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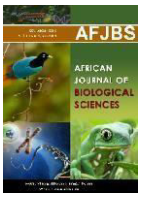

Cellulose Nanocrystals: A Brief Review On Structure, Methods Of Isolation And Application In Drug Delivery System

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Abstract
A combination of mechanical and chemical methods transforms cellulosic materials into cellulose nanocrystals (CNCs). CNCs have become a popular sustainable material owing to their enhanced features in response to the increasing global need for environmentally friendly, biodegradable and biocompatible products. Novel extraction processes, procedures, and resources are being developed to meet the increasing demand for cellulose nanocrystal-based products on an industrial scale. Cellulose nanocrystals show great potential as a biomaterial for drug delivery systems, catalysis, food processing, environmental investigations and heat transfer applications. The current review article examined the existing literature and presented a concise summary of the structural aspects, isolation methods and use of CNCs in the drug delivery system.

Keywords: Acid hydrolysis, Cellulose, CNCs, Enzymatic hydrolysis, Nanocrystal

1. INTRODUCTION

Cellulose (Figure 1.) is a naturally occurring polymer that may be derived from a variety of sources [1]. Worldwide funding agencies, governments and research communities are presently emphasizing the importance of nanotechnology and nanoscience, notably for cellulose nanocrystals (CNCs) also known as cellulose nanoparticles and cellulose nanowhiskers [2-5]. Several research organizations have recently shown an increased interest in cellulose nanocrystals as evidenced by the rising number of scientific research article publications on the subject of nanocellulose over the last decade [6-7]. Materials derived from bioresources have piqued researchers' interest in recent years due to their considerable potential for manufacturing a variety

of eco-friendly materials with minimal environmental impact [8-10]. Researchers utilized various biosources including tunicate, wood cotton, bacterial cellulose and others to produce a range of nanocrystalline cellulose (NCC) products with different dimensions [10]. The type of cellulosic material source and the hydrolysis conditions used for extraction determine CNC dimensions [11]. Nanocrystalline cellulose derived from bacterial cellulose and tunicate has been shown to have larger dimensions than nanocrystalline cellulose derived from wood and cotton [12-13]. CNCs are ideal materials for a new biopolymer composites industry because they have a higher axial elastic modulus than strength and their elastic modulus is comparable to other reinforcing materials [14-15]. Table 1. Mention some of the research initiatives and firms throughout the world that have recently begun commercial production of cellulose nanocrystals [16]. This review article begins with a summary of new findings in the structure of CNCs, followed by a detailed discussion of the leading methods for isolating CNCs from bioresources including applications.

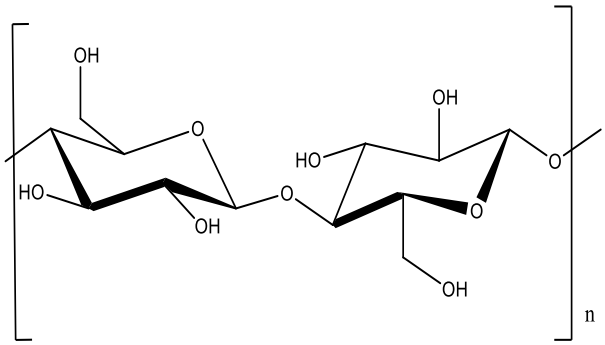

Figure 1. Structure of Cellulose molecule

Table 1. CNC producers with annual production capacity

Sr. No.	Company/Research Institute	Country	Production (tons/year)
1	CelluForce Inc.	USA	400
2	America Process Inc. (GranBio)	USA	200
3	Alberta Pacific Forest Industries Inc.	Canada	180
4	Anomera Inc.	Canada	11
5	Forest Products Laboratory (FPL)	USA	5
6	University of Maine	USA	4
7	Blue Goose Biorefineries Inc.	Canada	4
8	Cellulose Lab	Canada	4
9	Advanced Cellulosic Material Inc.	Canada	1
10	FPInnovations	Canada	0.5
11	InnoTech Alberta	Canada	0.3
12	Embrapa	Brazil	Pilot
13	Melodea	Israel	Pilot
14	Indian Council of Agricultural Research	India	Pilot (10kg/Day)

2. STRUCTURE OF CNC

With the elimination of the water molecule, the sugar units in the structure of CNCs are connected by -H and -OH groups [17]. It is believed to be in α configuration if the -OH group on the 1st carbon is on the same side of the ring as the -OH group on the 6th carbon. The first carbon of a cellulose molecule is oriented in the opposite way, resulting in the β arrangement [18]. Algae and tunicate cellulose microfibrils produce nanocrystals that are hundreds of micrometres long, whereas wood microfibrils produce a lot of shorter nanocrystals [19]. Cellulose is a linear chain of aldohexose molecules in which the continuation unit is made up of two anhydroglucose rings joined by a chemical element covalently attached to C1 of the aldohexose ring and C4 of the conterminous ring (1-4 linkage) and referred to as the glucosidic bond [20]. On one side of the molecule, the -OH group on the first carbon is an aldehyde hydrate group with increased activity. On the other hand, the -OH group on the fourth carbon on the other side of the chain is non-reducing [21]. Although cellulose does not exist as a single entity, a number of cellulose molecules (roughly 30 to 100 chains) can be arranged to form chain conformation using Van der Waals forces of attraction and hydrogen bonds to form the basic unit of cellulose fibres, protofibrils, which have dimensions ranging from 2 to 20 nm [22]. The presence of three -OH groups in equatorial sites all through the length of the cellulose chain was expected to make hydrogen bonding easy, according to some studies [23-24]. The cellulose microfibril and highly crystalline cellulose microfibril, may be isolated using a combination of mechanical, enzymatic and chemical processes, resulting in the production of desirable cellulose nanocrystals [25]. Surface modifications such as silane treatments, carboxylation, sulphonation, acetylation, polyelectrolyte adsorption, polymer grafting and 2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO) regioselective type oxidation can be used to optimize particle functionality [26-28]. Due to complex structural design that spans the nanoscale to macro range of the dimensions, biomaterials gain excellent mechanical strength and flexibility. It generates nanostructures with thin, seemingly limitless crystalline rods running along the cellulose microfibril [29-30]. Figure 2. shows schematic of hierarchical structure of plant cellulose.

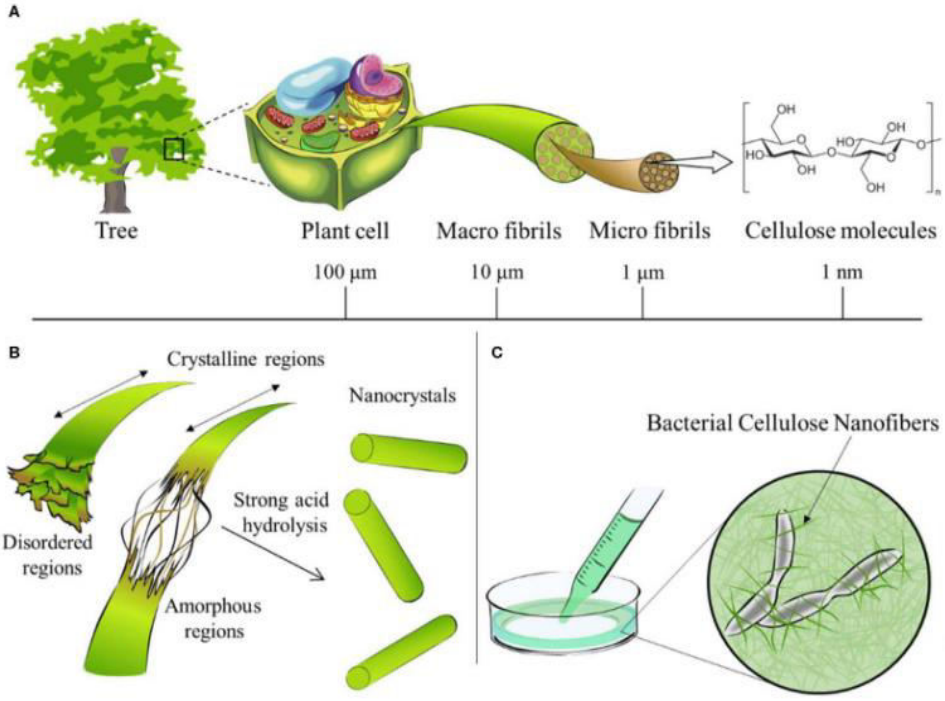

Figure 2. A) Plant based cellulose hierarchical structure from meter to nanometer scale. B) Schematic diagram of acid hydrolysis to obtain nanocellulose. C) Bionanocellulose cultured from

cellulose synthesizing bacteria [31].

3. METHODS OF ISOLATION

3.1. Acid Hydrolysis

Individual cellulose fibers can be isolated using pre-treatment methods that remove the matrix components out from feedstock completely or partially. Improving extraction techniques and improving integrated processes by combining two or more techniques from the list below might be the most effective way of enhancing CNC characteristics and solve the % yield limitation challenge. Mild acid hydrolysis yielded cellulose nanocrystals from cellulose fibres upon chemical treatment. On hydrolysis, concentrated sulfuric acid accesses the amorphous regions of cellulose polymeric chains and digests them, separating the crystalline regions [32]. The cellulose fiber's amorphous region is orientated randomly, resulting in a reduced density, making it highly susceptible to acid hydrolysis, particularly by hydronium ions H3O+, resulting in hydrolytic dissociation of glycosidic bonds. Figure 3. shows a schematic illustration of hydrolysis of cellulose fibre by sulfuric acid. Following the elimination of the amorphous region, an ultrasonic irradiation step (Sonication) isolates CNCs with half-sulfate ester groups on their surfaces. The charges are produced when sulfuric acid reacts with the hydroxyl groups of cellulose, causing repulsion between charged CNC (negatively charged) due to which it established colloidal stability in solution [33]. Other mineral acids or organic acid such as phosphoric acid, hydrochloric acid, hydrobromic acid etc. produce unstable suspensions, according to some studies [34-37]. Table 2 summarizes some of the reaction conditions for the acid hydrolysis technique.

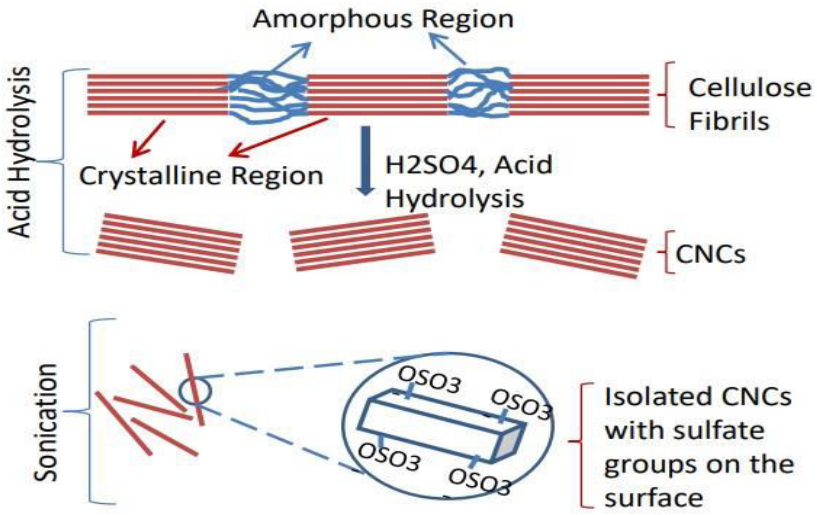

Figure 3. Schematic representation of sulfuric acid hydrolysis of cellulose fiber

Table 2. Reaction conditions for acid hydrolysis method of isolation

Sr. No.	Source	Purification	Treatment Procedure	Reference
1	Pineapple leaf	Grinding, NaOH, acetic acid, sodium chlorite treatments	Grinding, H_2SO_4 64% at 45 °C hydrolysis, dilution	[38]

2	Whatman filter paper	Blending	4N HCl solution at 100 °C for 120 min	[39]
3	Pseudostems of banana plants	Soxhlet extraction, bleaching with H_2O_2 and acetic acid	Dilution, blending, H_2SO_4 at 50 °C hydrolysis	[40]
4	Recycled Newspaper	Grinding, NaOH, sodium chlorite treatments at 125 °C	H_2SO_4 65% at 45 °C hydrolysis, dilution	[41]
5	Bleached kraft eucalyptus dry lap pulp	Soaking in water, disintegrating, drying	Anhydrous organic acid hydrolysis at 90–120 °C, dilution, filtration	[42]
6	Bacterial cellulose	Washing, homogenization, drying, grinding	H_2SO_4 /HCl mixture at 45 °C, dilution	[43]

3.2. Mechanical Treatment

Mechanical treatment is a physical approach for producing CNC that comprises of four steps: microfluidization, freeze shattering, fine grinding and homogenization [44]. The freezing medium in the freezing smashing method was liquid nitrogen, and the structure of the frozen fibres was fragmented into nanosize due to intense impaction resulting by mechanical smashing [45]. The kinetic friction of the two discs of a mechanical grinder divides the fibres into nanosize fibers via crushing, grinding, ripping, and shearing forces [46]. Figure 4. depicts the microfluidization under high pressure method for generating nanomaterial. The source material is compressed to high pressure around 4000 bars and then driven into an interactive tank, where a massive shear stress is developed, resulting in nanoparticle production [47].

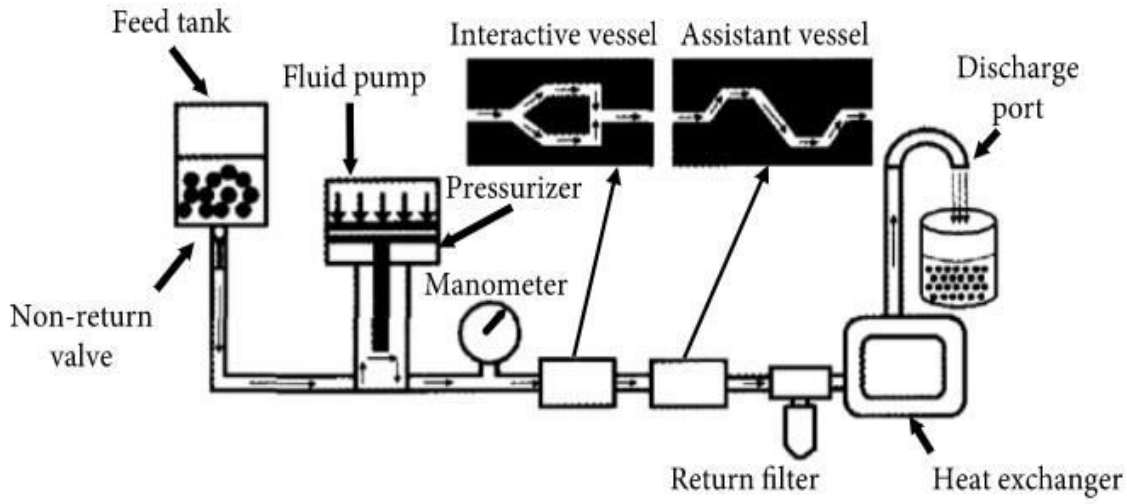

Figure 4. Schematic of microfluidization under high pressure method

3.3. TEMPO Oxidation Method

The CNC was harvested using TEMPO as an efficient oxidation method and common chemical reaction involved consist of green reagent TEMPO, NaBr, and NaClO [48]. According to current study, the C6-formyl group of cellulose was transformed to the C6-carboxyl group during TEMPO oxidation [49]. The NaBr was transformed to NaBrO by NaClO, which then oxidised TEMPO to nitrosonium. On oxidation, nitrosonium transformed the hydroxyl group in alcohol to carboxylic acid or aldehyde. Saito et al. used NaClO2 rather then NaBr in the TEMPO/NaBr/NaClO reaction system and reported that the aldehyde groups congregated in the newly produced CNC surface generated from the TEMPO/NaBr/NaClO reaction system, which didn't occur in the TEMPO/NaClO/NaClO2 system [50]. Figure 5 depicts the mechanism of primary hydroxyl to carboxyl oxidation by the TEMPO/NaClO/NaClO2 system. Though the TEMPO/NaClO/NaClO2 system had a few advantages, it also had some drawbacks, such as a lower rate of reaction and a relatively low carboxyl group content in newly produced CNC [51]. Table 3, lists several mechanical treatment conditions and oxidation procedures. The TEMPO-mediated oxidation approach has a number of major flaws, including hazardous TEMPO reagents, prolonged oxidation period, and restricted oxidation at C6 primary hydroxyl groups in CNCs. Another oxidation process was discovered utilising periodate-chlorite [52].

Table 3. Reaction conditions for Mechanical Treatment and Oxidation Methods

Sr. No.	Source	Purification	Treatment Procedure	Reference
1	Microcrystalline cellulose	-	Swilling in water, ultrasonication at power of 1500 W	[53]
2	Microcrystalline cellulose	-	Dispersion in water, ultrasonication for 50 minutes at an output of 500 W, frequency of 20 kHz	[54]
3	Wood	Ethanol solvothermal treatment, alkaline H_2O_2 treatment	Soaking in distilled water, ultrasonication	[55]
4	Jute fibers	Sodium hydroxide, washing, dimethylsulfoxide treatments	Treatment with TEMPO/NaClO/NaBr system	[56]

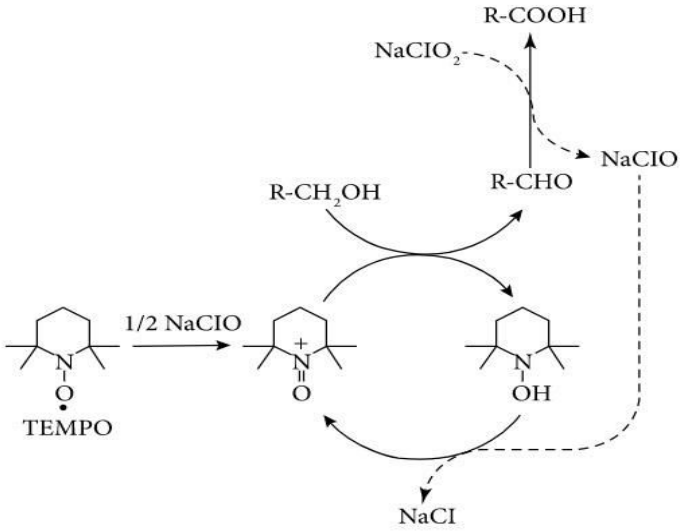

Figure 5. Mechanism of Oxidation of hydroxyl to carboxyl by TEMPO method [50]

3.3. Enzymatic Hydrolysis Method

Enzymatic CNC manufacturing is economical option of preparation methodology that does away with toxic chemicals and energy efficient method for mechanical fibrillation and heating process. Since enzymes selectively decompose the amorphous regions of cellulose fibres while ignoring the crystalline regions, CNCs with a hydroxyl group are produced. Figure 6. depicts the fundamental process of CNC manufacturing using the enzymatic hydrolysis technique.

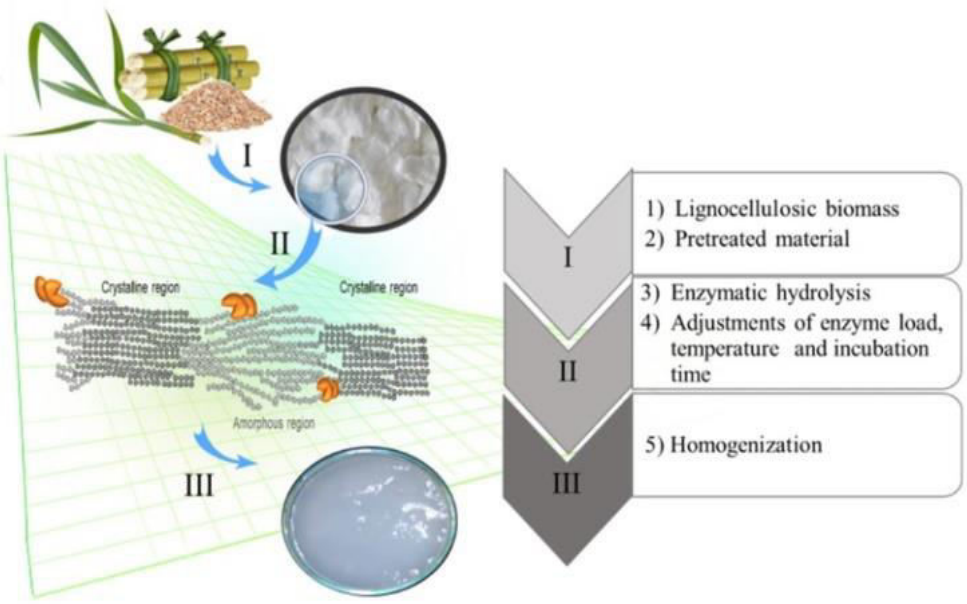

Figure 6. Schematic of enzymatic hydrolysis [57]

The first step (I) entails mechanically splitting the lignin and amorphous region inter - connectivity by trying to combine washing processes with pretreatment of the source material [58]. The processed material is hydrolysed in buffer solution with an enzymatic mixture in the second stage (II), followed by homogenization in the third phase (III) [59].

4. APPLICATION IN DRUG DELIVERY SYSTEM

CNCs were able to bind large amounts of the hydrophilic antibacterial drugs tetracycline and doxorubicin, allowing them to be released completely in one day. Due to the neutralising negative ionic conductivity property of CTAB (Cetyl trimethyl ammonium bromide), CTAB-modified CNCs bind a significant amount of water - insoluble chemotherapeutics, which were released in a predictable rate over several days [60]. The novel polyelectrolyte macro-ion complex (PMC) produced by cationic polysaccharide on the interface of CNCs (Binding chitosan) is critical for drug delivery applications particularly drug carriers [61]. Curcumin, a hydrophobic medication, may be contained in CD/ CNCs complexes, which are generated by ionic interaction between Cationic b-cyclodextrin (CD) and the surface of CNCs [62]. The coupling of folic acid molecules to the surface of CNCs provides another efficient mechanism for delivering chemotherapeutic compounds to folate receptor cancer cells [63]. Figure 7 depicts the hydrophobic and electrostatic relationships of surfactant molecules with cellulose nanocrystals, as well as the mechanism of drug adsorption. Table 4 lists numerous drug - delivery systems that rely on CNCs [64].

Table 4. Drug carrier systems based on CNCs

Sr. No.	Carrier form	Model drug	Release time and medium	Mechanism model
1	Microsphere or bead	Propranolol hydrochloride	12 h in pH 6.8 PBS	-
		Theophylline	16 h in pH 7.4, pH 6.8, pH 1.0 PBS	Ritger-Peppas equation
2	Suspension	Paclitaxel; docetaxel; DOX; TET; etoposide	1-4 d in PBS	-
3	Hydrogel or gel	DOX	6.5 d in water	Ritger-Peppas equation
		Bovine serum albumin	48 h in simulated body fluid	Fickian diffusion law
		Bovine serum albumin	20 h in pH 7.4 PBS	-
4	Nanofiber	Riboflavin	24 d in pH 7.4 PBS	-
		Hordein/zein	Riboflavin	24 d in pH 7.4 PBS

Abbreviations: DOX, doxorubicin hydrochloride; TET, tetracycline hydrochloride; PBS, phosphate-buffered solution.

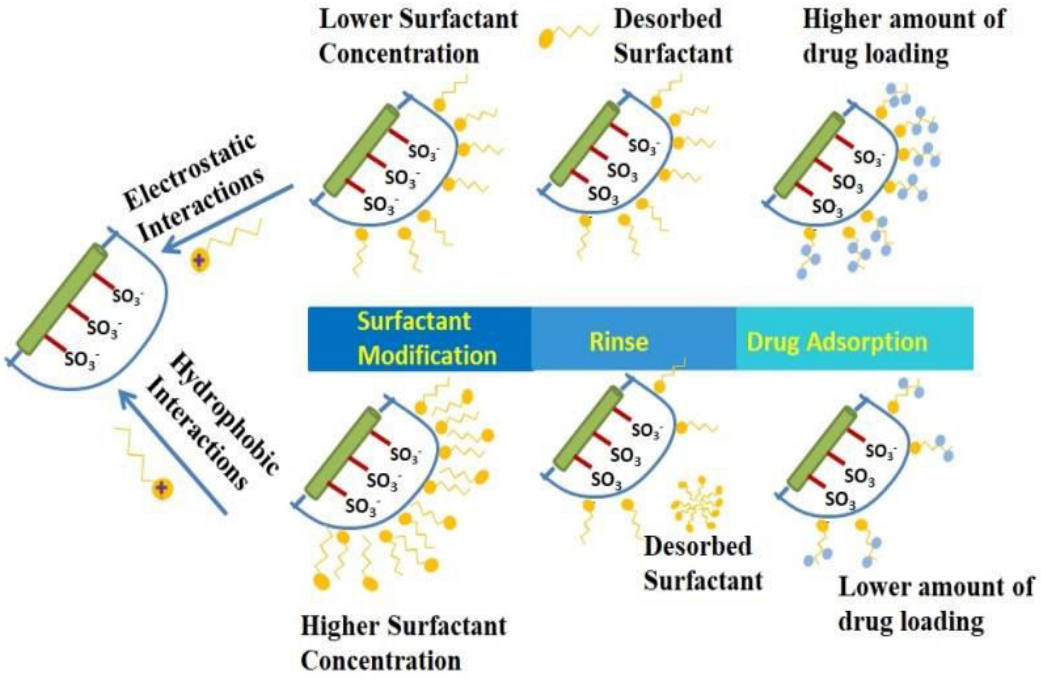

Figure 7. Schematic representation of surfactant and nanocrystalline cellulose mechanism and its effect on drug adsorption [65].

5. CONCLUSION AND FUTURE PERSPECTIVE

The extraction of CNCs from their parent biomaterials can disrupt their crystal structure, leading in qualities that differ from those anticipated for perfect cellulose crystal structures. CNC structural characterization will boost the development of models for better green industrial design. Because of the small size scale and present techniques' sensitivity is limited. Nano mechanics ofdiscrete CNC is still a huge difficulty. As a result, additional study is urgently needed to eliminate measurement uncertainty. Various chemical functionalization processes were investigated, and it was discovered that the majority of them focused on compatibilization. While much has been done to make CNCs compatible with matrix materials, the problem remains largely unsolved. Improving CNC dispersion in polymer matrix, reinforcing the composite's interfacial characteristics, and increasing strength and stiffness without sacrificing toughness are all major problems. One of the most significant obstacles in CNC chemistry is the high cost of production owing to low yield when starting with plant, animal, or tunicates-based raw materials and other bio sources. This may result in excessive waste output and energy consumption, in addition to higher manufacturing costs. Because cellulose is a cost-effective resource and the cellulose pulp and paper industry is particularly cost-conscious, if this problem is not solved, challenges to commercialization of the technology will arise in the near future.

Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

1. Cunha, A. G., Mougel, J. B., Cathala, B., Berglund, L. A., Capron, I., Preparation of Double Pickering Emulsions Stabilized by Chemically Tailored Nanocellulose, Langmuir, 30(31), 9327-9335 (2014).
2. Bledzki, A. K., and Gassan, J., Composites reinforced with cellulose based fibres, Prog. Polym. Sci. 24(2), 221-274 (1999).

3. Kumar, A., Vemula, P. K., Ajayan, P. M., John, G., Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil, Nature Materials, 7(3), 236–241(2008).
4. Lendlein, A., Jiang, H., Jünger, O., Langer, R., Light- induced shape- memory polymers, Nature, 434(7035), 879–882 (2005).
5. Pappu, A., Patil, V., Jain, S., Mahindrakar, A., Haque, R., Thakur, V. K., Advances in industrial prospective of cellulosic macromolecules enriched banana biofibre resources: A review, International Journal of Biological Macromolecules, 79, 449–58 (2015).
6. Abitbol, T., Kam, D., Levi-Kalisman, Y., Gray, D. G., Shoseyov, O., Surface charge influence on the phase separation and viscosity of cellulose nanocrystals, Langmuir, 34(13), 3925–3933 (2018).
7. Schyrr, B., Pasche, S., Voirin, G., Weder, C., Simon, Y.C., Foster, E. J., Biosensors based on porous cellulose nanocrystal-poly(vinyl alcohol) scaffolds, ACS Applied Materials and Interfaces, 6(15), 12674–83 (2014).
8. Sun, B., Zhang, M., Hou, Q., Liu, R., Wu, T., Si, C., Further characterization of cellulose nanocrystal (CNC) preparation from sulfuric acid hydrolysis of cotton fibers, Cellulose, 23(1), 439–450 (2016).
9. Braun, B., & Dorgan, J. R., Single-Step Method for the Isolation and Surface Functionalization of Cellulosic Nanowhiskers, Biomacromolecules, 10(2), 334–341(2009).
10. Cirtiu, C. M., Dunlop-Brière, A. F., Moores, A., Cellulose nanocrystallites as an efficient support for nanoparticles of palladium: application for catalytic hydrogenation and Heck coupling under mild conditions, Green Chemistry, 13(2), 288–291 (2011).
11. Cao, X. D., Habibi, Y., Lucia, L. A., One-pot polymerization, surface grafting, and processing of waterborne polyurethane-cellulose nanocrystal nanocomposites, Journal of Materials Chemistry, 19(38), 7137–7145 (2009).
12. Cheng, M., Qin, Z., Hu, J., Liu, Q., Wei, T., Li, W., Ling, Y., Liu, B., Facile and rapid one step extraction of carboxylated cellulose nanocrystals by H2SO4/HNO3 mixed acid hydrolysis, Carbohydrate Polymers, 231, 115701 (2020).
13. Coelho, C. C., Michelin, M., Cerqueira, M. A., Gonçalves, C., Tonon, R.V., Pastrana, L. M., Cellulose nanocrystals from grape pomace: production, properties and cytotoxicity assessment, Carbohydrate Polymers, 192, 327–336 (2018).
14. Nascimento, D. M., Almeida, J. S., Dias, A. F., Figueirêdo, M. C. B., Morais, J. P. S., Feitosa, J. P., Rosa, M. D. F., A novel green approach for the preparation of cellulose nanowhiskers from white Coir, Carbohydrate Polymers, 110, 456–463(2014).
15. De Souza Lima, M. M., Wong, J. T., Paillet, M., Borsali, R., Pecora, R., Translational and Rotational Dynamics of Rodlike Cellulose Whiskers, Langmuir, 19(1), 24–29 (2003).
16. Le Gars, M., Douard, L., Belgacem, N., & Bras, J., Cellulose nanocrystals: from classical hydrolysis to the use of deep eutectic solvents, Smart Nanosystems for Biomedicine, Optoelectronics and Catalysis. IntechOpen, France, 2019.
17. Dong, S., Cho, H. J., Lee, Y.W., Roman, M., Synthesis and Cellular Uptake of Folic Acid Conjugated Cellulose Nanocrystals for Cancer Targeting, Biomacromolecules, 15(5), 1560–1567 (2014).
18. Dong, X., Revol, J., Gray, D. Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose, Cellulose, 15(5), 19–32 (1998).
19. Errokh, A., Magnin, A., Putaux, J. L., Boufi, S. Morphology of the nanocellulose produced by periodate oxidation and reductive treatment of cellulose fibers, Cellulose, 25, 899–3911 (2018).

20. Beltramino, F., Roncero, M. B., Torres, A. L., Vidal, T., Valls, C., Optimization of sulfuric acid hydrolysis conditions for preparation of nanocrystalline cellulose from enzymatically pretreated fibers, Cellulose, 23, 1777–1789 (2016).
21. Favier, V., Chanzy, H., Cavaille, J., Polymer Nanocompsoites reinforced by cellulose whiskers, Macromolecules, 28(18), 6365–6367 (1995).
22. Foster, E. J., Moon, R. J., Agarwal, U. P., Bortner, M. J., Bras, J., Camarero-Espinosa, S. Current characterization methods for cellulose nanomaterials, Chemical Society Review, 47(8), 2609–2679 (2018).
23. Shaabani, A., Keshipour, S., Hamidzad, M., Seyyedhamzeh, M., Cobalt (II) supported on ethylenediamine- functionalized nanocellulose as an efficient catalyst for room temperature aerobic oxidation of alcohols, Journal of Chemical Sciences, 126(1), 111- 115 (2014).
24. Bandura, A. V., & Lvov, S. N., The ionization constant of water over wide ranges of temperature and density, Journal of physical and chemical reference data, 35(1), 15-30 (2006).
25. Fujisawa, S., Togawa, E., Kuroda, K. Nanocellulose-stabilized Pickering emulsions and their applications, Science and Technology of Advanced Materials, 18(1), 959–971(2017).
26. Chen, G., Roy, I., Yang, C., Prasad, P. N., Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy, Chemical Reviews, 116(5), 2826–2885(2016).
27. Ndong Ntoutoume, G. M. A., Granet, R., Mbakidi, J. P., Brégier, F., Léger, D. Y., Fidanzi-Dugas, C., Lequart, V., Joly, N., Liagre, B., Chaleix, V., Sol, V., Development of curcumin-cyclodextrin/cellulose nanocrystals complexes: New anticancer drug delivery systems, Bioorganic and Medicinal Chemistry Letters, 26(3), 941–945 (2016).
28. Gan, P., Sam, S., Abdullah, M. F., Omar, M. F., Thermal properties of nanocellulose-reinforced composites: a review, Journal of Applied Polymer Science, 137(11), 48544 (2020).
29. Gebald, C., Wurzbacher, J. A., Tingaut, P., Zimmermann, T., Steinfeld, A., Amine-based nanofibrillated cellulose as adsorbent for CO2 capture from air, Environmental Science and Technology, 45(20), 9101–9108 (2011).
30. Goussé, C., Chanzy, H., Excoffier, G., Soubeyrand, L., Fleury, E., Stable suspensions of partially silylated cellulose whiskers dispersed in organic solvents, Polymer, 43(9) 2645-2651(2002).
31. Miyashiro, D., Hamano, R., and Umemura, K., A review of applications using mixed materials of cellulose, nanocellulose and carbon nanotubes, Nanomaterials, 10(2),186 (2020).
32. Le Gars, M., Douard, L., Belgacem, N., & Bras, J., Cellulose nanocrystals: from classical hydrolysis to the use of deep eutectic solvents, Smart Nanosystems for Biomedicine, Optoelectronics and Catalysis. IntechOpen, France, 2019.
33. Habibi, Y., Lucia, L. A., & Rojas, O. J., Cellulose nanocrystals: chemistry, self-assembly, and applications. Chemical reviews, 110(6), 3479–3500 (2010).
34. Camarero Espinosa, S., Kuhnt, T., Foster, E. J., & Weder, C., Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis. Biomacromolecules, 14(4), 1223-1230 (2013).
35. Chen, L., Wang, Q., Hirth, K., Baez, C., Agarwal, U. P., & Zhu, J. Y., Tailoring the yield and characteristics of wood cellulose nanocrystals (CNC) using concentrated acid hydrolysis. Cellulose, 22(3), 1753-1762 (2015).
36. Viet, D., Beck-Candanedo, S., & Gray, D. G., Dispersion of cellulose nanocrystals in polar organic solvents. Cellulose, 14(2), 109-113 (2007).

37. Yu, H., Qin, Z., Liang, B., Liu, N., Zhou, Z., & Chen, L., Facile extraction of thermally stable cellulose nanocrystals with a high yield of 93% through hydrochloric acid hydrolysis under hydrothermal conditions. Journal of Materials Chemistry A, 1(12), 3938-3944 (2013).
38. Liu, D. G., Zhong, T.H., Chang, P. R., Li, K. F., Wu, Q. L., Starch composites reinforced by bamboo cellulosic crystals. Bioresource Technol., 101, 2529-36 (2010).
39. Hubbe, M., Rojas, O., Lucia, L., & Sain, M., Cellulosic Nanocomposites: A Review, BioResources, 3, 929-980 (2008).
40. Bercea, M., and Navard, P., Shear Dynamics of Aqueous Suspensions of Cellulose Whiskers, Macromolecules, 33, 6011-6016 (2000).
41. Tamo, A., Doench, I., Helguera, A., Hoenders, D., Walther, A., Madrazo, A. O., Biodegradation of Crystalline Cellulose Nanofibers by Means of Enzyme Immobilized-Alginate Beads and Microparticles, Polymers,f 12, 1522 (2020).
42. Kaushik, M., Li, A. Y., Hudson, R., Masnadi, M., Li, C.-J., Moores, A., Reversing aggregation: direct synthesis of nanocatalysts from bulk metal. Cellulose nanocrystals as active support to access efficient hydrogenation silver nanocatalysts, Green Chem., 18, 129-133 (2016).
43. Lima, M. M. D., Borsali, R., Rodlike Cellulose Microcrystals: Structure, Properties, and Applications, Macromol Rapid Commun., 25, 771-787 (2004).
44. Geng, L., Chen, B., Peng, X., and Kuang, T., Strength and modulus improvement of wet-spun cellulose I filaments by sequential physical and chemical cross-linking, Materials & Design, 136, 45-53 (2017).
45. Ma, J., Yu, F., and Wang,J. N., Preparation of water-dispersible single-walled carbon nanotubes by freeze-smashing and application as a catalyst support for fuel cells, Journal of Materials Chemistry, 20(27), 5742-5747 (2010).
46. LI, J., and LIU, Z.-m., Preparation and Hydrophobic Modification of Phyllostachys heterocycla cv. Pubescens NFC/SiO_2 Aerogel, Journal of Cellulose Science and Technology, 1, 8 (2016).
47. Yang, Y., Chen, Z., Zhang, J., Wang, G., Zhang, R., Suo, D., Preparation and Applications of the Cellulose Nanocrystal, International Journal of Polymer Science, 2019, 10 (2019).
48. Saito, T., Shibata, I., Isogai, A., Suguri, N., and Sumikawa, N., Distribution of carboxylate groups introduced into cotton linters by the TEMPO-mediated oxidation, Carbohydrate Polymers, 61(4), 414-419 (2005).
49. Saito, T., and Isogai, A., Introduction of aldehyde groups on surfaces of native cellulose fibers by TEMPO-mediated oxidation, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 289(1-3), 219-225 (2006).
50. Saito, T., Hirota, M., Tamura, N., Individualization of nano-sized plant cellulose fibrils by direct surface carboxylation using TEMPO catalyst under neutral conditions, Biomacromolecules, 10(7), 1992-1996 (2009).
51. Saito, T., Hirota, M., Tamura, N., and Isogai, A., Oxidation of bleached wood pulp by TEMPO/NaClO/NaClO2 system: effect of the oxidation conditions on carboxylate content and degree of polymerization, Journal of Wood Science, 56(3), 227-232 (2010).
52. Brinchi, L., Cotana, F., Fortunati, E., Kenny, J., Production of nanocrystalline cellulose from lignocellulosic biomass: technology and applications, Carbohyd. Polym., 94, 154-169 (2013).
53. Karim, Z., Mathew, A. P., Grahn, M., Mouzon, J., and Oksman, K., Nanoporous membranes with cellulose nanocrystals as functional entity in chitosan: removal of dyes from water. Carbohyd. Polym., 112, 668-676 (2014).
54. Kang, X., Kuga, S., Wang, C., Zhao, Y., Wu, M., and Huang, Y., Green preparation of cellulose nanocrystal and its application. ACS Sustain. Chem. Eng. 6, 2954-2960 (2018).

55. Mariano, M., El Kissi, N., Dufresne, A., Cellulose nanocrystals and related nanocomposites: Review of some properties and challenges, Journal of Polymer Science Part B: Polymer Physics, 52, 791-806 (2014).
56. Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., Dorris, A., Nanocelluloses: a new family of nature-based materials, AngewandteChemie International Edition 50, 24(2011), 5438-5466 (2011).
57. Zhu, J, Y., Sabo, R., Luo, X., Integrated production of nanofibrillated cellulose and cellulosic biofuel (ethanol) by enzymatic fractionation of wood fibers, Green Chem., 13, 1339-1344 (2011).
58. Camargo, L. A., Pereira, S. C., Pereira, S. C., Correa, A. C., Farinas, C. S., Marconcini, J. M., &Mattoso, L. H. C., Feasibility of manufacturing cellulose nanocrystals from the solid residues of second generation ethanol production from sugarcane bagasse, Bioenergy Res., 9(3), 894-906 (2016).
59. Barcelos, C, A., Rocha, V, A., Groposo, C., Castro, A, M., Pereira, N. Jr, Enzymes and accessory proteins involved in hydrolysis of lignocellulosic biomass for bioethanol production, Mycol. Curr. Futur. Dev., 1, 23-56 (2015).
60. Hamid, S. B. A., Zain, S. K., Das, R., Centi, G., Synergic effect of tungstophosphoric acid and sonication for rapid synthesis of crystalline nanocellulose, Carbohydrate Polymers, 138, 349-355 (2016).
61. Espinosa, S. C., Kuhnt, T., Foster, E. J., Weder, C., Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis, Biomacromolecules, 14(4), 1223-1230 (2013).
62. Dong, S., Cho, H. J., Lee, Y.W., Roman, M., Synthesis and Cellular Uptake of Folic Acid Conjugated Cellulose Nanocrystals for Cancer Targeting, Biomacromolecules, 15(5), 1560-1567 (2014).
63. Iwamoto, S., Nakagaito, A. N., Yano, H., Nano-fibrillation of pulp fibers for the processing of transparent nanocomposites, Applied Physics A- Materials Science & Processing, 89, 461-466 (2007).
64. Lin N., Dufresne A., Nanocellulose in biomedicine: Current status and future prospect, European Polymer Journal, 59 302-325 (2014).
65. Bundjaja, V., Sari, T. M., Soetaredjo, F. E., Yuliana, M., Angkawijaya, A. E., Ismadji, S., Cheng, K.-C., Santoso, S. P., Aqueous sorption of tetracycline using rarasaponin-modified nanocrystalline cellulose, J. Mol. Liq., 301, 112433 (2020).