.21	0.85
4	Holo Cellulose	38.4	1.42
5	Alpha Cellulose	35.2	1.23
6	Hemicellulose	3.2	0.11
7	Acid insoluble lignin	18.8	1.09

Ultimate analysis					
Specimen	**C (%)**	**H (%)**	**N (%)**	**S (%)**	**O[1] (%)**

Raw Stem	41.63	6.65	2.34	0.16	49.22
CPC_{BL}	39.16	6.42	1.51	0.06	52.85
CNC_{BL}	40.26	6.92	1.10	0.01	51.71
O^1 (%) = 100 – (%C + %H + %S + %N)					

Table S2 *Concentration of bonds and elements assessed through XPS analysis of CNC_{BL}, CD_{BL}, SCD_{BL}, and NCD_{BL}*

C 1s						
S.no.	**Chemical bond**	**Position of chemical bond (eV)**	**CNC_{BL} (% Area)**	**CD_{BL} (% Area)**	**SCD_{BL} (% Area)**	**NCD_{BL} (% Area)**
ChiSquared			*0.22*	*0.29*	*0.16*	*0.43*
1.	C=C	284.01	9.10	22.62	9.68	8.70
2.	C-C/H	285.4	56.51	53.24	61.02	58.74
3.	C-O/N/S	288.0	17.91	18.46	17.73	21.24
4.	C=O	288.9	16.48	5.69	11.58	11.32
O 1s						
ChiSquared			*0.30*	*0.30*	*0.21*	*0.40*
1.	C-O/OH	531.89	14.16	33.60	37.52	26.21
2.	C=O/ C-O-C	533.02	85.84	66.40	62.48	73.79
N 1s						
ChiSquared						*0.01*
1.	C-N-C	400.42	-	-	-	44.28
2.	$N-(C)_3$	402.12	-	-	-	46.06
3.	N-H	403.52	-	-	-	9.68
S 2p						
ChiSquared					*0.01*	
1.	$-C-S-(S2p_{1/2})$	162.54	-	-	12.12	-
2.	$-C-S-(S2p_{3/2})$	164.30	-	-	40.86	-
3.	$-C-SO_2-$	165.92	-	-	35.43	-
4.	$-C-SO_3-$	168.06	-	-	6.26	-
5.	$-C-SO_4-$	169.77	-	-	5.33	-
Elemental Analysis						

	Elements %	CNC_{BL}	CD_{BL}	SCD_{BL}	NCD_{BL}
1.	C	54.9	57.6	58.5	55.5
2.	O	36.9	34.4	36.5	35.6
3.	N	0.3	<0.1	0.7	2.4
4.	S	<0.1	<0.1	1.2	<0.1
5.	Si	5.1	6.7	1.7	3.7
6.	Others (Mg, P, Fe, Na, K, Zn, and Ca)	2.8	1.3	1.4	2.8

Table S3 *Antimicrobial assessment of the prepared sensors*

Nanocomposites	**MIC (mg/mL)**		**MKC (mg/mL)**	
	P. aeruginosa	**S. aureus**	**P. aeruginosa**	**S. aureus**
CD_{BL}	Not observed	Not observed	Not observed	Not observed
SCD_{BL}	6	6	10	10
NCD_{BL}	1.5	2.5	2.5	6

Table S4 *percentage solid content values, and pH change of the broccoli on different days, the biodegradability assessment of the prepared sensors.*

% solid content in the packed broccoli				
	CNC_{BL}	**CD_{BL}/CNC_{BL}**	**SCD_{BL}/CNC_{BL}**	**NCD_{BL}/CNC_{BL}**
2 Day	10.9	12.7	9.6	12.2
4 Day	6.87	9.4	9.4	10. 5
6 Day	6.7	9.2	8.2	9.0
8 Day	6.12	8.1	7.8	8.3
pH studies of the broccoli				
	CNC_{BL}/CNC_{BL}	**CD_{BL}/CNC_{BL}**	**SCD_{BL}/CNC_{BL}**	**NCD_{BL}/CNC_{BL}**

2 Day	6.87	6.55	6.48	6.78
4 Day	6.77	6.65	6.6	6.67
6 Day	6.21	6.93	5.95	6.48
8 Day	6.12	7.14	5.49	6.24

% Biodegradability of the prepared sensors			
% Biodegradability	**A**	**B**	**C**
CNC_{BL}/CNC_{BL}	99.90397	99.84879	99.85267
CD_{BL}/CNC_{BL}	98.85348	98.85744	98.75377
SCD_{BL}/CNC_{BL}	99.1579	99.8743	99.78349
NCD_{BL}/CNC_{BL}	98.46865	97.86592	98.78092

Supporting Information Section S3

S3 Energy and cost analysis for fabrication of aerogel

The following points were considered before estimating the cost:

- Waste Black Lentil Shell scraps are collected from local fields free of cost.
- Ethanol and toluene are also recyclable.
- The cost of manpower, establishment, and transportation is not considered.
- At the industrial level, costs may vary.

Table S5 *Prices of required chemicals*

Chemical name	Unit (kg or lit)	Price (INR/kg or INR/lit)
Distilled water	1	70
Ethanol	1	52
Toluene	1	40
Sodium chlorite	1	120
Sodium sulfite	1	22
Potassium hydroxide	1	25
Acetic acid	1	43
DMSO	1	113
Sulphuric acid	1	14
Ethylene diamine	1	155
Ammonium persulphate	1	85
Sorbitol	1	47

Table S6 *Energy calculation for fabrication of CNC-CDs/CNC_{BL} films*

1. Cellulose nanocrystals (CNC_{BL}) extraction	Dewaxing	Bleaching	Sulfite	Alkali	Hydrolysis	Centrifugation
Time consumed (h) (A)	8	10 (5+5)	2	4	0.75	1
Power (kW) (B)	0.11	0.06	0.06	0.06	0.06	0.2
Power consumed by each process (kWh)= (A×B)	0.88	0.6	0.12	0.24	0.045	0.2
Total power consumption (kWh)= 0.88+0.6+0.12+0.24+0.045+0.2= 2.085						
Chemicals	Ethanol:toluene (1:2= 150 ml)	Sodium Chlorite (5wt%-500ml) + acetic acid (400µl-500 ml) × 2	Sodium Sulfite (2wt%-500ml)	Potassium Hydroxide (7wt%-500ml)	Sulphuric acid (64 wt%-80ml)	-
Price (INR)	6.6	12.04	0.22	0.875	0.7392	
Total Chemical Cost (INR)= 6.6+6.02+0.22+0.875+0.7392= 20.47 **Distilled Water consumed in the process = 2 L, cost of distilled water = 140.0 INR**						
The cost for 4g CNC consumes 2.085 kWh, **The average electricity unit price (10 INR=P)** **4g CNC_{BL} consumes: 20.85(electricity) + 20.47 (Chemicals) + 140.0 (distilled water) = 181.32 INR**						
2. Formation of carbon dots (CDs) from waste shells of black lentils: • For the 150 ml CD_{BL} solution, only waste material and water are used (10.5 INR) • For 150 ml NCD_{BL} solution, ethylene diamine (7.5 ml) = 1.1625 + 10.5 = 11.6625 • For 150 ml SCD_{BL} solution, ammonium persulphate (1.5g) = 0.1275 + 10.5 = 10.627 • Drying with a vacuum oven consumes 1.45 kW/hour, so for 4 hours – 5.80 kW • Total cost of running vacuum oven for all three samples: 5.80 ×10= 58.0 INR • **Cost of 150 ml CD_{BL}- 29.83 INR** • **Cost of 150 ml NCD_{BL}- 30.99 INR** • **Cost of 150 ml SCD_{BL}- 29.957 INR**						
3. Formation of CDs/CNC_{BL} films: • **One CD_{BL}/CNC_{BL} film of 120 mm diameter and 2.5 mm thickness consumes 0.8 g CNC and 10 ml CD_{BL}** **Total cost of CD_{BL}/CNC_{BL} film= 36.26 + 1.988 = 38.25 INR** • **One NCD_{BL}/CNC_{BL} film of 120 mm diameter and 2.5 mm thickness consumes 0.8 g CNC and 2 ml NCD_{BL}** **Total cost of NCD_{BL}/CNC_{BL} film= 36.26 + 0.42= 36.68 INR** • **One SCD_{BL}/CNC_{BL} film of 120 mm diameter and 2.5 mm thickness consumes 0.8 g CNC and 2.5 ml SCD_{BL}** **Total cost of SCD_{BL}/CNC_{BL} film= 36.26 + 0.50 = 36.76 INR**						

References:

1 TAPPI T 550 om-08 - Determination of equilibrium moisture in pulp, paper and paperboard for chemical analysis, https://webstore.ansi.org/standards/tappi/tappi550om08, (accessed 27 December 2021).

2 Solvent Extractives of Wood and Pulp, Test Method T 204 cm-17, https://imisrise.tappi.org/TAPPI/Products/01/T/0104T204.aspx, (accessed 27

December 2021).

3 Acid-insoluble lignin in wood and pulp, Test Method T 222 om-21, https://imisrise.tappi.org/TAPPI/Products/01/T/0104T222.aspx, (accessed 28 December 2021).

4 C. Trilokesh and K. B. Uppuluri, *Sci. Reports 2019 91*, 2019, **9**, 1–8.

5 Alpha-cellulose in paper, Test Method T 429 cm-10, https://imisrise.tappi.org/TAPPI/Products/01/T/0104T429.aspx, (accessed 28 December 2021).

6 TAPPI 211 om-02 Cinzas - [PDF Document], https://fdocuments.in/document/tappi-211-om-02-cinzas.html, (accessed 28 December 2021).