Functionalized celluloses and their nanoparticles: Synthesis, properties and applications

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By

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August, 2014

CERTIFICATE

Certified that the work comprised in the thesis entitled "**Functionalized celluloses and their nanoparticles: Synthesis, properties and applications**" submitted by Miss. Priyanka R. Sharma was carried by her under my supervision/guidance. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

August, 2014

Dr. A.J. Varma

(Research Guide)

Declaration by the candidate

I declare that the thesis entitled **"Functionalized celluloses and their nanoparticles: Synthesis, properties and applications"** submitted for the degree of Doctor of Philosophy to the AcSIR-Delhi, has been carried out by me at the CSIR-National Chemical Laboratory, Pune, under the supervision of Dr. A. J. Varma. The work is original and has not been submitted as a part or full by me for any degree or diploma to this or any other university.

August, 2014 Priyanka R. Sharma

Dedicated to.....

My

Papa, Mummy

&

Bhaiya

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(Priyanka R. Sharma)

Contents

1.3. Carboxy Functionalized Celluloses

==========

Chapter 2: *Synthesis of 6-carboxycellulose and their nanoparticles*

==========

==========

Chapter 4: *Oxidized celluloses and their nanoparticles: Morphology, thermal properties and solubility studies*

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List of figures

Chapter1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter1

Chapter 2

Chapter 3

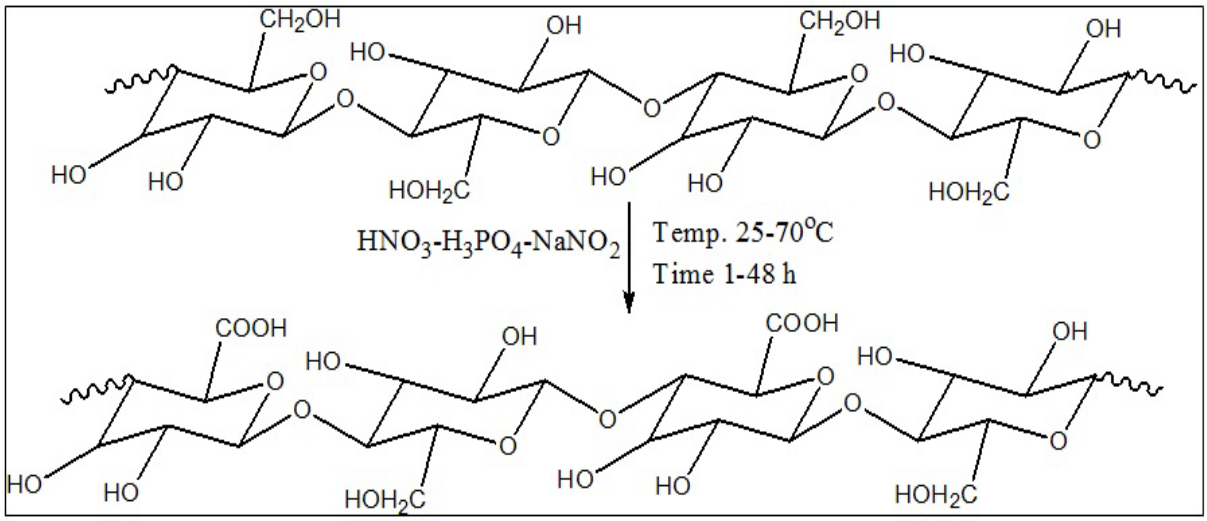
.

Chapter 4

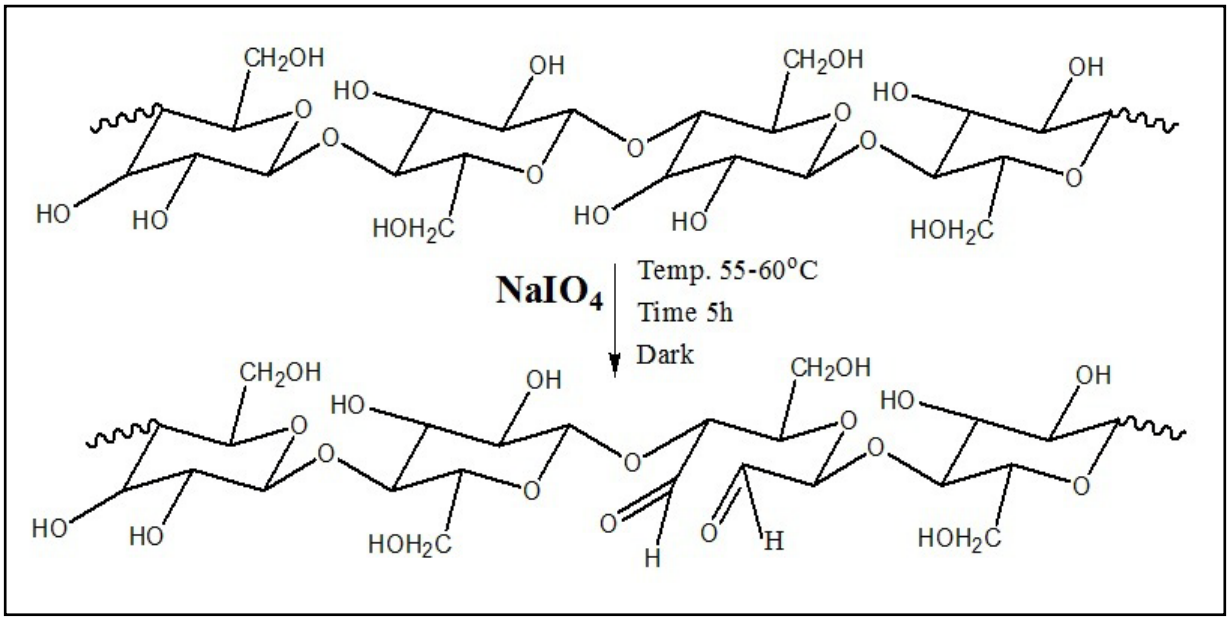
Chapter 5

List of Schemes

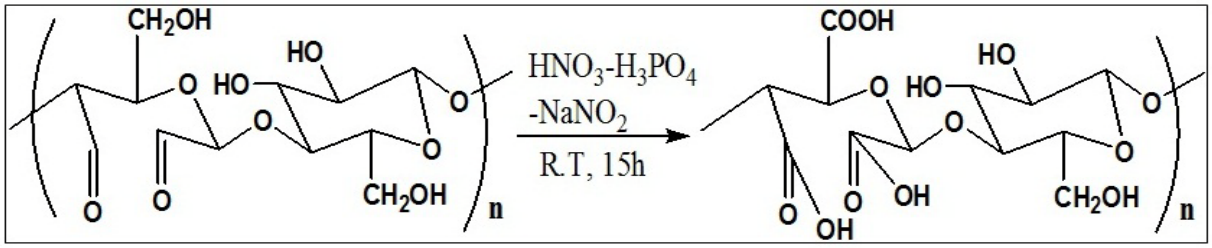
1. Preparation of 6-carboxycellulose



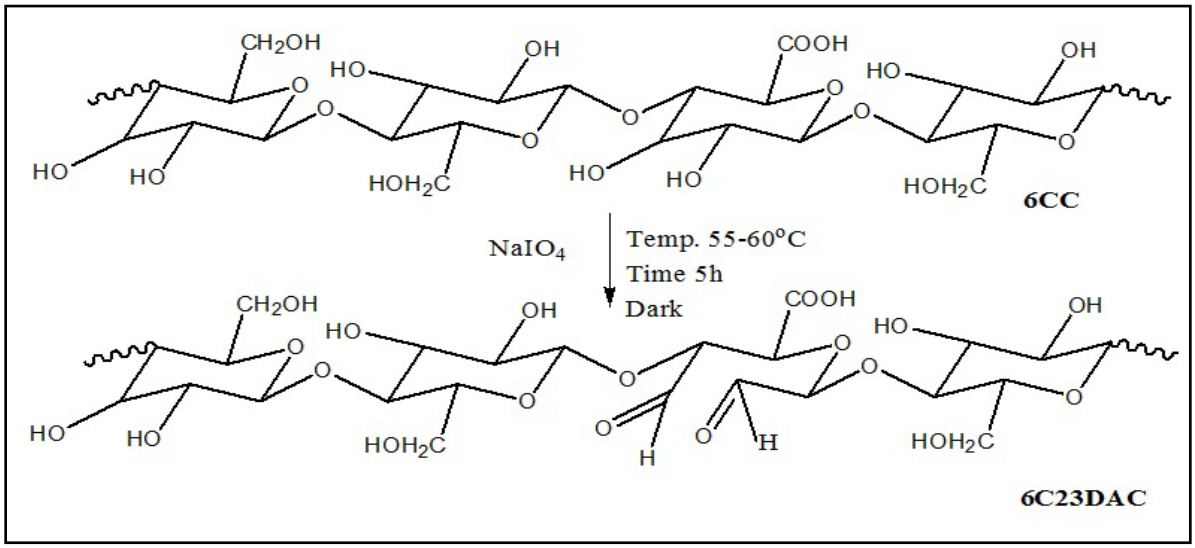
2. Synthesis of 2,3-dialdehyde cellulose



3. Synthesis of 2,3,6-Tricarboxycellulose.



4. Synthesis of 6-Caboxy-2,3-dialdehyde cellulose



Abbreviations

- 6CC: 6-Carboxycellulose
- TCC: 2,3,6-Tricarboxycellulose
- 6CC-NP: 6-Carboxycellulose Nanoparticles
- TCC-NP: 2,3,6-Tricarboxycellulose Nanoparticles
- DAC: 2,3-Dialdehyde Cellulose
- DCC: 2,3-Dicarboxycellulose
- 6C2,3DAC:6-Carboxy-2,3-Dialdehydecellulos
- DP: Degree Of Polymerization
- DLS: Dynamic Light Scattering
- AFM: Atomic Force Microscopy
- SEM: Scanning Electron Microscopy
- TEM: Transmission Electron Microscopy
- ATCC: American Type Culture Collection
- MIC: Minimal Inhibition Concentration
- nm: Nanometer
- *E. coli: Escherichia coli*
- *B. subtilis: Bacillus subtilis*
- *S. aureus: Staphloccocus aureus*
- CFU: Colony Forming Units
- DS: Degree of Substitution
- FTIR: Fourier transform infrared spectroscopy
- C: Carbon

- H: Hydrogen
- O: Oxygen
- nm: Nanometer
- ^{13}C-NMR: Carbon-13-Nuclear Magnetic Resonance
- OH : Hydroxyl
- H-bond: Hydrogen bond
- WAXRD: Wide angle X-ray diffraction
- L: Length
- W: Width
- MCC: Microcrystalline cellulose
- MFC: Microfibrillated cellulose
- NCC: Nanocrystalline cellulose
- BNC: Bacterial nanocellulose
- NFC: Nanofobrillated cellulose
- CMC: Carboxymethylcellulose
- MC: Methylcellulose
- HMC: Hydroxylmethylcellulose
- HPC: Hydroxylpropylcellulose
- NP's: Nanoparticles
- NaOH: Sodium hydroxide
- HCL: Hydrochloric acid
- H_2SO_4: Sulphuric acid
- H_3PO_4: Phosphoric acid
- HNO_3: Nitric acid

- PDI: Poly Dispersity Index
- $NaNO_2$: Sodium Nitrite
- FAS: Ferrous Ammonium Sulphate
- RT: Room Temperature
- CFC: Chloro Fluoro Carbon
- APS: Ammonium Persulphate
- TEMPO: 2,2,6,6-Tetramethylpiperidinium-1-Oxyradical

Abstract of the thesis

Introduction

This research thesis is based on developing a hypothesis and proving experimentally the possibility of synthesizing **shape and size tailored nanoparticles of carboxy celluloses using sugarcane bagasse derived cellulose as well as cotton cellulose**. Most published literature in this field is based on using softwood, hardwood, or cellulose or cotton linters. However, agricultural residue derived cellulose, also known as non-wood, have advantages over the wood cellulose as it does not contribute to de-forestation and can be obtained annually. The oxidized derivatives of cellulose such as 6-carboxycellulose and 2,3,6-tricarboxycellulose are well known biomedically important polymers. Since the 1940's, these have been extensively used as haemostatic scaffolding material, wound gauzes, bandages, bioresorable material etc. The properties of the molecules greatly depend on their shape, size, and molecular weight and these factors affect applications of various grades of these materials. Therefore, the biomedical properties of carboxy celluloses can be enhanced by converting them to nanoforms of uniform shape and size. So far, only nanofibres and nanowhiskers forms of carboxy cellulose are reported in literature. The spherical nanoparticles of cellulose are found more stable in aqueous dispersion media. Hence, the process of synthesis of carboxy cellulose is finely tuned by controlling the reaction conditions to obtain specific shapes and sizes of carboxy cellulose nanoparticles; in the present case, spherical shaped nanoparticles were obtained. The nanoparticles obtained were of uniform size (25-35nm for 6-carboxy cellulose and 20-50nm for 2,3,6-tricarboxycellulose) with low polydispersity (PDI) ratio (0.045 for 6-carboxy cellulose and 0.165 for 2,3,6-tricarboxycellulose). The obtained products 6-carboxy celluloses, 2,3,6-tricarboxycelluloses, 6-

carboxy-2,3-dialdehydecelluloses and their quasi-spherical nanoparticles are well characterized using FTIR, 13C CPMAS-NMR, WAXRD, TGA-DTG, SEM, TEM and AFM. The thesis also includes the study of supramolecular transitions occurring in the polymeric chain during the oxidation of cellulose by using HNO_3/H_3PO_4-$NaNO_2$ system. The transitions observed were proved by analysis using DTG, ^{13}C CPMAS-NMR and WAXRD techniques. The oxidation of cellulose by HNO_3-H_3PO_4-$NaNO_2$ system, coverts the cellulose I to cellulose II for <14% carboxy content 6CC and >14 % carboxyl content 6CC to amorphous cellulose.

The present thesis comprises of six chapters as follows:

CHAPTER 1: Introduction and Literature Survey

This chapter covers the detailed study on historical perspective of cellulose chemistry, cellulose structure, properties of cellulose (chemical, physical, and thermal properties), oxidized cellulose and nanoparticles of cellulose along with the table of major chronological events in cellulose chemistry. The section '**oxidized celluloses'** is nicely structured to cover the detailed description on several aspects which have had not covered in previous literature. This includes the history of oxidized celluloses, their classification, oxidizing agents-merits and demerits, methods of determination of oxidized group, synthesis method of mono and multi oxidized celluloses, industrially viable process of oxidized cellulose, possible oxidized cellulose derivatives, their applications, CAS No. for commercially available oxidized cellulose. Similarly, the section '**cellulose nanoparticles'** is configured to include the classification into sub-topic of nanoparticles of wood, cellulose, functionalized cellulose. Here we present, for the first time, detailed studies on nanoparticles on the basis of shape, size and functionalization are presented, revealing the significance of these factors in high-tech applications.

CHAPTER 2: Synthesis of 6-carboxycellulose and their nanoparticles

Carboxy functionalized celluloses, prepared by oxidation of cellulose, are amongst the most investigated derivatives of cellulose, due to their specific biomedical applications in surgical gauzes, wound dressing material, and several other related applications for over 70 years. The earliest report on oxidation of cellulose was reported way back in 1883. Since then there have been continuous streams of papers and patents on various methods of oxidation of cellulose, and the properties of such materials. In recent years, specifically around the period 1998-2000, nanofibres of oxidized celluloses have also been synthesized and their properties and applications vigorously investigated. However, we have seen no reports of spherical shaped carboxy functionalized celluloses. As observed for other cellulose nanoparticle derivatives, spherical shapes are more likely to show stability in solvent dispersions as compared to nanofibres. Similarly, the geometrical shape of the nanoparticle can play a role in drug delivery and biomedical applications. Herein we report, for the first time, devising a simple method to obtain spherical shaped polymer nanoparticles of 6-carboxycellulose (6CC) where so far only rod-shaped particles are reported. Since 6CC of varying carboxyl group content are an extremely important class of commercial biomaterials, we decided to investigate methods to prepare spherical nanoparticles of such materials as a function of carboxy content. We also demonstrate for the first time that these spherical functionalized nanoparticles are extremely efficient in stabilizing carbon nanotubes with minimal ultrasonication, thereby saving energy. Both these factors could be a key development for paving the way to many new applications for that material. The efficiency of these carboxycellulose quasi-spherical nanoparticles in anti-microbial applications was studied for E. Coli. It was found that the nanoparticles were more efficient than their larger sized analogs in eliminating E.Coli from aqueous solutions.

CHAPTER 3: Synthesis of multi-oxidized celluloses and their nanoparticles: A biomedically important polymer

In this chapter, we report for the first time the synthesis of quasi-spherical shaped TCC using cellulose I, having different contents of carboxy groups (high molecular weight cotton linters and low molecular weight sugarcane bagasse derived cellulose molecules). These new materials have been found to have sizes in the range 20-50 nm with narrow polydispersity (0.169). TCC molecules, due to their relatively high charge density as compared to 6CC, can expand the range of applications of 6CC. A previous report of carboxylated cellulose nanocrystals (11-19% carboxyl content, similar to ours) prepared by a different procedure did not produce spherical nanoparticals. Thus, the shape of the nanoparticle is highly dependent on the synthetic method employed for oxidation.The efficiency of these TCC quasi-spherical nanoparticles in anti-microbial applications was studied for E. Coli. We also demonstrate for the first time that these spherical functionalized nanoparticles are extremely efficient in stabilizing carbon nanotubes with minimal ultrasonication, thereby saving energy. TEM and AFM images of these nanoparticles shows fairly uniform size of the particles in the range 20-50 nm and with low DP (50-70), that can extend the range of applications of TCC made by other oxidation systems and using either Cellulose I or Cellulose II as starting material. TCC, either alone or as a combination with other materials, has been reported to be an extremely important biomedical polymer. It is manufactured by several industries under different brand names, each referring to a different formulation for specific applications. Thus, perhaps due to their high industrial value, there are hardly any publications describing their detailed synthesis, properties, and morphology; however, most information is available only in patent literature. A search of commercial literature shows that many products based on TCC are produced for high value medical

applications, and are referred to a “oxidized regenerated cellulose” (ORC). ORC has been used clinically for well over 50 years. The role in surgery has been reported in in several publications.

CHAPTER 4: Oxidized celluloses and their nanoparticles: Morphology, thermal properties and solubility studies

Agricultural residues derived cellulose was used to synthesize a new series of carboxy functionalized cellulosic nanoparticles (quasi-spherical shaped, 13.2% - 21.5% carboxyl content) and macro-sized 6-carboxycelluloses (long-fibril shaped, 1.7% - 22% carboxyl content). The DP (50-70) and yield (upto 46%) of nanoparticles were manipulated by controlling the reaction temperature and time. TGA/DTG thermographs of the carboxycelluloses gave thermostability data and co-related well with the residual crystalline, amorphous, and anhydroglucuronic acid content. The particle shape and size had no effect on the thermal stability. Some derivatives were fully or partially soluble in aqueous alkali and non-aqueous solvents, which can lead to increased versatility of these polymers. This chapter also includes the study of transition occurring in the polymeric chain during oxidation and was proved by our analysis using DTG. It was found that oxidation of cellulose by HNO_3-H_3PO_4-$NaNO_2$ system, coverts cellulose I to cellulose II for <14% carboxy content 6CC and >14 % carboxyl content 6CC to amorphous cellulose.

CHAPTER 5: Structure-property relationship of 6-carboxycellulose and their spherical nanoparticles by using ^{13}C CPMAS-NMR and WAXRD

A hypothesis was formulated and successfully proved to show that native cellulose-I can convert to cellulose-II during partial oxidation reaction to produce 6-carboxycellulose (6CC), using phosphoric acid as a component of the reaction medium. Previously reported oxidation studies

of cellulose are based on using cellulose-II (for the production of 6CC) due to its higher absorption capacity for body fluids. Since cellulose-I is known to swell considerably in phosphoric acid and converts to amorphous cellulose via a cellulose-II transition state, it was felt that conversion of cellulose-I to cellulose-II was possible during the oxidation process by use of phosphoric acid in the reaction medium. Thus, during the oxidation of cellulose-I to 6CC using the HNO_3-H_3PO_4-$NaNO_2$ oxidation system over periods ranging from 1-48h and temperatures ranging from 25-70^oC, 6CC's of varying carboxyl contents (1.7%-22%), shapes and sizes (macro-sized fibrils of several micron length and/or spherical nanoparticles of 25-35nm) could be produced selectively. It was found that 6CC's having <14% carboxyl content were largely in cellulose-II form, whereas at >14% (upto 22%) the 6CC's were largely amorphous, with trace crystallinity observed at 19% and 22% carboxyl 6CC, evidenced by a small 101 peak in the WAXRD. Interestingly, a comparison between spherical nanoparticles and macro-sized fibrils of 6CC's containing similar carboxyl group contents showed that the nanoparticles retained a high degree of crystallinity having cellulose-I structure, whereas the macro-sized fibrils were converted to the more absorbent cellulose-II structure. WAXRD results were well complimented by CP-MAS ^{13}C NMR studies, which confirmed amorphous cellulose for >14% carboxyl 6CC compounds (C6 peaks reducing and shifting, broadening of C2,3,5) and increased crystallinity of nanoparticles (sharp C4 and C6 peaks at ~63 and ~81 ppm respectively).

CHAPTER 6: Conclusions and Suggestions for future work

This chapter presents the summary of the results obtained in this thesis research. It includes relevant conclusions, along with predicting new applications and suggestions for future work.

Chapter 1

Introduction and Literature Survey

Cellulose, Nanocellulose and Carboxyl Functionalized Cellulose /Nanocellulose

1.1. Cellulose

1.1.1. Earliest developments in cellulose chemistry

The journey of cellulose technology has been assumed to have started around 4500 BCE, with the discovery of cotton. The evidences for spinning cotton into cloth were known during the Indus Valley Civilization, around 3000 BCE (Chaurasia, 2008). The great Indian epic Ramayana also depicts the existence of high quality writing material. In 326 CE, Nearchos, an admiral of Alexander the Great, mentioned in his writings that the Indians wrote letters on well beaten cotton cloth. It has been widely reported that around 105 CE, Ts'ai Lun of the imperial court of China found a way to make paper from mulberry and other bast fibers (Edwin, 1954). During the 2^{nd} to10^{th} century CE, the paper-making process had expanded in many European countries. In the 14^{th} and 15^{th} century, paper production had rapidly expanded with the invention of printing press, which ushered the printing revolution (Boruchoff, 2012).

1.1.2. Birth of modern cellulose chemistry

The systematic development of cellulose began with the isolation of cellulose from plant material by Sir Anselme Payen in 1838 (Payen, 1838). He concluded that the extracted fibrous material must contain glucose molecules and could be an isomeric form of starch due to the elemental analysis (44.4% C, 6.2% H and 49.4 % O) (Payen, 1842). In 1839, this fibrous material was named as cellulose by the French academy (Brongniart, 1839). In 1833 before the discovery of cellulose, Bracconet had produced a mixture of nitrated cellulose products named 'xyloidine' by dissolving wood in concentrated nitric acid. This was the first cellulose derivative 'nitrocellulose', which led to several industrial applications of cellulose (Schoenbein, 1846;

Pierre et al., 2003). In 1844 Mercer succeeded in dissolving cotton fibers in strong alkali, and this was the beginning of the now famous mercerization process of cotton (Parnell, 1886; Mercer, 1850; Arthur et al., 1898; Trillat, 1928). The discovery of mercerized cellulose fibres opened up the area of cellulose polymorphism. The first "artificial silk Chandronnet" was discovered in 1884. This was prepared by treating mulberry leaves with concentrated nitric and sulphuric acids to form soluble cellulose nitrate (Chandronnet, 1884). A momentous period of cellulose chemistry came about in 1865, when Hyatt Manufacturing company prepared the first man-made highly transparent and flammable plastic film "celluloid" (cellulose nitrate with camphor as plasticizer) (Hyatt, 1865). This celluloid was used for making photographic films, cinema films, etc. After this, successful commercialization of celluloid new cellulose derivatives continued to be developed and exploited industrially. Cellulose acetate was patented in 1865 (Schuetzenberger, 1865; Weber et al., 1899). In 1857, Schweizer dissolved cellulose in cupric hydroxide - ammonia solution (Schweizer's reagent cuoxam) (Schweizer, 1857). British chemists Charles Cross, Edward Bevan, and Clayton Beadle were also active in this research; they treated cellulose with xanthate solution to prepare the 'viscose cellulose' (Simonsen and Einar, 1890; Siegmund, 1899; Mitchell, 1949). The discovery of cellulose xanthate was a big triumph in the field of cellulose "solution" chemistry, and till date the viscose cellulose continues to be the basis of multi-billion dollar rayon, rayon staple and cellophane manufacturing companies (North American Raron Corporation, 2004). In the early 1900's Cross and Bevan had characterized cellulose into various forms based on the dissolution of plant components in a 20% aqueous sodium hydroxide solution. The un-dissolved part was considered as α-cellulose while the soluble materials assigned as β-cellulose and ϒ-cellulose. The α-cellulose was considered as pure cellulose while β-cellulose and ϒ-cellulose was referred to as a part of pentosans (Cross and

Bevan, 1907). The polymeric form of cellulose was elucidated in 1920-30 by Herman Staudinger (Staudinger, 1920). He proved his discovery by X-ray diffraction studies.

1.1.3. Cellulose structure

1.1.3.1. Early era of structure determination

Since hydrolysis of cellulose as well as cellobiose produced glucose as the main hydrolysis product, it was soon realized that cellobiose, consisting of two glucose moieties linked by β-glycosidic bond, could be considered to be the repeating unit of cellulose (Fisher, 1909; Richtmyer and Hudson, 1939, Haworth and Hirst, 1921). Representation of glucose as a six membered pyranose ring was propounded by Haworth (Haworth, 1925; Charlton, Haworth and Peat, 1926); this facilitated the representation of cellulose as a linear chain of glucose molecules connected by β-1,4-glycosidic bonds. The molecular structure of cellulose is shown in Figure 1.1. Further, this interpretation was confirmed by X-ray crystallography and nuclear magnetic resonance studies of cellulose (Brown and Levy, 1965; Chu and Jeffrey, 1968; Bergmann and Knehe, 1925; Sundararaajan and Rao, 1968; Michell, 1970; Koch and Peterlin, 1970; Ham and Williams, 1970).

1.1.3.2. Molecular structure: Current status

Further work showed that the pyranose units are present in an energetically favourable $^4C_1$ chair conformation (Michell and Higgins, 1965; Ellefsen and Tonnesen, 1971). The glucan chain consists of a non-reducing group at one end and a reducing group at the other end of the chain. The cellulose molecule was found to have a twofold helical conformation (French et al., 2003);

this means that the units are oriented with each other at 180° with respect to their mean plane. This conformation gives a flat ribbon like structure to each molecule that is stabilized by intra-molecular H-bonds (Nishiyama et al., 2003; Nishiyama, Chanzy and Langan, 2002; Cousins and Brown, 1997; Ritcey and Gray, 1988). Additionally, the intermolecular H-bonds present between the chains caused the molecules to have crystalline features. The twisting of microfibrils due to intramolecular H-bondings leads to amorphous regions in cellulose, making it a semi-crystalline polymer (Meyer and Mark, 1929).

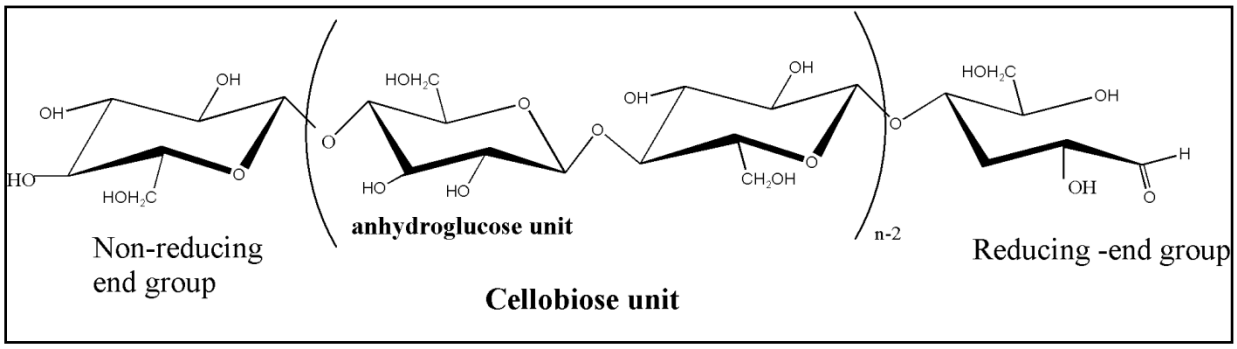


Figure1.1: Molecular structure of cellulose polymer

The degree of polymerization (DP) of cellulose molecules varies with the origin and extraction procedures adopted. The DP reported for bacterial cellulose is 4000-10,000, for purified cotton linter is 1000-5000, wood pulps 500-2000, algal cellulose Valonia 25000, and agricultural residues 200-1000. Regenerated cellulose, prepared by first dissolving and then precipitating cellulose, has much lower molecular weight; rayon (300-500) and cellophane (300) are examples (Zugenmaier, 2008; Stamm, 1964). Microcrystalline cellulose, produced by acid hydrolysis, has a very low DP of 200-300 (Dumitriu, 2005). Interestingly, cellulose with a DP of as low as 20-50 also shows the properties of a cellulose polymer, such as its thermal degradation features and its solubility in alkali solutions (Kobayashi, Sakamato and Kimura, 2001).

1.1.3.3. Supramolecular structure

The supramolecular structure of cellulose can be surmised as follows: each linear glucan chain has a width 0.4 nm and length 500 nm. The single glucan chains H-bond with neighbouring chains to form microfibrils of 3-4 nm width and about 2000 nm length. Thirty to forty cellulose chains H-bond closely to form a microfibril. The microfibrils are in turn H-bonded with other microfibrils to form bundles of microfibrils. Extraction procedures generally yield microfibril bundles. These fibres have a width of 20-30 microns, length of 1-3 mm, and 70-90% crystalline regions (Isogai, Saito and Fukuzumi, 2011). This can be visualized by the Figure 1.2.

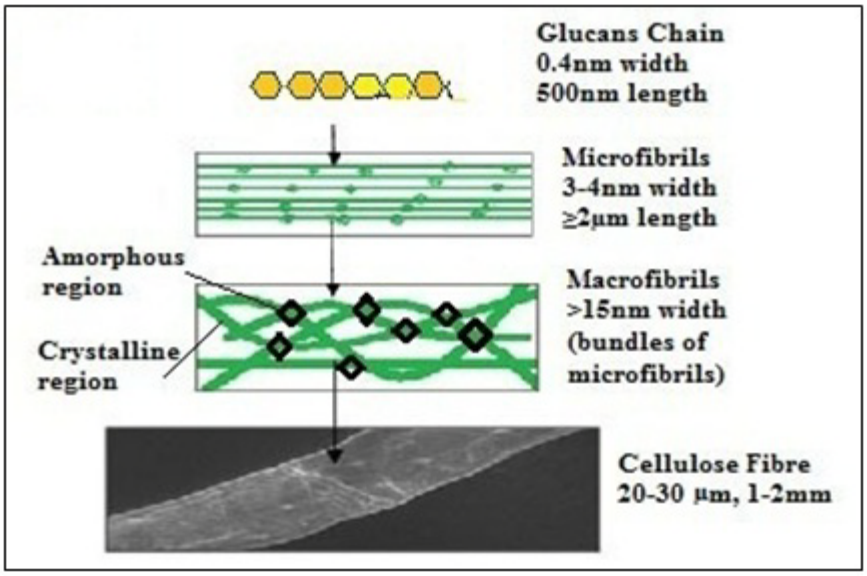


Figure1.2: Hierarchical structure of wood biomass.

1.1.3.4. Polymorphs of cellulose

Native cellulose occurs as cellulose I, the most crystalline and abundant form of cellulose. Further, cellulose I has two variants, Iα and Iβ (Atalla and Vanderhart, 1984). The ratio of Iα and Iβ in cellulose depends on its source. The crystalline form Iα was found to be analogous to the model proposed by Gardner and Blackwell (Nishiyama et al., 2003). But, the crystalline structure

of Iβ is still under investigation due to the problem of extracting it in pure form (Lejeune and Deprez, 2010). Cellulose Iβ is thermodynamically more stable than Iα, and the latter can be transformed into Iβ by annealing at 260-280°C in various medium (0.1 NaOH, glycol, ethanol, glycerol, PEG 300, helium, heptane). The Iα and Iβ crystalline forms of cellulose can be easily differentiated by solid state ^{13}C-NMR, Raman spectra and electron diffraction studies (Sugiyama, Vuong and Chanzy, 1991; Atalla and Vanderhart, 1987). Solid state ^{13}C-NMR demonstrates that Iα form has singlet at C1 and C6 while Iβ has doublet at C1, C4, C6 carbon of anhydroglucose units. Similarly electron diffraction studies indicate that the Iα form has a triclinic unit cell with one chain per unit cell with non-equivalent anhydroglucose units while Iβ form appears as monoclinic unit cell of space group P_{21} with two chains per unit cell with symmetrical anhydroglucose units. Recently, FTIR spectroscopic techniques along with the practice of intra-crystalline deutereration and rehydrogenation were used to investigate the localization of Iα and Iβ in cellulose microfibrils of Iα-rich algal cellulose (Horikawa and Sugiyama, 2009). The FTIR band at 3240cm^{-1} corresponding to –OH stretching band is more prominent in Iα-rich cellulose I. Similarly, FTIR band at 750cm^{-1} is also present in Iα-rich cellulose I but these band disappeared on converting Iα to Iβ cellulose **(**Debzi et al., 1991; Hardy and Sarko, 1996). The bonding patterns of Iα and Iβ are still a point of discussion as different patterns of bonding have been observed in Iα and Iβ. This could be due to different stacking patterns of cellulose sheets in both Iα and Iβ forms (Jarvis, 2003). The native cellulose exists mostly as cellulose I and also known to be possesses the highest crystallinity as compared to any other polymorph. Cellulose I can be transformed into cellulose II by mercerization, regeneration, and treatment with super critical solvents (Langan, Nishiyama and Chanzy, 2001; Langan, Nishiyama and Chanzy, 1999; Zhao et al., 2006). This transformation is irreversible as cellulose

II is found to be thermodynamically more stable than cellulose I (Kroon-Batenburg and Kroon, 1997). Cellulose I has parallel chains, while cellulose II has anti-parallel chains. Cellulose I has two intramolecular H-bonds at (O)5-(OH)3^{1} and (OH)2-(O)6^{1} and an interchain H-bond between (O)6-(O)3^{11} as shown in figure 2 (Liang and Marchesssault, 1959; Mann and Marrianna, 1958; Marchesssault and Liang, 1962). Cellulose II is reported to have an intrachain hydrogen bonding at (OH)3-(O)5^{1} and intermolecular hydrogen bonding at (OH)6-(O)2^{11} for corner chains and (OH)6-(O)3^{11} for centre chain. In cellulose II one more hydrogen bond is observed which is absent in cellulose I. This is intersheet-intersheet interaction between (OH)2 (corner chain)-(O)2^{11} (center chain) (Warwicker, 1967). (The superscript ^{1} and ^{11} are used for explaining intra and intermolecular hydrogen bonding in the molecule, respectively). The H-bonding in cellulose I and cellulose II are shown in figure 1.3.

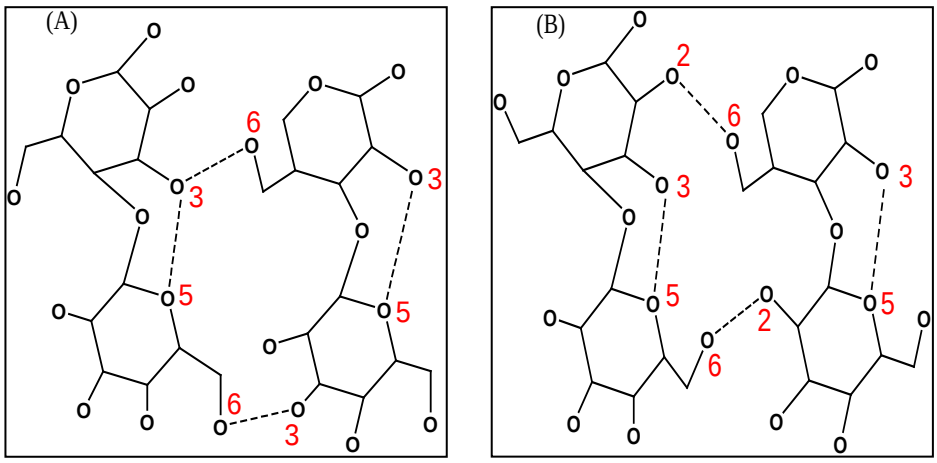


Figure1.3: Hydrogen bondings pattern for (A) Cellulose I and (B) Cellulose II

X-ray diffraction studies have shown cellulose to possess different polymorphic forms, depending on the chemical treatment to which the native cellulose is exposed; these are known as cellulose I, cellulose II, cellulose III, cellulose IV. Each of them possesses different

arrangements of lattice planes in the unit cell. These can be well studied by electron diffraction and solid state ^{13}C-NMR (Isogai, 1994). Cellulose III and cellulose IV can be derived from both cellulose I and cellulose II through different treatments. However, cellulose III and IV constitute a very specialized field of research and are beyond the scope of this paper. Most practical applications of cellulose are limited to cellulose I and II. The crystallinity of cellulose I and II can be destroyed by various techniques to produce largely amorphous cellulose. Some of the common methods are phosphoric acid treatment, ball milling of cellulose (Hermans and Weidinger, 1946), deacetylation of cellulose acetate under nonaqueous alkaline condition (Wadehra and Manley, 1965), regeneration of cellulose solution into nonaqueos media (Jezirny and Kepka, 1972), and regeneration of cellulose in aqueous media (Isogai and Atalla, 1991).

Non crystalline forms of cellulose can be clearly differentiated from the crystalline form by several characterization techniques such as solid state ^{13}C-NMR, WAXRD, thermal analysis, etc. It is reported in literature that the chemical shift for the C4 resonance of amorphous cellulose appears at about 84 ppm while for other forms, (cellulose I, II, and III) it appears about 89-90 ppm (Isogai and Atalla, 1991)**.** Cellulose I and II have distinct peaks in the WAXRD spectrum (Sebe et al., 2012, Wang, Ding and Cheng, 2008, Sharma and Varma, 2014b).

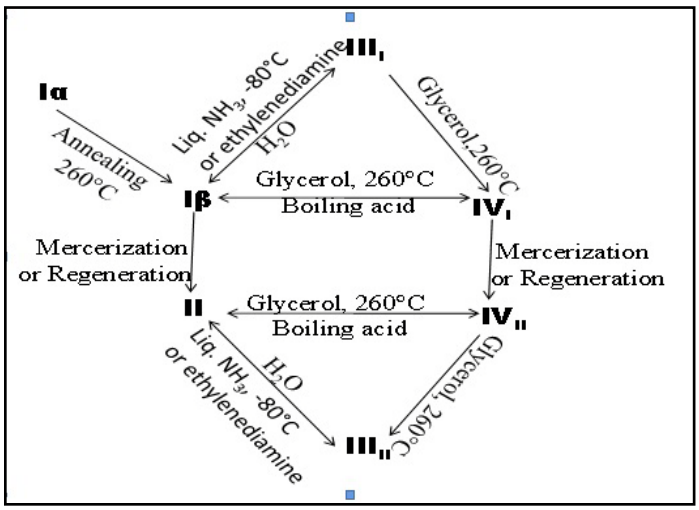


Figure1.4: The diagrammatic representation for various allomorphs of cellulose (Lejeune and Deprez, 2010)

1.1.3.5. Properties of cellulose

Cellulose is biodegradable, non-toxic, and hygroscopic material. It is insoluble in water and dilute acids. Aqueous alkali solutions cause swelling and dissolution, depending on the concentration of alkali. The surface hydroxyl groups of cellulose are highly reactive, and can be used for preparing cellulose esters, ethers, etc. However, in order to get uniform products, the cellulose has to be first swollen to allow reactants to penetrate the fibrils. The elastic modulus (GPa) and tensile strength of cellulose are relatively high (e.g. for cotton the elastic modulus is 5-13 GPa and the tensile strength is 0.28-0.6 GPa; these values increase with increase in degree of polymerization, crystallinity, and degree of orientation (Krassig, 1993; Gilbert,1994). Cellulose fibres obtained from different sources such as hemp, kenaf, jute, sisal, bamboo, cotton, coir, etc. have different physical properties due to different fibril sizes, crystallinities, and molecular weights (Saheb and Jog, 1999). The thermal degradation of cellulose starts at 180°C. Thermal data on cellulose is shown in the table 1 (Klemm et al., 1998; Krassig et al., 2004).

Pure cellulose is a good insulator with resisitivity of $10^{18}\Omega$cm (D. Klemm et al., 1998). All these properties are appropriate for using cellulose fibres as reinforcement for composites. Due to the hydrophilic nature of cellulose, chemical modifications by esterification, etherification, oxidation etc. are favoured to make cellulose fibre more suitable for composites.

Table1.1: Thermal and electrical properties of cellulose (Klemm et al., 1998; Krassig et al., 2004)

Thermal decomposition	>180°C
Glass transition temperature	230-245°C
Ignition Point	>290°C
Heat of combustion	17.46 J g^{-1}
Heat of crystallization	18.7 – 21.8 J g^{-1}
Specific heat	1.00 – 1.21 J $g^{-1}K^{-1}$
Heat of transition Cell I → Cell II	38.1 J g^{-1}
Coefficient of thermal conductivity	0.255-0.920 $KJm^{-1}h^{-1}K^{-1}$

1.2. Cellulose Nanoparticles

1.2.1. Introduction

In recent years, nanoscience and technology has taken centre stage of new developments in the chemistry of materials. Cellulose chemists have not been far behind in adapting this science. The first report of nanocrystal of cellulose was by Ranby in 1949 (Ranby et al., 1949; Bengt and Ranby, 1949). In addition to this, Battista had successfully prepared MCC from cellulose by hydrochloric acid hydrolysis followed by ultrasonication treatment (Battista, 1950; Battista et al.,

1956). Subsequently, the unique properties of MCC had increased its application in the areas of food additives, food binders, pharmaceutics exciepients, paper, and composites industries.

1.2.2 Classification of family of nanocellulose

The classification of cellulose nanoparticles depends on many factors. For example, bacterial celluloses have very high length/diameter compared to plant derived nanocelluloses, and a very different morphology and physical structure which gives it several unique properties, such as high hydration, shape retention and antibacterial properties (Lin et al., 2013). Similarly, if hydrolysis of cellulose is used for preparing nanoparticles, then the surface of cellulose gets endowed with functional groups. Consequently, charged nanocrystals obtained from H_2SO_4 hydrolysis are more stable in colloidal dispersions (Araki et al., 1998). Klemm et al. differentiated cellulose nanoparticles in three categories: microfibrillated cellulose (MFC), nanocrystalline cellulose (NCC), and bacterial nanocellulose (BNC) on the basis of their dimension, functions, and preparation methods (Klemm et al., 2011). However, the latter classification neither included the nanoparticles of cellulose derivatives, and nor the different shapes such as fibriller, ribbon shaped, spherical, etc. Therefore, we now propose the following as a more complete classification of various nanocelluloses.

1.2.2.1. Nanoparticles obtained from different cellulose sources

Celluloses can be obtained as commercial crops such as cotton, hemp, sisal, kenaf, etc., extracted from woods such as soft woods and hardwoods, extracted from grasses and agricultural residues, or extracted from animals like tunicates, produced by fungi, algae and bacteria. Nanofibres can also be obtained from potato tuber cells, banana rachis, pea hull, corn cobs, coconut husk, jute,

jute plant wsates, cotton plant waste, castor plant wastes, mulberry plant, palm leaves and cuttings, soybean plant wastes (Khalil et al., 2012; Peng et al., 2011; Cao et al., 2008). Each of these celluloses has characteristic differences due to changes in DP and polydispersity, morphology, fibre bundle sizes, and so on.

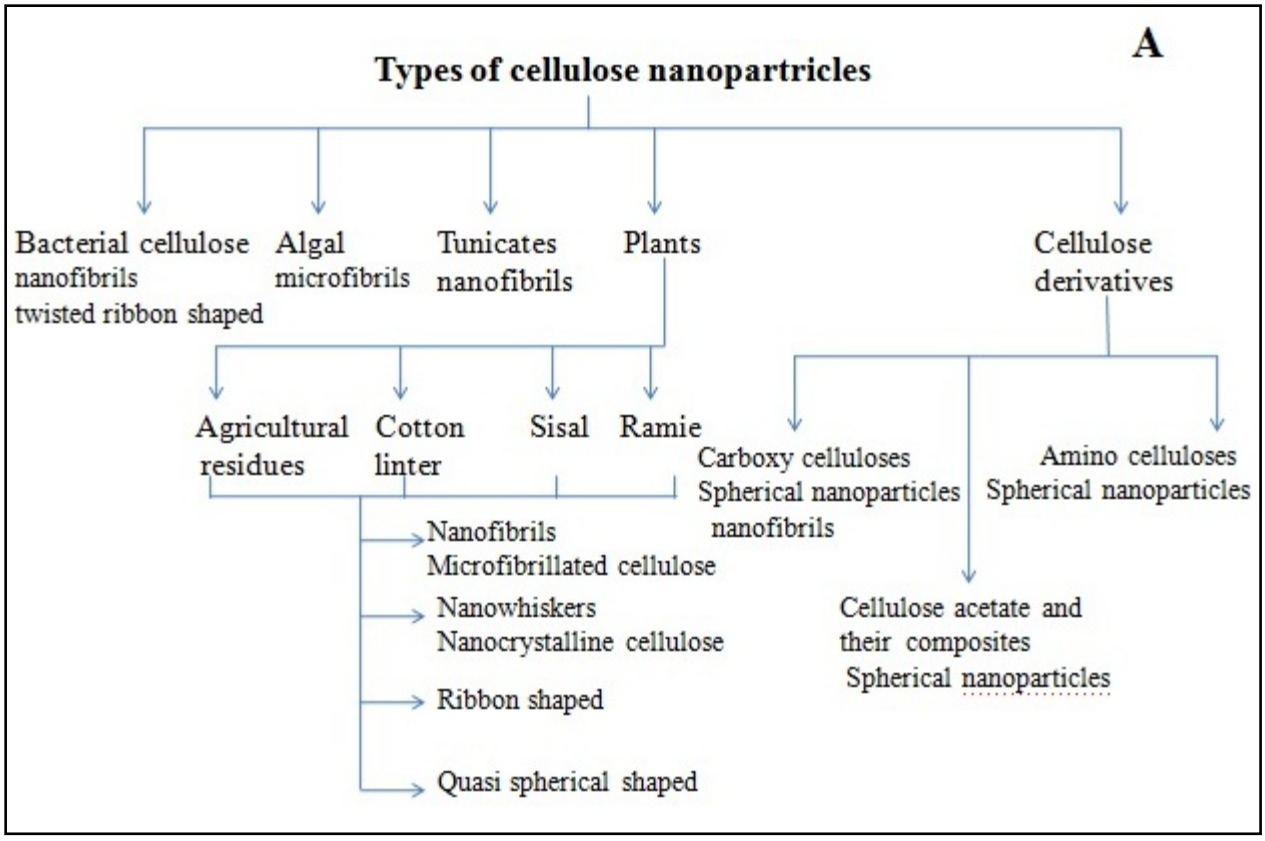


Figure1.5 (A): Various shapes of nanoparticles of cellulose and their derivatives from different sources of cellulose, as reported in literature (see refs. in text).

1.2.2.1.1 Nanofibrils / Microfibrillated celluloses (MFC)/ Nanofibrillated cellulose (NFC)

Individual separated microfibrils of cellulose are known by many terms, like microfibrillated cellulose (MFC), microfibrils, and nanofibrillated cellulose (NFC). Various methods like mechanical treatments like cryocrushing (Wang et al., 2007), high pressure homogenizing

(Angles and Dufresne, 2000; Habibi et al., 2010), enzymatic pretreatment (Malainine et al., 2005; Nakagaito and Yano, 2004; Alemdar and Sain, 2008), surface modification (Saito et al., 2009) and ultrasonic technique are used to obtain microfibrillated cellulose/ nanofibres/ nanofibrillated celulose. Such treatments cause defibrillation (separation) of microfibrils bundles to separate fibrils. These fibrils are 2-40 nm in diameter and >10,000 nm in length with aspect ratio (L/D) generally >1,000. In the period 1970-1980, Sandberg et al. started manufacturing MFC at ITT, Rayonnier, USA (Turbak, Snyder and Sandberg, 1983; Herrick, 1983). The defibrillation of cellulose fibres requires either multiple step homogenization under high pressure or long time sonication treatment. It is reported that ~25,000 kwh/ton electrical energy is required for defibrillation of soft wood cellulose fibres (Turbak et al., 1983; Herrick et al., 1983). Therefore, to develop energy efficient process, pretreatment is one of the methods for "loosening" the bonds between the fibres and facilitating the process of de-fibrillation. The main problem encountered with defibrillation under high pressure was the blocking of homogenizer due to wet fragments of cellulose fibres (Turbak et al., 1982); later it was found that the clogging of homogenizer could be avoided by use of hydrophilic polymers such as CMC, MC, HMC HPC, poly (acrylic acid), carrageenan, and guargum (Turbak et al., 1982). Several advancements are in progress to develop new low energy processes for nanofibres (Lavoine et al., 2012; Khalil, Bhat and Yusra, 2012). The MFC/ nanofibres/ NFC posses both crystalline as well as amorphous regions while NCC have much high crystallinity. The latter are discussed in greater details in the following section.

1.2.2.1.2. Nanowhiskers / Nanocrystalline cellulose (NCC)

Rods like crystalline nanoparticles are known as nanowhiskers. In general, nanowhiskers of cellulose are 2-20 nm in diameter and 100-600 nm in length with aspect ratio (L/D) 10-100 (Habibi et al., 2010). NCC/ nanowhiskers preferentially have crystalline region. These are produced by acid hydrolysis of cellulose which results in removal of noncrystalline regions, and crystalline region remains intact (Angles and Dufresne, 2001; Ruiz et al., 2000). Thus, NCC has greater crystallinity than cellulose fibre but a drastically reduced DP. However, it was noticed that after a sudden decrease in DP, no further significant change in DP occurred on further hydrolysis (Battista et al., 1956; Sharples, 1958). A number of papers mention the production of NCC from various sources such as cotton (Dong, Revol and Gray, 1996; Araki et al. 2000) hemp , flax (Cao et al., 2007), wheat straw (Helbert, Cavaille and Dufresne, 1996), ramie (Habibi and Dufresne, 2008; Habibi et al., 2007), avicel, tunicin, algae (Angles et al., 2001; de Souza Lima and Borsali, 2002; Angles and Dufresne, 2000) and bacteria (Grunert and Winter, 2002). These are mostly used as reinforcing materials for nanocomposites. The examples of L and D of cellulose nanoparticles from various sources and obtained by different techniques are presented in a paper by Habibi (Habibi, Lucia and Rojas, 2010).

1.2.2.1.3. Ribbon shaped nanoparticles

Recently, Sebe reported the formation of ribbon shaped cellulose nanoparticles by acid hydrolysis of MCC (Sebe et al., 2012). The shape of these particles was controlled by the amount of concentrated sulphuric acid added and time of reaction. On analysis, it was found that two types of NP's were obtained: cellulose nanowhiskers of cellulose I structure and ribbon shaped nanoparticles of cellulose II structure (Sebe et al., 2012). It was concluded that due to increase in

surface area of ribbon shaped cellulose nanoparticles, these could improve the barrier properties of nanocomposite films.

1.2.2.1.4. Quasi spherical nanoparticles

In general, spherical shaped nanoparticles of cellulose can be obtained by several methods; NaOH treated cellulose under ultrasonication (Adsul et al., 2012), mixed acid (HCl + H_2SO_4) treated cellulose followed by ultrasonication (Wang, Ding, and Cheng, 2008; Zhang et al., 2007), ionic liquid (Han et al., 2013) and controlled hydrolysis of cellulose in presence of anaerobic bacteria (Satyamurthy and Vigneshwaran, 2013).

The quasi spherical shaped cellulose nanoparticles have been obtained from different sources such as MCC (Wang, Ding, and Cheng, 2008**;** Zhang et al., 2007; Han et al., 2013) and from microwhiskers of sesame husk by homogenization (Sekhar et al., 2011). The spherical nanoparticle formation might be the result of swelling action by acid mixtures, alkaline solutions or ionic liquids. It seems that in all the reported data, swelling agents such as aqueous NaOH, ionic liquid, concentrated H_3PO_4, mixture of concentrated H_2SO_4 and HCl. This fact seems to suggest that swollen cellulose takes up spherical shape on drying, though the exact reason for that is still a mystery. The diameters of spherical cellulose nanoparticles vary with the methods of preparation; sizes obtained from NaOH treatment produce 60-570 nm, ionic liquids 118 ± 32 nm, and acid mixtures of concentrated H_2SO_4 and HCl and produce 20-90 nm size particles. Two recent papers throw useful new light on the reasons for the supramolecular changes occurring on cellulose I as as it undergoes reaction in a strongly acid medium to produce a product with spherical nanoparticles of carboxy functionalized cellulose (Sharma and Varma, 2014a; Sharma and Varma, 2014b). These aspects need to be investigated further, and as more work is published

on supramolecular transitions of cellulose in strongly acidic media undergoing various reactions, the reasons will undoubtedly unfold.

1.2.2.1.5. Bacterial cellulose nanofibrils (BNC)

In 1886, it was found for the first time found that cellulose can be produced from *Acetobacter xylium* bacteria in the form of extracellular matrix (Brown, 1886). Bacterial cellulose is also termed as microbial cellulose and biocellulose. Many gram-negative and gram-positive bacteria such as *Acetobacter*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella*, *Alcaligenes*, and *Sarcina ventriculi* respectively produced cellulose (Shoda and Sugano, 2005). But, *Gluconacetobacter xylinus* which was formerly known as *Acetobacter xylinum*, have the ability to form cellulose in high yield. *Gluconacetobacter xylinus* form biofilms in the presence of sufficient supply of oxygen (air) and a carbon source (glucose). Bacterial synthesis of cellulose leads to a cellulose with diameter of 20-100 nm and length of several micrometers, and consist of different types of nanofibre networks. Thus, bacterial cellulose does not have to undergo any further treatment process to convert to a nanofiber, it is designed by nature to be a nanofibre.

Applications

BNC's possess properties such as high purity, high tensile strength, high water absorption capacity, hydrophillicity, etc. which are different from celluloses obtained from woods, agricultural residues, and grasses. Nature produces them in the form of nanofibers and they have very high DP (4000-10000) and crystallinity above 60% (Klemm, 2006; Salmon and Hudson, 1997; Yano et al., 2005). BNC hydrogels have several applications: as bioactive implants ('in vivo' scaffold material), as biofabricated material in the field on personal care and medicine (Klemm et al., 2006), as immobilizing agent for cells, as base for the production of cellular

matrices (Bodin et al., 2008), as a wound healing material, and as a material for tubular implant (Wiegand et al., 2006; Wang et al., 2007)

There are several limitations of the above products for their large scale manufacture. A cost efficient process is required for the mass production and continuous harvesting of cellulose filaments with uniform nanofibre lengths (Sakairi et al., 1998). Many research papers and reviews have been published dealing with biofabrication of bacterial cellulose (Chawla et al., 2009), on it's status and prospects (Tan, Hong and Shao, 2007; Dahman, 2009), adhesion and surface characteristics (Gardner et al., 2008), physiochemical properties and the potential for medical applications, (Luong, Lee and Nam, 2008; Thomas, 2008), technical applications, (Wang, Liu, Gao and Li, 2007) and uses in veterinary medicine (Angles and Dufresne, 2001; Angles and Dufresne, 2000; Cao et al., 2008).

1.2.2.2. Nanoparticles of cellulose derivatives: Cellulose acetate, Aminocellulose, and Carboxycellulose

Spherical hydrophobic cellulose acetate composites with other polymers has been recently reported and found to be useful in pharmaceuticals and food technology (Kulterer et al., 2012; Hornig and Heinze, 2008). Cellulose acetate nanofibrils were used for loading high capacity of silver NP's for various biomedical applications (Luong, Lee and Nam, 2008). Spherical shaped amino functionalized cellulose derivatives of size 80-200 nm are found to be useful in gene delivery (Nikolajaski et al., 2012). However, amine functionalized polymers polyethyleneimine used for such studies posses toxicity while polysaccharides are non-toxic (Nikolajaski et al., 2012). The carboxymethylcellulose-quaternary ammonium cellulose spherical NP's were found efficient as protein carriers showing high degree of cellular uptake (Song et al., 2012).(The detail

descriptions of carboxycellulose NP's are given separately in section 1.3.4 due to significant biomedical applications of carboxycelluloses over several decades).

1.3. Carboxy Functionalized Celluloses

1.3.1 Introduction to oxidation chemistry of celluloses

The oxidation reaction is one of the most important methods for functionalizing cellulose with ketone, aldehyde, and carboxylic groups by use of appropriate reagents. Moreover, the physiochemical properties of the oxidized products greatly vary with the DS, randomness of distribution of substituted groups, molecular weight, and polydispersity of the products. Since 1880, several types of oxidizing agents were used, but only a selected few gave useful products. In this section, the reactivity of various oxidizing reagents on cellulose is explained. Initially, oxidation of cellulose was mainly carried out for bleaching cotton fibres (Edmund and Leonard, 1920; Harold, 1915). Oxidation chemistry of cellulose was founded by Witz in 1883 with their first oxidation reaction using concentrated HNO_3 (Witz, 1883; Cross and Bevan, 1883). The product obtained was termed "oxycellulose" with empirical formula $C_{18}H_{26}O_{16,}$ but it was not fully characterized. In 1899, Faber and Tollen observed that oxycellulose is the composition of unreacted cellulose units and reacted celloxin units (defined by Tollen as the cellulose repeating unit containing aldehyde, ketone or carboxyl groups). The reaction of oxidized products with phenylhydrazine proved that the molecule contained reducing groups. In order to find the degree of oxidation, the oxycellulose was acetylated with acetic anhydride (Vignon and Gerin, 1900). The developments in oxidation of cellulose were modest till the 1940's, and the products obtained were heterogeneous. It was observed that degradation of product increased with

increase in oxidant quantity and reaction time, giving low yields (Heuser, 1938; Rowen, Hunt and Plyler, 1947). At that time, periodic acid was the only known selective oxidant which attacked the secondary hydroxyl groups at C2, C3 position of anhydroglucose unit (Jackson and Hudson, 1932; Jackson and Hudson, 1937; Rutherford et al., 1942). The oxidation of cellulose with nitrogen dioxide came to light in 1941, and was found to be a promising oxidant for cellulose oxidation at C6 primary hydroxyl group, selectively giving rise to carboxyl groups (Yackel and Kenyon, 1942). This product was found to have anti-microbial properties and had applications in wound gauzes. This led to the systematic development of 6CC (Yackel and Kenyon, 1942). Figure 1.5 (B) shows the selective and non-selective oxidants of cellulose. The type of oxidized celluloses is shown in Figure 1.5 (C).

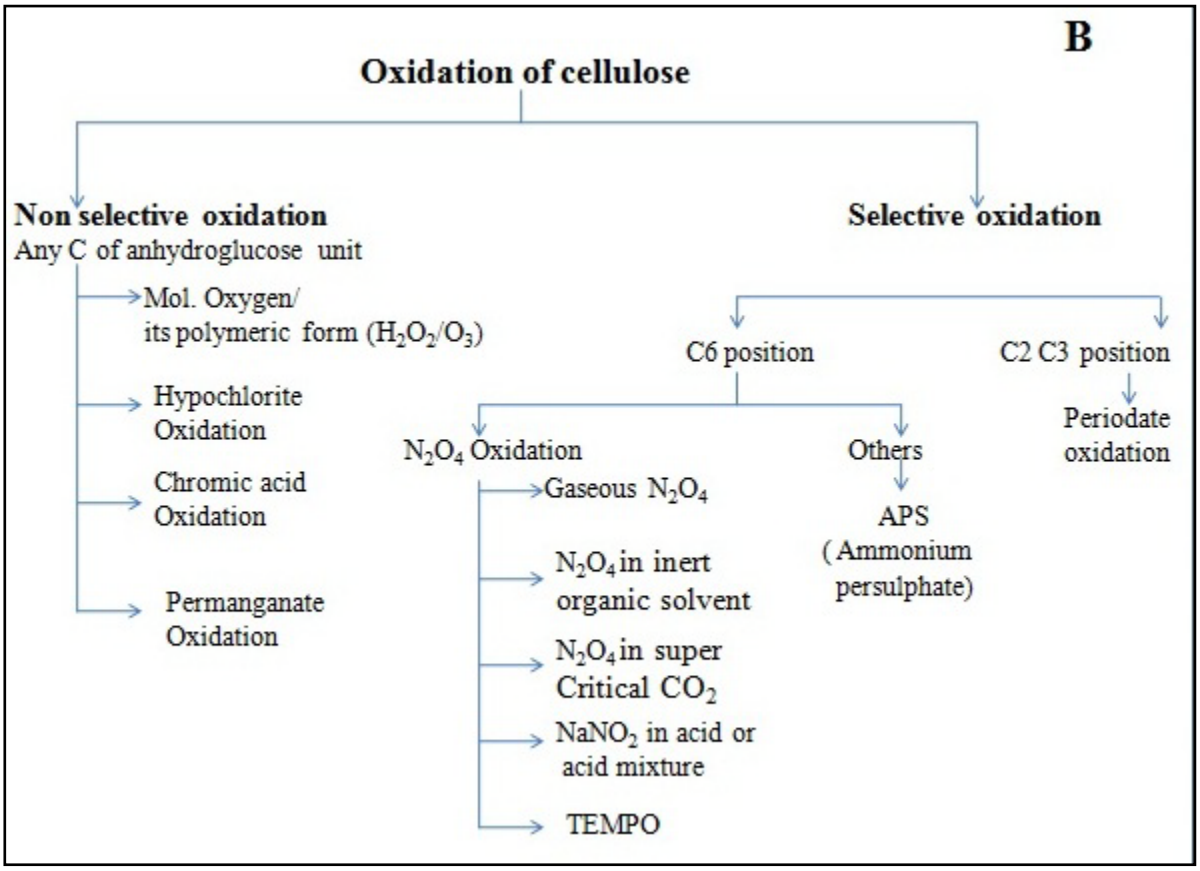


Figure1.5 (B): Types of oxidation of cellulose

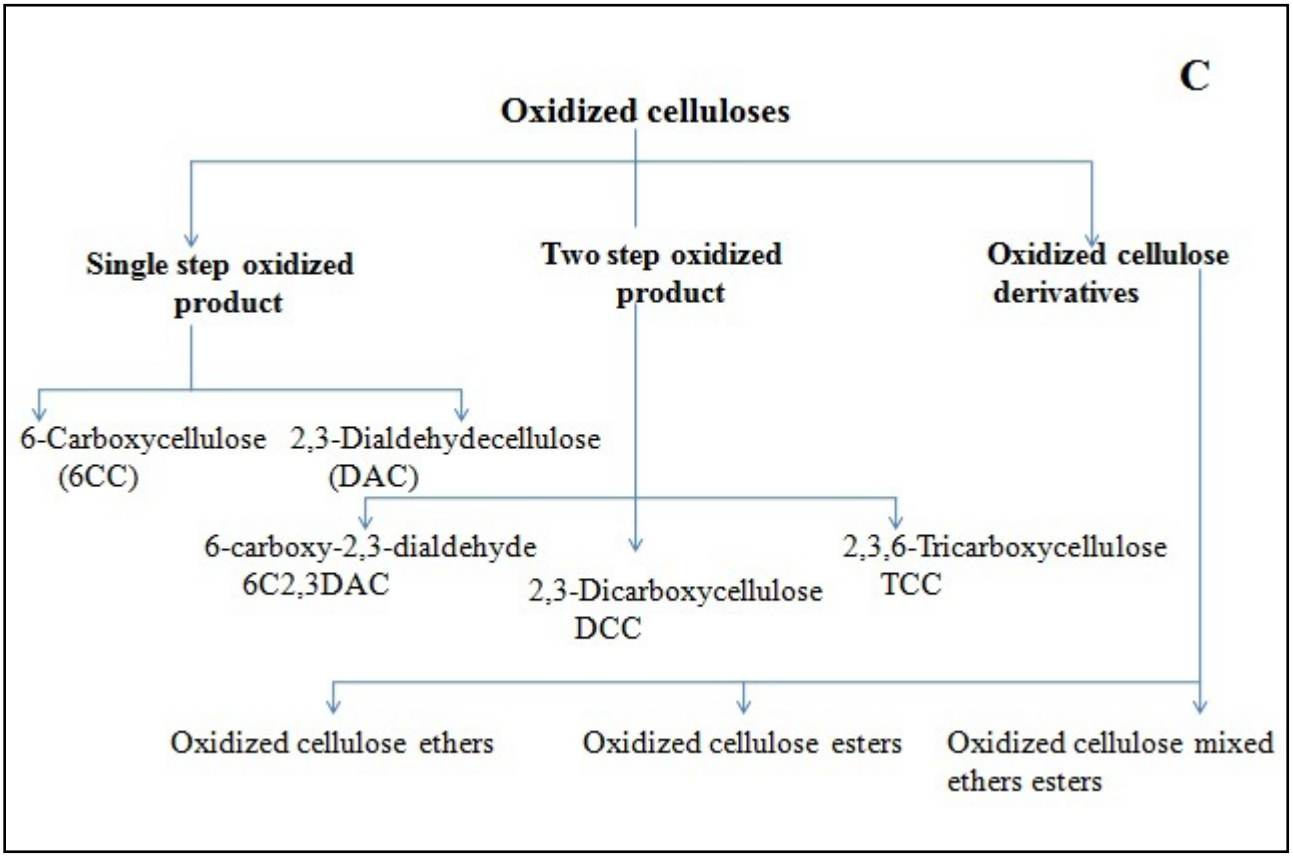


Figure1.5 (C): Types of oxidized cellulose.

1.3.2. Non-Selective Oxidation

Non-selective oxidation of cellulose refers to the random oxidation of primary and secondary hydroxyl groups of cellulose. Non-selective oxidation of cellulose, using reagents such as molecular oxygen, ozone, sodium chlorite, chlorine, and hydrogen peroxide, had been used for bleaching purposes rather than for synthesizing functionalized cellulose derivatives. The common mechanism for all these reactions includes the liberation of free radicals (oxygen radical, chlorine radical or peroxide radical species), which can remove the color (mainly lignin) of wood pulp. Other reagents like chromic acid and potassium permanganate are strong oxidizing agents which can oxidize primary as well as secondary hydroxyl groups of cellulose; however,

they have not been explored sufficiently. However, chromic acid with sulphuric acid at high temperatures has been used for complete oxidation of organic debris like hospital wastes into carbon dioxide.

1.3.2.1. Oxygen

The oldest known oxidizing agent is molecular oxygen, which liberates free oxygen radical in presence of acid; similarly ozonide liberates oxygen radical in the presence of acid. The liberated oxygen radical is highly reactive and readily oxidizes wood pulp by reaction with the functional groups of residual lignin responsible for the color of wood pulp (Bogaty, Campbell and Appel, 1952; Holden, 1954). It has been reported in literature that oxidation with ozone causes drastic decrease in the fibre strength, upto $1/12^{th}$ of the original strength (Charles and Mary, 1913; Charles, 1913). The oxidation of cotton with molecular oxygen in presence of the catalyst N-hydroxy pthalamide had no affect on DP and strength of cellulose fibre (Biliuta et al., 2011).

Similarly peroxide and inorganic forms of peroxide are also good oxidizing agents. In presence of acid, peroxides release peroxide radical (ȮOH), which helps in bleaching process via oxidation. Since 1899 peroxides and other inorganic peroxides have been preferentially used for bleaching of cotton (Bumke and Richard, 1899; Haskins and Hogsed, 1950).

1.3.2.2. Hypochlorite Oxidation

Both chlorites and chlorate oxidants have also been used for bleaching of cellulose fibre (Oranskii, 1930). In the presence of acids, hypochlorite liberates nascent oxygen which causes bleaching. However, the strong oxidizing property of hypochlorite also led to the reduction of fibre strength by 33%, and disrupted uniformity of color (Kramer, 1915; Edmund, 1920).

1.3.2.3. Permanganate oxidation

Oxidation of cellulose with permanganate was first carried out in 1890 by Cross and Bevan (Cross and Bevan, 1890). They observed that the oxidation of flax with permanganate produced carbonyl groups and oxalic and carbonic acid as by-products (Knecht et al., 1920; Mehta and Turner, 1947). The oxidation of cellulose with permanganate occurred in two steps. The first step was fast and involved formation of transient complexes comprising green manganate (VI) / blue hypomanganate(V) intermediates. The second step involved the break down of the above intermediates into keto or keto-acid derivatives. In 1949, Ant-Wuorinen attempted to improve the permanganate oxidation process by first soaking the cellulose in alkali (0.1 NaOH) and then reacted with permanganate solution in acidic medium. The product contained 0.79% carboxy group with a reduced DP of ~35. The main problem associated with permanganate and chromic acid oxidation lies in the removal of the reduced manganese and chromium metal ions embedded in the fibre (Davidson, 1948). The reaction of permanganate in presence of H_2SO_4 is a vigorous reaction leading to formation of Mn_2O_7.

Recently, researchers have shown great interest in studying the kinetics and mechanism of oxidation of methylcellulose (Hassan et al., 2012; Shaker, 2001), alginates (Hassan, 1993), pectates (Khairou, Hassan, 2000), CMC (Shaker, 2001), K- Carragenan (Hassan et al., 2011)**,** and amino acids (Hassan et al., 1988)**.** The mechanism of permanganate reaction is shown in Figure 1.6.

Permanganate oxidation was found more suitable for water soluble products such as alginates, pectates, CMC and amino acids. However, due to toxicity of potassium permanganate, disposal of effluent is an issue.

Mechanism:

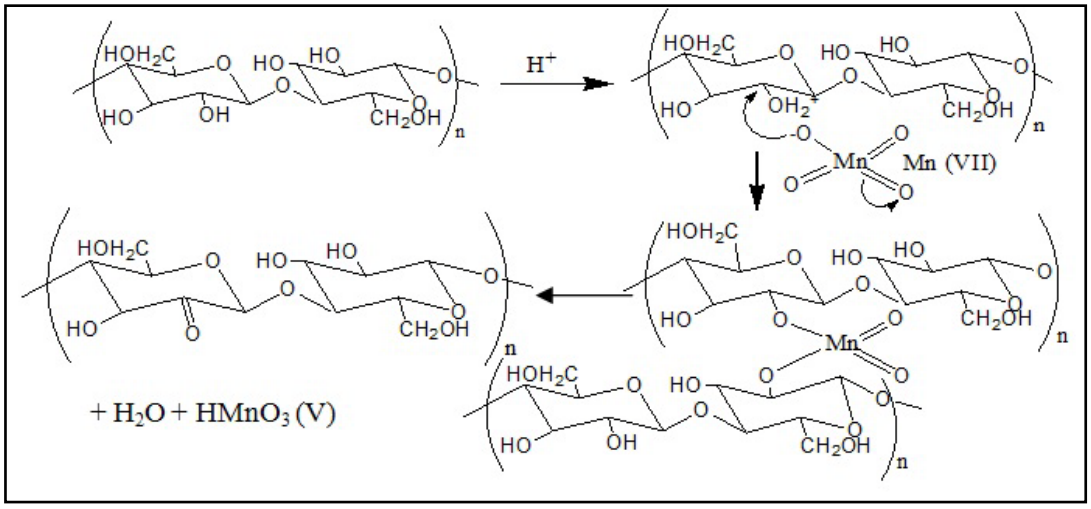


Figure1.6: Mechanism of oxidation of cellulose by permanganate.

1.3.3. Selective oxidation

1.3.3.1. Oxidation at C-2, C-3 Positions: Periodate oxidation

1.3.3.1.1. Introduction

Periodate oxidation of cellulose led to 2,3-dialdehyde cellulose (DAC) by C2-C3 diol cleavage (Jackson and Hudson, 1932; Jackson and Hudson, 1937; Heidt et al., 1945; Rutherford et al., 1942; Davidson, 1940; Yashunskaya, Shorygina and Rogovin, 1949; Navell, 1963). DAC is also known as aldehydocellulose or dialdehydocellulose. The phenylhydrazine derivative was prepared to show the presence of aldehyde groups (Bumke and Richard, 1899). There are some other reagents such as HIO_4, $Na_3H_2IO_6$, $Pb(OA)_4$, and $NaBiO_3$ which were also used for oxidation to produce DAC (Heidt et al., 1945). The reaction with periodate is photo-sensitive (periodate is decomposed to iodine in presence of light); hence high temperatures and light has to

be excluded from its synthesis procedures. In general, synthesis is performed at 50-55°C under acidic pH (~4.4) in a dark environment. Several parameters such as relative concentration of periodate to cellulose, reaction temperature, pH and morphology of cellulose affect the degree of oxidation (Varma and Kulkarni, 2002, Kim et al., 2000). To improve the efficiency of periodate oxidation, metal chlorides such as LiCl have been used as they help to reduce inter and intra H-bonding between the fibrils (Sirvio et al., 2001). Some side products are also formed during periodate oxidation through β-alkoxy fragmentation (Calvini and Gorassini, 2012). DAC is extremely sensitive to alkali and acidic treatment, as being a open ring dialdehyde it rapidly degrades to 2,4-dihydroxybutanoic acid in alkali (Nevell, 1985) and d-erythrose in acidic medium (Guthrie, 1961; Jackson and Hudson, 1937). The quantification of the carbonyl group was been done by standard iodometric titrations (Maekawa and Kohijima, 1984). It is difficult to analyze the functionality of DAC through spectroscopic techniques. This is because DAC molecule easily undergoes acetal and aldal formation in presence of moisture. Nevell et al. suggested that DAC exists in hydrated forms [-CH $(OH)_2$], the 2,3-hemialdal form [-CH(OH)-O-CH(OH)-], the 2,6 or 3,6 hemiacetal forms [-C(OH)- O-CH_2-], as well as free aldehyde form (Nevell, 1985; Spedding,1960). It was noted that free aldehyde or hydrated aldehyde groups react three hundred times faster than hemialdal and hemiacetals group. Similarly, the reactivity observed for the hemialdal group was five time faster than the reducing end groups in hydrocellulose (Nevell, 1985). The dialdehyde group can easily undergo Schiffs' base reactions at room temperature. Recently, many derivatives such as dioximes, hydrazones, and hydrazides were reported and have been found to posses antimicrobial properties (Maekawa and Koshijima, 1991; Verma et al., 2008; Shaikh et al., 2011). The schematic mechanism is shown in Figure 1.7.

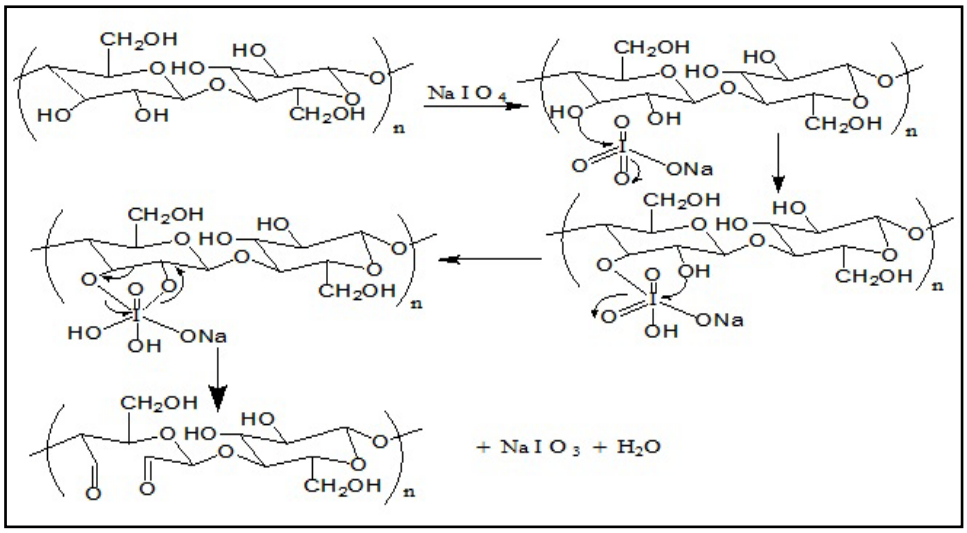


Figure1.7: Mechanism of oxidation of cellulose by periodates.

Applications of 2, 3-dialdehyde celluloses (DAC)

DAC can be applied for ion exchange after converting aldehyde to carboxylic acid (Casu et al., 1984). Dialdehyde groups substituted with aromatic amines are an important class of biologically active substances (Schmidt et al., 1998), for immobilization of β-galactosidase (Qingqi et al., 2011) and proteolytic enzymes during radiaton treatment (Svetla et al., 2010), for flourescent and photometric analysis (Ye, Xiong and Sun, 2012), tissue scaffolding material (Verma et al., 2008), chronic wound care dressing material (Ignatjuk et al., 1999), treatment of gastro-intestinal functional disorder (Betty and Wen-Yang, 2005), hemostatic textile material (Filatov et al., 2008), biosoluble carrier (Singh, Ray, Vasudevan, 1979), insulin delivery system (Singh, Vasudevan and Sinha, 1981), as fillers in epoxy composites (Varma and Chavan, 1994), as a builder in detergents (Varma and Chavan, 1995), sewage waste-water treatment (Magda et al., 2005), and a host of other applications. This shows the wide range of applications of DAC.

1.3.3.2. Oxidation at C-6 Position: N_2O_4 Oxidation

1.3.3.2.1. Gaseous N_2O_4

Literature reports both NO_2 and N_2O_4 as oxidizing agents for cellulose; however, they are both essentially the same and exist in equilibrium form. They can be used either in gaseous or solution form after dissolving in suitable solvents (Ashton, 1968; Bertocchi et al., 1995; Nevell. 1985). The oxidation of cellulose in presence of nitrogen (IV) oxide requires high pressure (around 70 atmospheres). The process is slow and produces many side products with incorporation of N (Zimnitski, Yurkshtovich and Bychkovsky, 2004). Both the adsorption of N_2O_4 gas on to cellulose fiber as well as oxidation are exothermic processes, therefore it is important to control the temperature during processing.

1.3.3.2.2. N_2O_4 in inert organic solvent

The oxidation of cellulose using dissolved N_2O_4 was developed in order to overcome the problems with gaseous N_2O_4 under high pressure. In solution phase energy dissipation is more facile and homogenous reaction occurs. Therefore, inert solvents like CFC were explored where the products were found to contain carboxyl groups ranging from 1-25%. However, due to the environmental hazardous effects of CFC, the process was not implemented further (Kenyon and Yackel, 1948; Mcgee, Fowler, Unruh and Kenyon, 1947; Hirota et al., 2009; Dong et al., 2012).

1.3.3.2.3. N_2O_4 in polar acidic solvent

CFC solvents were replaced with several acids for dissolving N_2O_4. However, these too gave some undesirable results. For example in the chromic acid/N_2O_4 system: removal of chromium metal ion from fiber was difficult. The HNO_3/N_2O_4 system required heating at 50-100°C; this

cause drastic hydrolysis and degradation of cellulose. The H_2SO_4/N_2O_4 system caused extensive hydrolysis, even at low temperature and the H_3PO_4/ N_2O_4 system caused phosphorylation at high temperature. Therefore acid//N_2O_4 systems seems to have in-built fault-lines. However, as later research has shown, the reactions can be controlled for specific products. This is discussed in the section on functionalized nanocelluloses (see section 1.3.4).

1.3.3.2.4. N_2O_4 in supercritical CO_2

The oxidation of cellulose by NO_2 dissolved in pressurized CO_2 (supercritical CO_2) has also been studied. However, it was observed that degree of oxidation is too low in supercritical CO_2 (Camy et al., 2009).

1.3.3.2.5. $NaNO_2$ in acid or acid mixture

HNO_3/ $NaNO_2$ oxidation: Strong acids cause hydrolysis and degradation of cellulose. To avoid this, several metal ion catalysts such as potassium, calcium, barium, aluminium, chromium etc. were used with the $HNO_3/NaNO_2$ oxidation system (Gert et al., 1995; Oskar, 1949). The drawback was the presence of trace metal ion in the fibre and low degrees of oxidation (Yackel and Kenyon, 1942; Pigman et al., 1949; Nabar and Padmanabhan, 1950). Changing the system to $HNO_3/H_3PO_4/NaNO_2$ had several advantages. For example, 22% oxidation of cellulose with $NaNO_2/HNO_3$ system required 64 hours while with $HNO_3/H_3PO_4/NaNO_2$ system it required 48 hours (Yackel and Kenyon, 1942; Kumar and Yang, 2002a).

$H_3PO_4/NaNO_2$: The oxidation of amylase at C_6 using $NaNO_2/H_3PO_4$ produced a series of glucuronoglucans (Painter et al., 1985; Painter, 1977). The $HNO_3/H_3PO_4/NaNO_2$ oxidation system was found more convenient as compared to $NaNO_2/HNO_3$ system as it caused less

hydrolysis. Efficient swelling of cellulose by H_3PO_4 plays a role in uniform substitution of carboxyl groups along the cellulose chain. When the cellulose was oxidized using simple $H_3PO_4/NaNO_2$ system, the cellulose obtained had similar carboxyl content (16-25%); detection of hydrolysis products like glucuronic acid, erythronic acid, glyoxalic acid, and glucaric acid were also obtained (Arendt et al., 1973). Thus it appears that H_3PO_4 is a useful solvent for cellulose oxidation. The mechanism of acid/ $NaNO_2$ oxidation of cellulose is shown in figure 5 (A).

HNO_3-HNO_2-NO_2 system: In 1949, Pigman observed that of oxidation of cellulose with H_3PO_4/NO_2 produced 26% uronic acid units (Pigman et al., 1949). These results are almost similar to the H_3PO_4-$NaNO_2$ system.

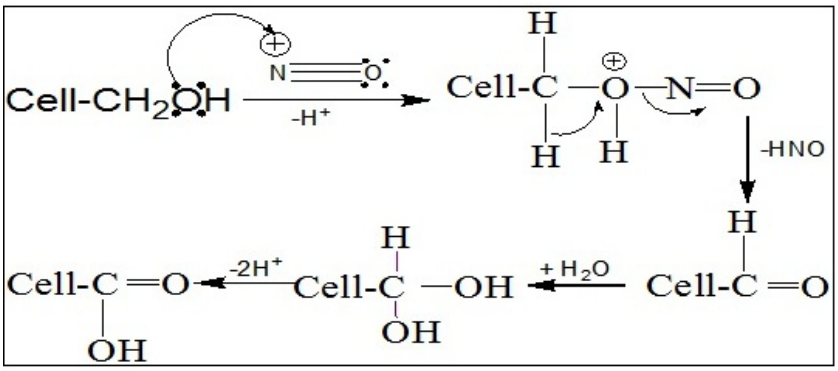


Figure1.8: Mechanism acid-$NaNO_2$ oxidation system

1.3.3.2.6 Ammonium persulphate (APS)

APS is a strong oxidizing agent with high water solubility and low cost. It can be easily broken into ammonia and peroxysulphuric acid in aqueous media, which act as radical initiator. The oxidation of cellulose with APS involved vigorous heating at 60°C for 16 hours. It is found that APS oxidation can also be applicble for oxidation of raw wood material or cellulose containing lignin. Here, free radical anion $2SO_4^{\cdot -}$ formed on heating of persulphate $S_2O_8^{-2}$ removes the lignin as well as the amorphous region in cellulose fibre, leading to the formation of nanocrystals. In

acidic solution persulphate liberates peroxide radical, which helps in bleaching of wood pulps. The oxidized cellulose obtained using APS contain carboxyl percent in the range of 14 to 19 % **(**Leung et al., 2011).

1.3.3.2.7. TEMPO

TEMPO is well known stable radical oxidizing agent used for selective oxidation of primary hydroxyl groups. The reagent used commonly for the oxidation of cellulose is 4-acetamide-TEMPO, which is a derivative form of TEMPO. TEMPO oxidation is comparatively mild and loss of DP is lesser than the other systems described.

The following are some of the features of TEMPO oxidation:

(1) TEMPO can work with and without use of NaBr/ NaBrO co-catalyst (Bragd, Besemer and Bekkum, 2000); other primary oxidants like ($NaClO_2$, ClO_2, / H_2O_2) can also be used with TEMPO (Weerawarna, Komen and Jewell, 2003). Also reported are enzyme based systems such as O_2/laccase/TEMPO (Vilkari, Kruus and Buchert, 1999). In fact, to avoid the use of hazardous chemicals like NaClO/NaBrO, a new enzyme based O_2/laccase/TEMPO system had been proposed (Vilkari, Kruus and Buchert, 1999). In this system, laccase act as co-catalyst and molecular oxygen is the primary oxidant. Even though the O_2/laccase/TEMPO is an environment friendly system, but due to limitations such as long reaction time, high consumption of TEMPO and deactivation of enzyme after reaction, this system has been found unsuitable for practical purposes (Lejeune and Deprez, 2010). The hydrogenated derivative TEMPO-H, has low dissociation energy of 70kcal for O-H bond which is 30% lower than the typical O-H bond, hence the TEMPO formed acts as relatively stable radical. Additionally, hyper-conjugation

between nitrogen atom and two carbon atoms through two-carbon atom three-electron systems ($2C3e^-$) contributes significantly to the stability of TEMPO radical. TEMPO is expensive and highly toxic (radical form is hazardous for environment), and has to be stored at low temperature (2-8ºC). It is not re-cyclable and preferable reaction temperature is around 10ºC. For these reasons TEMPO systems are not amenable for large scale industrial production.

Mechanism: In TEMPO oxidation system, NaClO acts as a primary oxidant (used in excess), NaBr as a secondary oxidant and NaBrO as a stoichiometric oxidant. The mechanism proceeds in two steps as shown in Figure 1.9. In the first step TEMPO selectively attacks the primary hydroxyl group and converts it into a aldehyde group. In the second step, aldehyde group converts to carboxylic acid group by NaClO or NaBrO. The reaction is performed in alkaline medium. The TEMPO reaction is performed in alkaline since TEMPO decomposes in acidic media to hydrogenated derivative TEMPO-H, rather than its nitronium ion form. Alkaline degradation is avoided by carrying out the reaction at low temperature (~10ºC) Figure 1.10.

At higher pH>10.5, the secondary oxidant used in the reaction (NaOCl) starts consumption of OH^- ion to generate NaOH and $\dot{O}Cl$ radical, which retards the reaction as shown in Figure 1.11. In some cases the use of buffer solutions were reported to maintain the pH of reaction media (Isabel et al., 2006)

TEMPO/O_2/laccase system

Laccase is an oxidoreductase enzyme having multiple copper containing oxidases. It contains the catalytic reductive site (cluster of four copper atoms), responsible for the monoelectronic oxidation of suitable substrate on the expense of molecular oxygen (Burton, 2003; Mayer and

Staples, 2002).The overall catalytic cycle includes the consumption of one oxygen molecule to two water molecules and simultaneously oxidizes four substrates molecule to generate four radicals (Solomon, Sundaram and Machonkin, 1996). The mechanism of oxidation of cellulose by TEMPO/laccase/O_2 is explained in scheme Figure 1.11.

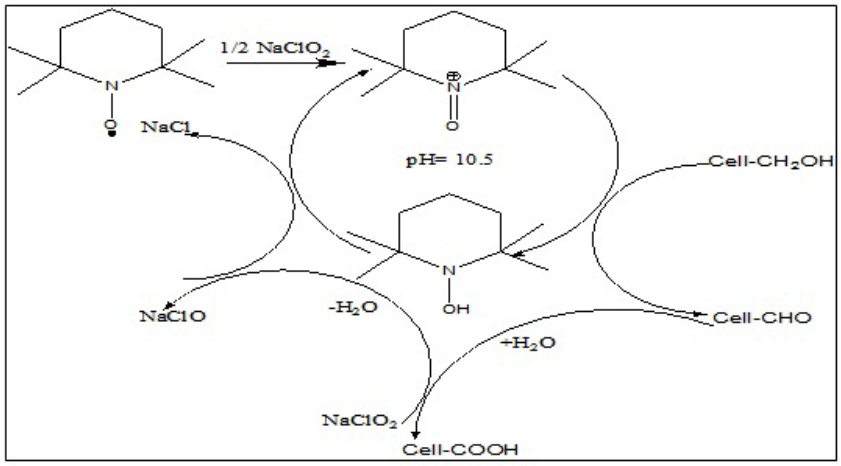


Figure1.9: Mechanism of TEMPO/NaBr/NaClO oxidation system.

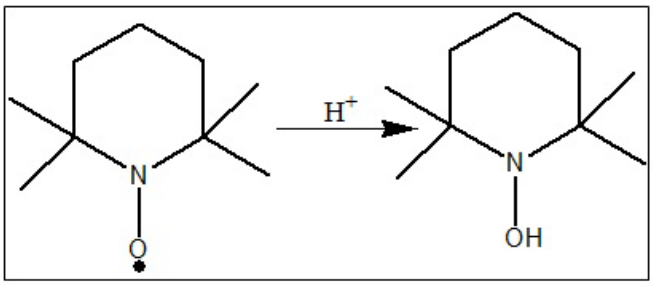


Figure1.10: TEMPO in acidic media.

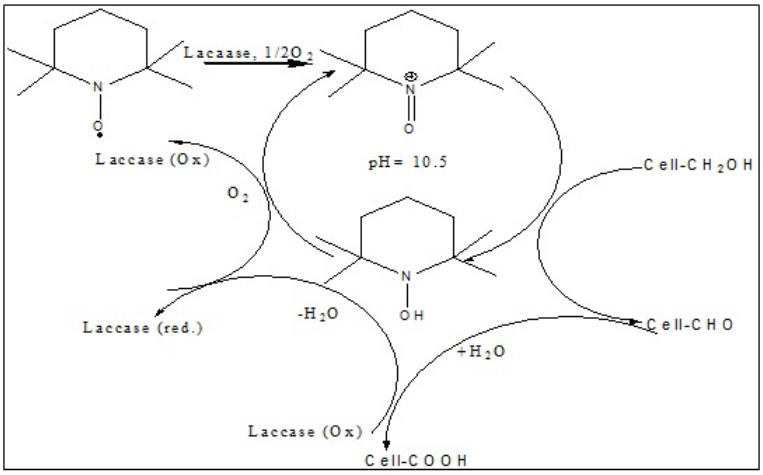


Figure1.11: Mechanism of TEMPO/O_2/ Laccase oxidation system.

1.3.3.2.8. Methods used for analysis of oxidized functional group

In 1917, the copper number method was applied for the estimation of oxycellulose (Kita, 1917). This method was based on the calculation of weight of precipitated red cupric oxide formed in the reaction of cupric tartarate with aldehyde, which represents the percent oxidation of cellulose. It was observed that with increase in the amount of oxidant, Copper number also increases (Edmund and Thompson, 1922). On the basis of solubility, oxidized celluloses were differentiated into 3 types: α-oxycellulose (insoluble in 18% aqueous alkali), β-oxycellulose (soluble in <18% alkali) and ϒ-cellulose soluble in H_2O (Bancroft and Wilder, 1915).

Several new methods have been developed for determining more accurate oxidation percentages, such as: esterification, amine binding (Matiya et al., 1946), CO_2 evolution (Nevell, 1947), Ag adsorption (Davidson and Nevell, 1947), Benizidine number (Krajcinovic and Arhiv za Kemiju, 1947), iodometric titration (Nabar and Padmanabhan, 1950; Hall, 1930), and calcium acetate titration method (Heymann et al., 1942). The detailed description on these methods is given in Table 1.2.

Table1.2: Methods used for analysis of oxidized functional group

Sr. No.	Method	Determination of percent oxidation in oxidized cellulose	Remarks	References
1.	Copper number method	Cellulose is treated with the cupric tartarate (equivalent mixture of $CuSO.5H_2O$ and tartaric acid in alkaline solution) at 100°C. Cupric tartarate reacts with aldehyde groups to generate cuprous oxide (red colour ppt.). The weight of red ppt., indicates the copper number of cellulose material.	This method is generally used to determine the carbonyl content in cellulose.	Staud and Gray, 1925
2.	NaOH consumption method	0.5 gm oxidized cellulose is treated with 75% alcohol (20 ml) and 20 ml of 0.5N sodium hydroxide and allowed to stand for 5 minutes. The solution is titrated with acid to determine the consumed alkali.	The methods gives variable oxidation percent depends on the time period of alkali treatment to oxidized cellulose and on the temperature	Yackel and Kenyon, 1942.
3.	Ca-acetate method	The carboxyl group of fixed amount (0.5 gm) oxidized cellulose reacts with the weaker base 2% calcium acetate (50ml) for 30minutes. The released weak acid is titrated with 0.1 NaOH solution using phenopthalin indicator.	This investigated method several times modified carefully to obtained accurate carboxyl content. The equation used here is N x 45 x V of NaOH consume/ wt. of sample (mg). This method is found more reliable as compared to other methods.	Ludtke, 1935; USP, 1995.
4.	Schiff's base	Accurately weighed oxidized cellulose (0.5 gm) is treated with 50 ml hydroxylamine hydrochloride in an inert atmosphere. The reaction mixture is heated at 50°C for 2h. After cooling at room temperature, a 25ml aliquot is titrated with 0.1N HCl solution to pH 3.2	The method is used to calculate the carbonyl content of the oxidized cellulose. The precaution used during the reaction is maintaining the inert condition. The equation used to measure carbonyl content is: MWCo(B-S)/10 x Wt of sample x 100 B and S –Volume of 0.1 N HCl consumed during blank and sample solutions.	Green, 1963
5.	Dische carbazole method	Hydrolysis of oxidized cellulose is performed with 1.2 M HCl in inert condition at 99°C for 1hr. The TLC should show complete hydrolysis of the sample. After cooling, the uronic acid is determined by Dische carbazole method. This method is based on colorimetric determinations of the constituent sugars liberated by sulphuric acid.	The method use to determine the uronic acid concentration in the oxidized cellulose. This includes the assay containing phenol-, the orcinol-, the anthrone-, the naphto- the resorcinol-, and the L-cysteine-sulphuric acid.	Dische, 1962.
6.	Conductimetry titration	The oxidized cellulose sample (30-40mg) is suspended into 15 ml of 0.01 M hydrochloric acid solution and allowed for 10 minutes stirring. The suspension is titrated with 0.01 M NaOH. The titration curves shows the presence of strong acid corresponding to the excess of HCl and weak acid corresponding to carboxyl content.	The method includes the calculation: % carboxyl=$162(V2-V1)c[w-36(V2-V1)c]^{-1}$ V2 and V1 are amount of NaOH consumed where conductivity of solution remains constant, w= weight of sample; C=concentration of NaOH used (mol/l). Before, titration sample should be properly dispersed in HCl, otherwise the reading observed will be inaccurate.	Saito and Isogai, 2004.
7.	Methylene Blue Adsorption	A fixed amount of oxidized cellulose (10-15mg) is dispersed in 25ml of a borate buffer solution (pH=8.5). A 25ml of a 300mg/L solution of methylene blue is added to above suspension and allowed stirring for 1h. The solution is centrifuged and 1ml of decanted portion containing the unreacted methylene blue is added to 1ml of 0.1M HCl and water is added to make it 10ml. After this, methylene blue concentration is measured by photometry apparatus.	The carboxyl content measured by the equation: COOH (mmol/g)= $(7.5-X)0.00313w^{-1}$ X is nonadsorbed methylene blue (mg); W= weight of sample.	Neale and Stringfellow, 1940.

1.3.3.2.9. Applications of carboxycelluloses

Oxidized cellulose with carboxyl functional group has been a special focus of interest for the last 70 years due to its several desirable properties: the products are bioresorbable, biocompatible and easily degradable under normal physiological conditions. Therefore, carboxyl functionalized celluloses have wide range of applications in various fields, especially biomedical and pharmaceutical fields. Frantz was the first to test the effect of oxidized cellulose on tissues (Frantz, 1943), and observed the haemostatic properties of oxidized cellulose (Frantz, Clarke and Lattes, 1944). Putman had reported the application of oxidized cellulose as a carrier of thrombin in neurosurgery (Putman, 1943). It was found that the acidic nature of oxidized cellulose facilitated hemostasis by decreasing the pH and thus generating artificial clot (Correll and Wise, 1947). Oxidized regenerated cellulose (ORC) has been reported to be an exceedingly good material for controlling the oozing from injuries. It can be directly applied in brain surgery for controlling bleeding from small vessels (Voormolen, Ringers Bots et al., 1987; Schonauer et al., 2004). Commercially available 6-carboxycelluloses (6CC) are Oxycel® produced by Eastman Kodak and sold by Becton, Dickinson & Co., Surgicel® produced by Ethicon; new advanced products are Surgicel® Fibrilar (wool like form), Gelitacel® (Gelita medical), Curacel® (Curamedical), calcium salt of 6CC (Traumacel®P) calcium-sodium salt of 6CC (m.doc®), biodegradable, haemostatic and wound healing formulation (HemostasenT®), antimicrobial bandages Linkomicin® and Oxycelanim® produced by Borimed, and tissue reinforcement material (Proceed®), consisting of monofilament polypropylene mesh containing 6CC fibre.

Several other applications have been investigated, such as:

(i) Oxycellulose filters for tobacco filters (Harry, 1953).

(ii) Cleansing creams for removing dirt from the hands without the use of water (formulation

consists of casein, limewater, NH_3, soda, oxycellulose or hydrocellulose, perfume, water) (Georges and Rodolphe, 1930).

(iii)Treatment of mineral oil to improve the dielectric constant (Sylvester, 1930).

(iv) Ion exchange with dye (Davidson, 1950).

(v) Absorbable laproscopic cigarette sponge, used in open surgery in retracting, dissecting and controlling small area of hemorrhage.

(vi) Dental surgery as dental sponge (Wall, 2004).

(vii) Carrier of pharmacological substance (Dolberg et al., 1973; Kumar et al., 2001).

(viii) Component of insecticide (Tomonori et.al, 2012).

(ix) Haemostatic material to stop bleeding during surgery (Frantz, 1948) and to prevent post-surgical adhesion layer (Marta et al., 2012; Johnson & Johnson, 1989; Wiseman, Saferstein and Wolf, 2002).

(x) Excellent scaffolding material (Dias, Peplow and Teieira, 2003; Galgut, 1990).

(xi) Bilrubin adsorption (Du Xnyao et al., 2011).

(xii) Drug delivery by oxycellulose beads (Martina et al., 2011).

(xiii) Carrier for macromolecular prodrug delivery system (Zhu, Kumar and Banker, 2001).

(xiv) Diethylene triamine oxycellulose as urea, uric acid, arsenic (III) adsorbent (Po et al., 2009).

(xv) Synthesis of diethylene triamine oxycellulose and its adsorbility of uric acid and As (III)); Pharmaceutical and medical application (Martina et al., 2009).

(xvi) Hydrophilic gel system (Martina et al., 2008).

(xvii) Antibacterial agent (Kaputskii et al. 1986; http://www.okcel.eu/design/okcel/images/Okcel_antimicrobial%20effect.pdf),

(xviii) Antitumor agent (Sano, Kojima and Naruse, 2000; Tokunga and Naruse, 1998).

(xix) Wound healing properties (Finn, Schow and Schneiderman, 1992).

(xx) Immumostimulant (Otterlei et al., 1992).

Table1.3: Oxidizing reagents used for oxidation of cellulose.

Oxidation System	**Reactant species**	**Products Obtained**	**Remarks**	**Reference**
Mol. O_2	Oxygen radical	Non specific	Used for bleaching of cotton fiber	Smith, 1957.
O_3	Oxygen radical	Non specific	Used for bleaching of cotton fiber	Charles and Mary, 1913; Charles, 1913.
H_2O_2	Oxygen radical, Peroxide radical	Non-specific	Used for bleaching of cotton fiber	Bumke and Richard, 1899.
$NaClO_2$	Cl radical, nascent oxygen	Non-specific	Used for bleaching of cotton fiber	Oranskii, 1930.
CrO_3^- in acid	Chromic acid	Primary alcohol to acid Primary alcohol to ketone	Toxic, non specific, strong oxidizing agent, reduce fiber strength, cause non-uniform bleaching due to deposition of Cr ion.	Davidson, 1941.
$KMnO_4$	Permanganate ion	Primary alcohol to acid Primary alcohol to ketone	Toxic, non specific, strong oxidizing agent, reduce fiber strength, cause non-uniform bleaching due to deposition of Mn ion.	Hassan et al., 2011.
Gaseous N_2O_4	NO_2	Primary alcohol to acid	Reaction temperature 343 K and pressure 70atm. Slow process, and produced many N containing by-product. At large scale, controlling of temperature is major issue due to exothermic process.	Yackel and Kenyon, 1942; Zimnitski, Yurkshtovich and Bychkovsky, 2004.
N_2O_4 in inert Organic solvent	NO_2	Primary alcohol to acid	Inert solvent used CFC or fluorinated solvents but were banned due to environment hazard.	Kenyon and Yackel, 1948.
N_2O_4 in supercritical CO_2	NO_2	Primary alcohol to acid	It has been noted that CO_2 inhibits the process of oxidation by interacting with NO_2	Camy et al., 2009.
$NaNO_2$ in acid or acid mixture	NO^+	Primary alcohol to acid	Industrially viable process if proper neutralization of effluent done. Affects the DP of fibre.	Yackel and Kenyon, 1941; Painter et al., 1985.
Nitroxyl Radical (NO) (TEMPO)	NO+	Primary alcohol to acid	Expensive, required low temperature for storage 2-8 ºC, toxic (radical form is hazardous for environment), preferable reaction at low temperature ~10 ºC	Chang and Robyt, 1996.
APS (Ammonium persulphate	peroxysulphuric acid	Primary alcohol to acid	Low long term toxicity, high water solubility, and low cost. Formed sulphate radical removes off the amorphous as well as lignin part of wood pulp.	Leung et al., 2011.

1.3.3.3. Oxidation of cellulose to multi-oxidized cellulose (Two step oxidized products)

When cellulose undergoes sequential oxidation processes with different types of oxidants, then oxidized celluloses with multiple functional groups are produced. These highly oxidized celluloses contain carboxyl and aldehyde functional groups in place of free hydroxyl group of anhydroglucose units. The commonly derived highly oxidized cellulose are 2,3-dicarboxycellulose (DCC), 2,3,6- tricarboxycellulose (TCC) , 6-carboxy-2,3-dialdehyde cellulose (6C2,3DAC) etc.

1.3.3.3.1. DCC: 2, 3-dicarboxycellulose

DCC is derived from DAC. The DAC can undergo further oxidation with $NaClO_2$ at room temperature and pH~5. This appeared to be most facile method for preparing DCC's with varying degree of oxidation (Varma and Chavan, 1995).

1.3.3.3.2. TCC: 2,3,6-Tricarboxy cellulose (based on cellulose I) or 2,3,6-Tricarboxy cellulose (Supercel, based on cellulose II)

In 1948, Head and Frank prepared TCC for the first time. The NO_2 oxidized cellulose (i.e., 6CC) was reated with NaIO4. The product formed was used to produce the meso-tartaric acid on hydrolysis (Head and Frank, 1948). This established the structure of 6C2,3DAC. The latter was further oxidized with $NaClO_2$ to produce TCC. In another variant of the synthesis, first DAC is produced, which is then further oxidized by gaseous NO_2 to produce TCC. It was found that in presence of dehydrating agent P_2O_5, nitration of cellulose occurred while in absence of P_2O_5 oxidation of cellulose occurred to yield TCC (Kuznetsova, Ivanova and Shorygina, 1966).

Applications of 2,3,6-Tricarboxycellulose (TCC)

TCC is commercially available as Surgicel® (oxidized regenerated cellulose), Supercel®, INTERCEED®, TC7. The biomedical studies showed that TCC interacts with blood for facile blood clotting through release of platelets and acts as a promising haemostatic material (Sinha et al., 1984). Tartaric acid having two carboxy groups has a plethora of applications in pharmaceutics and food additives. Therefore, scientists were targeting the hydrolysis of the carboxy rich TCC for the production of tartaric acid. Nevertheless, being a complex cellulose molecule it was difficult to selectively produce a single desired product and many more products such as erythronic acid, glyoxylic acid and oxalic acid were also produced on hydrolysis of TCC (William et al., 1975; William et al., 1976). Of the many variants of oxidized celluloses, TCC was found to be the most important biomedical polymer. As a result, most of the studies are found only in patent literature (Kiyohiko and Yoshimi, 1979). Applications reported so far include:

(i) Selective ion exchange properties (Ivanov et al., 1960)

(ii) Haemostatic material (Sinha et al., 1984)

(iii) TCC metal crystal formation with transition metal complex $YBa_2Cu_3O_7$ used as superconductor (Novikov, 1991; Kaputskii, 1994)

(iv) TCC as intraoperative hemostatic material (Young and Paulson, 1993)

(vi) Enterosobent (Alam et al., 2012)

1.3.3.3.3. 6-Carboxy 2,3-dialdehyde cellulose (6C2,3DAC)

The 6-carboxy-2, 3-dialdehyde cellulose (6C23DAC) was synthesized from 6-carboxy cellulose. The 6-carboxy cellulose was further oxidized at C-2 and C-3, by using the well established procedure using sodium metaperiodate, described in the above section (Head, and Frank, 1948).

1.3.3.3.4. Oxidized cellulose ethers

The introduction of hydrophobic group in hydrophilic polymers opens up the application of these polymers as emulsifier, liposomes, drug delivery agent, stabilizing agent and nanoparticles through the formation of supramolecular structures. Carboxycellulose ethers behave like a polyelectrolyte and are found to form gels with chitosan and copper sulphate solution (Yin, Koschella and Heinze, 2009).

1.3.3.3.5. Oxidized cellulose esters

The 6CC has wide applications in the biomedical field but other applications are limited due to its insolubility in organic solvents. The oxidized cellulose esters with 20% carboxyl groups and esterified to a DS 1.1-2.3 showed solubility in a wide range of organic solvents such as ethyl acetate, methylenechloride, acetone, dimethylsulphoxide and dimethylformaamide. Therefore, oxidized cellulose esters can overcome the problem of insolubility. These are prepared by reaction of 6CC with acetic anhydride and glacial acetic acid in presence of catalytic amount of concentrated sulphuric acid. The DS of oxidised cellulose esters depends on concentration of anhydride, reaction time and reaction temperature (Kumar and Yang, 2002b). It is reported in literature that partially acetylated oxidized cellulose can also be obtained during fermentation of *Rhizobium meliloti* (M5N1 mutant strain) but had low carboxylic content <1.7% (w/v) and hence

not biodegradable. Walker had proposed the application of partially carboxylated cellulose esters in coatings, printing inks, castings etc.Gelation of oxidized cellulose occurs on esterification, and this gel could be an excellent material as reinforcement for nonpolar polymer matrices (Benkaddour et al., 2014).

1.3.3.3.6. Oxidized cellulose mixed ethers esters

The functionality and applicability of oxidized cellulose has been further increased by insertion carboxy group in already functionalized (ethers and esters) cellulose derivatives. The haemostatic property of 6CC had improved on increase in solubility by introduction of esters and ethers. Except solubility, other properties are the same as 6CC. The oxidized cellulose mixed esters and ethers also showed good gelation (Looney et al., 2004; Buchanan et al., 2005; Kumar and Yang, 2002b).

1.3.4. Carboxy functionalized nanocelluloses

1.3.4.1. Nanofibrils oxidized celluloses

Nanofibrils of carboxycelluloses with nanometer size diameters and micrometer size lengths were prepared by TEMPO oxidation followed by sonication (Saito et al., 2006; Saito et al., 2007; Fukuzumi et al., 2009; Saito et al., 2009; Isogai, Saito and Fukuzumi, 2011; Nachtkamp et al., 2012). Recently, nanocrystals of carboxycellulose (0.11 to 0.18 mmol/gm) were prepared from different sources such as flax, flax shives, hemp, triticale, MCC, wood pulp, bacterial cellulose by using APS oxidant (Leung, 2013). Nanofibrils of TCC have also been recently reported in literature (Van de Ven et al., 2012). These fibres have high carboxyl content and have been used

as superabsorbent material (Van de Ven et al., 2012). The nanofibres with high aspect ratio were prepared by TEMPO-mediated oxidation of DAC molecule.

1.3.4.2. Spherical nanoparticles of carboxy celluloses

Recently, spherical shaped 6CC with narrow polydispercity (PDI=0.045) having uniform size (25-35 nm) was prepared from sugarcane bagasse cellulose. The process included treatment of cellulose by using HNO_3 and H_3PO_4 followed by $NaNO_2$ oxidation (Sharma and Varma, 2013). It was concluded that the uniform size of (25-35 nm) of these nanoparticles could have potential applications as drug delivery material. Also, due to spherical surface it possess high surface area as compared to carboxycellulose nanofibrils, which might be more useful in nanocomposites whereby the increased surface area increases the interaction between the two polymer surfaces. We have recently prepared quasi-spherical shaped TCC nanoparticles of 20-50 nm in diameter with 0.0169 PDI ratio. These particles were prepared by using HNO_3-H_3PO_4-$NaNO_2$ system at room temperature. Quasi spherical shaped 6CC-NP, TCC-NP and their microfibers were tested against a wide range of bacteria *E. coli*, *B. Subtilis*, *S. aureus* including *Mycobacterium tuberculosis* and found that these are excellentent anti-microbial and anti TB material (Varma, Sharma and Dhiman, 2013).

1.3.4.3. Applications

Functionalized cellulose nanoparticles with charged groups have been investigated as delivery system for drugs and bioactive molecules (Panyam and Labhasetwar, 2003; Hamidi, Azadi and Rafiei, 2008; Sonaje et al., 2009; Soppimath et al., 2001; Hans and Lowman, 2002). Published studies show that small and uniform size diameter of the nanoparticles affects the properties of

polymer (Harton et al., 2010). It has been shown that nanoparticles of polyelectrolyte complex polysaccharides (PEC) play a vital role in delivery of drugs, proteins, nucleotides (DNA and RNA's) and other bioactive molecule (Song, Ying Zhou and Chen, 2012), in which the functionalized cellulose nanoparticles such as carboxy, amino, acetate, quaternized ammonium naoparticles prove to be highly proficient for these applications (Sharma and Varma, 2013; Kulterer et al., 2012; Hornig and Heinze, 2008; Luong, Lee and Nam, 2008; Nikolajaski et al., 2012; Song, Zhou and Chen, 2012). The functionalized cellulose nanoparticles in the field of cellular bioimaging by tagging with flourescent molecule have been also reported (Lin, Huang and Dufresne, 2012). In addition, these were also used for several other niche applications such as nanocomposites (Klemm et al., 2005; Klemm et al., 2011), brain tumor tracing (Yoonhwa, 2013), endocytosis (Liebert, 2011), fabrication of recyclable solar cell (Zhou et al., 2013) and in pharmaceutical and food technology industries. The modifications, conversions and decoration of the most abundant natural polymer cellulose into nanoforms have definitely opened up multitude paths for new high tech applications. Still, developments are lacking in industrially viable processes for such nanomaterials.

1.4. Future perspectives

Oxidation of cellulose using various reagents can be used for synthesizing a great variety of well controlled structures having ketonic, aldehydic, and carboxyl groups in various combinations. This will provide building blocks for many products. One possible set of new carboxyl functionalized celluloses is shown in Figure 1.12. New oxidation catalysts (metallic, non-metallic, enzymatic, and reagent based) can be developed which can make the synthesis of various building blocks mentioned above more facile.

We expect that researches into size and shape selective cellulose and functionalized celluloses will intensify in the years to come. New synthetic methods for preparing oxidized celluloses will provide avenues to accomplish such goals. Applications of nanocelluloses and functionalized nanocelluloses of various shapes, sizes and molecular weights in bionanocomposites, electronic applications, tissue engineering scaffolds, drug delivery vehicles, biosensors, and other biomedical applications are likely to be investigated with increasing fervour.

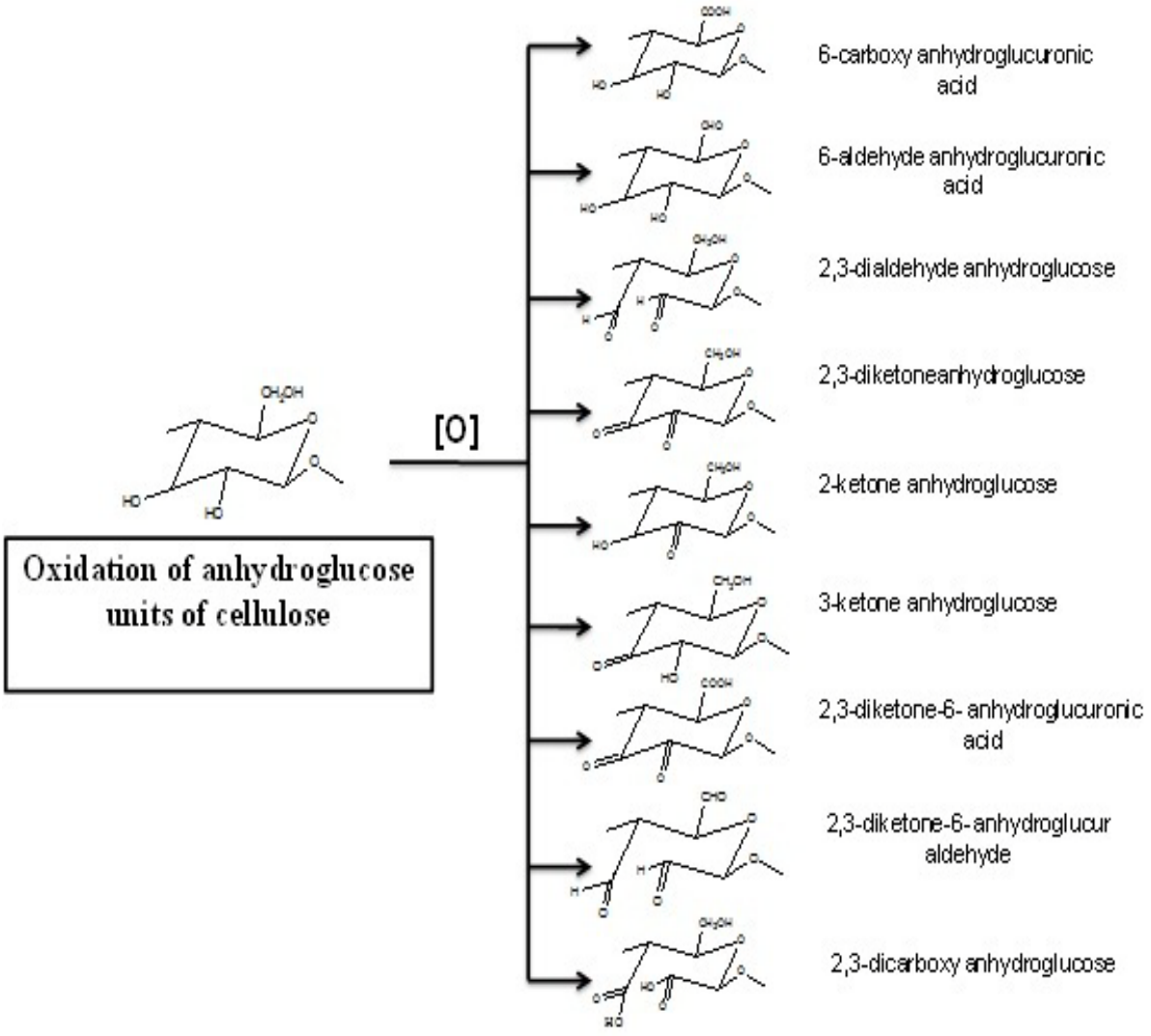


Figure1.12: Possible set of new carboxyl functionalized celluloses.

1.5. References

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1.6. Appendix: Chemical Abstracts Service number for oxidized celluloses.

CAS numbers for oxidized celluloses

Chemical Name	CAS number
6-Carboxycellulose	9032-53-5
2,3,6-Ticarboxycellulose	9032-54-6
2,3-Dialdehyde cellulose	9032-52-4
2,3-Dicarboxycellulose-Na salt	51281-05-1
Oxidized regeneratec cellulose	9032-54-6
6-Carboxy cellulose-Na salt	9069-12-9
6-Carboxy cellulose-Ca salt	52001-91-9
6-Carboxy cellulose-Zn salt	138264-20-7

1.7 Appendix II: Golden periods in cellulose chemistry

Year	Inventor	Invention	References
BC 100-500	Indian Valmiki	Ramayan	-
AD 105	Chineese	Paper making	Edwin, S. (1954). R.R. Bowker Company, New York.
1819	Braconnet	Conversion of wood and straw into sugar by using H_2SO_4.	Labrude, P. (2003). *Rev. Hist. Pharm.* 51, 61.
1833	Braconnet	Nitrocellulose 'xyloidine'	Labrude, P. (2003). *Rev. Hist. Pharm.* 51, 61.
1838	Sir Anselme Payen	Isolated cellulose from plant parts.	Payen, A. (1838). *Compt. Rend. Acad. Sci.* 1052, 7.
1844	J. Mercer	Mercerization of cellulose.	Mercer, J. (1850). *British Patent 13296*.
1865	Wesley Hyatt	First man made plastic celluloid.	Hyatt, J. W. (1865). *US Patent 50359*.
1865	Paul Schuetzenberger	Cellulose Acetate.	Schuetzenberger, P. (1865). *Compt. Rend*., 61, 485.
1883	Witz	First oxidation of cellulose at C-6 position.	Witz, G. (1883). *Bull. Soc. Ind. Mulhouse*. 43, 334.
1884	Chandronnet	Founder of regenerated cellulose fibre industry.	Chandronnet, A. M. (1884). *French Patent 165349*.
1900	Cross and Bevan	First time classification of cellulose as α, β, γ cellulose.	Cross, C. F., Bevan, E. J. (1907). Researches on Cellulose 1895-1900. IInd edition, Longmans, Green And Co. London, NY.

1929	Andress	Unit cell for mercerized cellulose (Cellulose II).	Andress, K. R. (1929). *Z. Phys. Chem.*, 34,190.
1929 1937	Meyer and Mark Meyer and Misch	Unit cell for cellulose I. Unit cell for cellulose I.	Meyer, K. H., Mark, H. F. (1929). *Z. Phys. Chem. B2*, 115. Meyer, K. H., Misch, L. (1937). *Ber. 70B*, 266.
1942	Yackel and Kenyon	First industrial technology developed for the production of carboxy cellulose.	Yackel, E. C., Kenyon, W.O. (1942). *J. Am. Chem. Soc.*, 64, 121.
1949	Ranby	Cellulose nanocrystal reported by acid hydrolysis of cellulose.	Ranby, B. G. (1949). *Acta Chem. Scand*. 3, 649. Ranby, B. G. (1951). *Discuss Faraday Soc*. 11, 158.
1950	Jorgensen & Ribi	X-ray analysis of transformation of cellulose I lattice plane to cellulose II lattice plane on mercerization.	Jorgensen, L., Ribi, E. (1950). *Nature*. 166, 148.
1959	Marchessault et al.	Observed bifringes property in cellulose nanocrystals.	Marchessault, R. H., Morehead, F. F., Walter, N. M. (1959). *Nature*. 184, 632.
1983	Herrick et al.	Isolated microfibrillated cellulose by mechanical homogenisation from hardwood.	Herrick, F.W., Casebier, R.L., Hamilton, J.K., Sandberg, K. R. (1983). *J. Appl. Polym. Sci. Appl. Polym. Symp.* 37, 797.
1984	Atalla & Vanderhart	Iα and Iβ composition of native cellulose through solid state ^{13}C-NMR.	Attala, R. H., Vanderhart, D. L. (1984). *Science*. 223, 283. Vanderhart, D. L., Attala, R. H. (1984). *Macromolecules*. 17, 1465.
1991	Sugiyama	Determine the unit cell parameter of Iα and Iβ phase.	Sugiyama, J., Vuong, R., Chanzy, H. (1991). *Macromolecules*, 24, 4168.
1995	De Nooy et al.	TEMPO oxidation on water soluble polysaccahride such as maltose, starch, pectin.	De Nooy, A. E. J., Besemer, A. C., Van Bekkum, H. (1995). *Carbohydr. Res.* 269, 89.
1996	Chang & Robyt	TEMPO oxidation on cellulose and chitin.	Chang, P.S., Robyt, J.F. (1996). *Carbohydr. Chem.* 15, 819.
2001	Wada	Determine unit cell of cellulose III.	Wada, M., Heux, L., Isogai, A., Nishiyama, Y., Chanzy, H., Suziyama, J. (2001). *Macromolecules*. 34, 1237.
2007	Zhang et al.	First time synthesized the spherical cellulose nanoparticles.	Zhang, J., Elder, T.J., Pu, Y., Ragauskas, A. J. (2007). *Carbohydr. Polym.* 69, 607.
2008	Hornig et al.	First time synthesized spherical nanoparticles of cellulose acetate.	Hornig, S., Heinze, T. (2008). *Biomacromolecules*. 9, 1487.
2013	Sharma & Varma	First time synthesized spherical carboxycellulose nanoparticles.	Sharma, P.R. and Varma, A.J. (2013). *Chem. Commun*. 49, 8818.

Chapter 2

Synthesis of 6-carboxycellulose and their nanoparticles

2.1. Introduction

A very large fraction of published literature on cellulose is devoted to softwood, hardwood, and cotton based cellulose, as these have higher molecular weights and are generally more suited for synthesizing various polymeric derivatives. Most cellulose production industries worldwide are still based on wood. Today, however, sugarcane bagasse and other agricultural residues/non-woods derived celluloses (Ververis et al., 2004; Barba et al. 2002; Varma, 2008) are considered key raw materials for producing cellulose pulp, as they are annually renewable and considered more environment-friendly in comparison to wood. The latter takes much more time to get replenished by nature, and separate land need not be set aside for their cultivation. While sugarcane bagasse and other non-wood biomass are known to possess lower molecular weights than wood celluloses, cellulose derived from these sources can substitute wood cellulose in several applications, particularly as oxidized celluloses in wound dressing gauzes, oxidized nanocelluloses for use in biocomposites, antimicrobial coatings, certain low molecular weight grades of high-volume cellulose ethers like CMC, HMC etc. Our research group has been active for over two decades in conducting researches pertaining to oxidation and oxidation products of cellulose based on hardwood cellulose (Varma and Kulkarni, 2002; Chavan, Sarwade and Varma, 2002; Varma et al., 1997; Varma and Chavan, 1995a; Varma and Chavan, 1995b; Varma and Chavan, 1995c; Varma and Chavan, 1994; Varma and Jamdade, 1985**)**. Recently we took up work pertaining to developing technologies for extracting high α-cellulose from sugarcane bagasse (Varma, 2008**).** This technology has been transferred to an industrial company for production. In this chapter, instead of cotton linters or wood cellulose of relatively high molecular weights, we have used low molecular weight and lowcrystalline cellulose derived from sugarcane bagasse (94% α-cellulose) prepared by our proprietary process (Varma, 2008**).**

Indeed, there exists a plethora of patents and papers published over the past six decades on synthesis, manufacturing processes and applications of oxidized celluloses (Kumar and Dang, 2010; Kumar, 2004; Moser, 1968; Anderson and McIntyre, 1946; Houser, 1946)**.** Nanofibers of oxidized celluloses are a subject of intense recent interest due to their potential for applications in several areas high performance materials, such as gas barrier films, for example coatings for polyesters like poly(lactic acid) with very low oxygen permeability (Isogai et al., 2011)**,** hemostatically efficient materials with no pathological response (Wu et al., 2012)**,** as a material to fractionate and purify proteins, enzymes, hemoglobins, hormones etc. (Coseri et al., 2012)**,** use in shear thinning gels (Crawford et al., 2012), and so on. Functionalized nanocellulose technology appears to be destined to remain a key area of cellulose research due to innumerable possible applications.

Controlling the shape, size and surface functionality of nanoparticles can be a potent tool to manipulate the properties, and thereby the applications of nanoparticles. In the case of metal nanoparticles, intense research efforts have been directed towards controlling these parameters (Watt, Cheong, and Tilley et al., 2013; Wang et al., 2013; Xia et al., 2009; Niu and Xu, 2011; Quan et al., 2013; Sun and Xia, 2002; Burda et al., 2005; Anker et al., 2008; Wang et al., 2008; Liu, Wang and Li, 2012)**.** Metal nanomaterials have dimensions similar to several biomolecules such as proteins, DNA, etc., and the integration of metallic nanoparticles with biopolymers (such as cellulose and it's functionalized derivatives) can lead to hybrid nanomaterials having a combination of properties of the two constituent materials. Cellulose is well recognized as the most abundant, annually renewable, and extremely versatile polymer with a multitude of applications (Katz and Willner, 2004). Conversion of cellulose to nanocellulose, in forms generally referred to as nanofibres, nanofibrils, nanowhiskers, nanocrystals, etc., (Okita et al.,

,2010; Tanpichai et al., 2012) have led to many new high-end applications of cellulose in fields as diverse as nanocomposites, nanobiomaterials, cosmetics and medical devices (Klemm, 2005; Klemm, 2011). Cellulose nanomaterial substrates have also been utilized in the fabrication of recyclable organic solar cells, and further development in this field can lead to their increasing use in energy production technologies (Zhou, 2013). Cellular bioimaging applications by attaching fluorescent molecules onto cellulose nanocrystals have been reported (Lin et al., 2012), in addition to several other niche applications such as drug delivery (Jager et al., 2012). Important reviews have recently appeared on composites produced from cellulose nanoparticles, new applications of cellulose nanowhiskers and self-assembly of cellulose nanocrystals (Moon, 2011; Eichhorn, 2011; Habibi et al., 2010) .There are also reports of obtaining spherical shaped cellulose nanoparticles (Zhang et al., 2007), often under very special reaction conditions which includes sonication for ten hours during the course of mild acid hydrolysis. However, in the latter case the polydispersity obtained was very high, with the size of the nanoparticles ranging from 10-180 nm (Wang, 2008). One of the major advantages of spherical cellulose nanoparticles is its stability in aqueous suspensions for several months (Liebert et al., 2011). Investigations on cellular uptake by such nanoparticles have revealed the profound effect of geometry of biocompatible nanomaterials on endocytosis (Liebert et al., 2011). In a detailed review paper, Katz and Willner had stated: “The importance of functionalized nanoparticles for biomedical applications cannot be overestimated. For instance, targeted entry into cells is an increasingly important area of research” (Katz and Willner, 2004). Spherical nanoparticles (80-200 nm) of amino functionalized celluloses have also been recently reported by them (Nikolajaski et al., 2012). Such materials have important implications in biomedical applications, such as gene delivery. Amine functional polymers such as polyethyleneimine used in such studies possess

toxicity, whereas polysaccharides are non-toxic (Nikolajaski et al., 2012). Spherical hydrophobic cellulose acetate composite nanoparticles with several polysaccharides have been prepared for potential applications in the pharmaceutical and food technology industries (Kulterer et al., 2012; Hornig and Heinze, 2008).

Carboxy functionalized celluloses, prepared by oxidation of cellulose, are amongst the most investigated derivatives of cellulose, due to their applications in wound dressing gauzes and several other related biomedical applications for over 70 years (Houser,1946; Anderson et al. , 1946; Kennedy et al., 1947; Scarff et al., 1949). The earliest report on oxidation of cellulose was way back in 1883 (Witz, 1883). Since then there have been continuous streams of papers and patents on various methods of oxidation of cellulose, and the properties of such materials (Yackel and Kenyon, 1942; Nooy et al., 1997; Kumar and Yang, 2002; Okita et al., 2010; Shinoda et al., 2012; Nachtkamp et al., 2012). In recent years, specifically around the period 1998-2000, nanofibres of oxidized celluloses have also been synthesized and their properties and applications vigorously investigated (Okita et al., 2010; Shinoda et al., 2012; Nachtkamp et al., 2012; Crawford et al., 2012). However, we have seen no reports of spherical shaped carboxy functionalized celluloses. As observed for other cellulose nanoparticle derivatives, spherical shapes are more likely to show stability in solvent dispersions as compared to nanofibres. Similarly, the geometrical shape of the nanoparticle can play a role in drug delivery and biomedical applications. Herein we report, for the first time, devising a simple method to obtain spherical shaped polymer nanoparticles of 6-carboxycellulose (6CC) (Figure 2.1) where so far only rod-shaped particles are reported. Since 6CC of varying carboxyl group content are an extremely important class of commercial biomaterials, we decided to investigate methods to prepare spherical nanoparticles of such materials as a function of carboxy content. We also

demonstrate for the first time that these spherical functionalized nanoparticles are extremely efficient in stabilizing carbon nanotubes (Figure 2.5) with minimal ultrasonication, thereby saving energy. Both these factors could be a key development for paving the way to many new applications for that material. The efficiency of these carboxycellulose quasi-spherical nanoparticles in anti-microbial applications was studied for *E. coli* (Figure 2.5). It was found that nanoparticles were more efficient than their larger sized analogs in eliminating *E. coli* .

2.2. Experimental

2.2.1. Materials

Sugarcane bagasse cellulose containing ~94% α-cellulose and 0.08% residual lignin was prepared by this method (Varma, 2008)**.**

2.2.2. Chemicals

Analytical grade nitric acid (65%), ortho-phosphoric acid (85.0%), sodium nitrite (98.0%), and calcium acetate (99.0%) were procured from Thomas Baker, Mumbai, India. Sodium metaperiodate (99.5%), sodium thiosulphate (99.5%), and sodium bicarbonate (99.5%) were obtained from S.D. Fine Chemicals, Mumbai, India. Soluble starch (Merck), sodium hydroxide, methanol GR grade, potassium iodide and potassium dichromate were purchased from Rankem, Mumbai, India. All chemicals were used without further purification.

2.2.3. Synthesis of 6-carboxycellulose in non-nano and spherical nanoparticle forms

In general, to finely powdered sugarcane baggase cellulose (10 g) was added 140 ml acid mixture (2:1 ratio, v/v) of 65% HNO_3 and 85% H_3PO_4 over a period of 5 minutes. The acid mixture was allowed to get absorbed in the cellulose for 10-15 minutes. This was followed by slowly adding 1.96 g of $NaNO_2$ (1.4 w/v %). As soon as the $NaNO_2$ was added, reddish fumes of NO_2 gas were evolved. The reaction was performed at three different temperatures: 25°C, 40°C, 50^{o}C and 70 ^{o}C, as shown in Table 2.1. The reaction mixture was quenched by diluting with distilled water (5 times the volume of acid mixture), allowed to settle down for 30 minutes, then decanted off. The solid part was washed with water (3 times) then with water –methanol mixture (2:1 v/v), then centrifuged at 2000 rpm to remove the solid.

The reaction products at 25°C were obtained as single crops, none of which were nanoparticles. In the specific case of the reaction at 40^{o}C (24h and 48h), after centrifugation of the initial solid at 2000 rpm for 15 minutes, the supernatant liquid was cloudy. This cloudy supernatant liquid was separately centrifuged at 12000 rpm for 15 minutes to separate out a second crop, which on analysis showed up to be quasi-spherical shaped nanoparticles of narrow size distribution (25-35 nm). For the reactions at 50^{o}C (12h) and 70^{o}C (8h) centrifugation had also to be carried out at 12000 rpm for 15 minutes to allow the nanoparticles to settle down and be collected. In both these cases the entire product consisted of nanoparticles in a single crop.

2.2.4. Preparation of 6-carboxycellulose

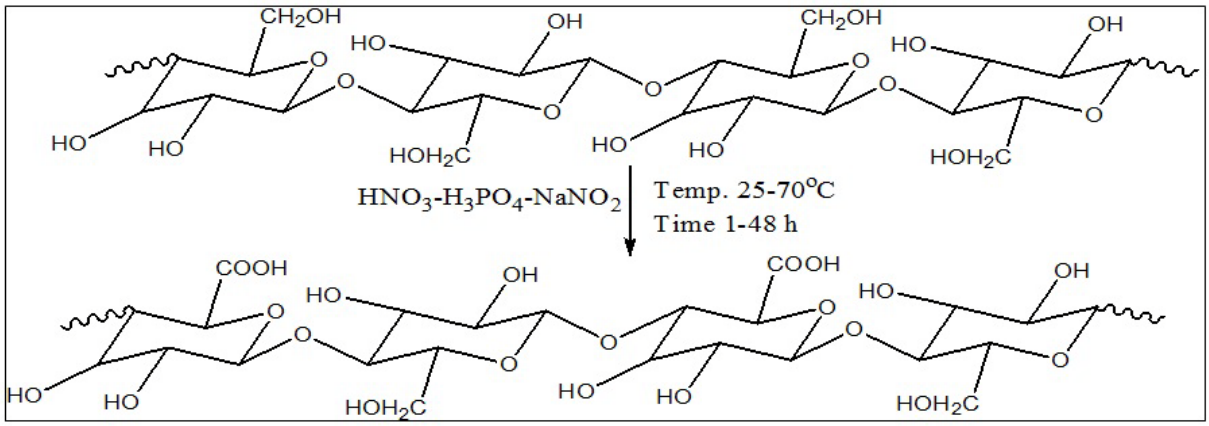

Figure 2.1: Reaction scheme of 6-carboxycellulose synthesis.

2.3. Methods

2.3.1. Determination of carboxyl content

The carboxyl content of oxidized cellulose was measured according to the method described by United state Pharmacopoeia (USP, 1995). A 0.5 g of sample was taken and emerged in 50 ml of 2% calcium acetate solution for 30 minutes. The mixture is then titrated with 0.1N NaOH (Standardized) by using Phenolphthalein as an indicator. The volume of NaOH used was corrected by blank titration. The % Carboxyl content in sample was calculated by following formula:

$$\text{Carboxyl Content (\%)} = \frac{\text{N x V x MW (COOH)}}{\text{Wt. of Sample (mg)}} \times 100$$

Where N is the normality of NaOH , V is the volume of NaOH used in titration and MW is the molecular weight of COOH.

2.3.2. Degree of Polymerization (DP)

The particles were not soluble in water due to low DS (13.2% for 6CC nanoparticles). Therefore, it was convenient to evaluate the DP of the celluloses by viscosity studies in cupriethlenediamine 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 o.2500g o.d. of cellulose. The weighed cellulose was solvated with cupriethylenediamine and passed through SCHOTT (kapillar-Vis Kosimeter) at 25^{o}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 viscocity was converted to the degree of polymerization (see Morton ,J.H,; Viscocity /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.

2.3.3. Fourier transforms 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 resolution of 4 cm^{-1}.

2.3.4. 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.

2.3.5. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) studies of cellulose nanoparticles were carried out by using FEI –Technai G^2-20 instrument. A 10µL 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)

2.3.6. 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 10µl sample drop of 0.01% concentration was cast on silicon wafer.

2.3.7. Dynamic Light Scattering (DLS)

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

2.4. Results and Discussion

This chapter has attempted to prepare 6CC from waste agricultural non-wood sources of cellulose I, avoiding the use of forest-wood which can cause environmental damage due to depletion of forest cover. Further, availability of nanoparticles of 6CC could result in improved performance of the currently used macro-sized 6CC in certain high-technology applications, especially biomedical applications (Varma and Sharma, 2013). Most synthesis of 6CC is based on the reaction of nitrosonium ion (NO^+) with cellulose in a heterogenous reaction medium. Dinitrogen tetraoxide gas in carbon tetrachloride, TEMPO with sodium bromide/sodium chlorite, and sodium nitrite in nitric acid/phosphoric acid media have been the most investigated methodologies for the synthesis of 6CC. Synthesis of 6CC using dinitrogen tetraoxide gas requires high pressure (70 atm), oxidation reaction is slow and produces many by-products (Coseri et al., 2013; Zimnitski, Yurkshtovich and Bychkovsky ,2004). Other oxidation systems based on TEMPO/NaBr/NaClO are of more recent origin, and produce long fibrils (Okita, Saito and Isogai, 2010; Isogai and Kato, 1998). In this work we adapted the nitric acid/phosphoric acid solvent system to produce a range of 6CC, including nanoparticles of quasi-spherical

shapes, narrow size dispersion (25-35 nm) and low DP 50-70. The strongly acidic medium is known to be highly temperature sensitive and causes loss of molecular weight. Hence most reports have limited the temperature range from 20-25^{o}C (Arendt, Carrier et. al, 1973; Kumar and Yang, 2002). However, in our experience, we found that up to 40^{o}C, the yields were comparable to that obtained at 25^{o}C for reaction times up to 12h (Table 2.1). As expected, the kinetics of the reaction is highly dependent on the temperature. The initial rates of oxidation reaction were much higher at 40^{o}C, for eg., 6CC of 13.2% was achieved after 3h reaction time, as compared to 3.0% carboxyl content for the 25^{o}C reaction temperature. At 12h reaction time, the carboxyl content was about the same for the reactions at 25^{o}C and 40^{o}C. Thereafter, the yields as well as differential rates of carboxylation decreased, and only a 25% yield (16% carboxyl) was obtained at 40^{o}C/24h reaction time at as compared to 63% yield (19.7% carboxyl) for the 25^{o}C/24h reaction (Figure 2.2, Table 2.1). A major change was observed beyond the 24h reaction at 40 ^{o}C: now nanoparticles started to be observed, but with a yield of only 5% (Table 2.1). This encouraged us to attempt higher temperatures and lower reaction times, so as to maximize the nanoparticles yield. Above 40^{o}C reaction temperature only single crops were obtained, all entirely consisting of nanoparticles. The carboxy content of the 6CC was 13.2% (i.e., 13.2% of the C6 groups of cellulose were replaced by carboxy groups), with DP ~70. Details of yields obtained for various reaction times and temperatures are shown in the (Table 2.1). At higher temperatures (50 and 70^{o}C), the entire product consists of nanoparticles. Only fifteen minutes of low power ultrasonication**,** (50Hz) produced stable aqueous dispersions of nanoparticles. This is in contrast to published reports where much longer sonication times are needed. We observed that at 50^{o}C/12h, we obtained nanoparticles, with a yield of 46%. Going higher, at 70 ^{o}C/8h, we again obtained only nanoparticles, but with a reduced yield of 16%. This

shows that a temperature range around 50^{o}C and low reaction times of upto 12h is needed to maximize nanoparticle yields. In order to obtain only macro-sized fibrils and no nanoparticles, the lower reaction temperature of 25^{o}C is favourable. The morphologies were confirmed by SEM and TEM studies, and representative pictures are shown in Figure 2.5. The detailed description is given in chapter 4. A study of the molecular weights of the 6CC products showed that upto 50 ^{o}C, DP was in the range of 70-87 (lower DP at higher reaction times), followed by a significantly reduced DP of 50 at 70^{o}C. Thus, it was possible to control the molecular weights and yields of the 6CC by controlling the reaction temperature and time. It may be mentioned, that lower molecular weights may be beneficial for cellular uptake studies, especially for bioimaging studies after attaching fluorescent probes.

The products were characterized by FTIR, ^{13}C-NMR, and Wide-angle XRD; they all show that the overall spectral features of the carboxy functionalized cellulose nanoparticles correspond to the starting cellulose even after reaction, with the carboxy functional groups at C6 clearly identified and corresponding changes in the original unreacted C6 peaks (Figure 2.3). The work-up procedures after the oxidation reaction were carefully tuned so as to obtain two crops at temperatures upto 40^{o}C (where the second crop consisted of quasi-spherical nanoparticles; the first crop consisted of larger particles). The spherical morphology of 6CC nanoparticles is clearly seen by SEM, TEM, and AFM images (Figure 2.5). TEM and AFM images of these nanoparticles show fairly uniform size of the particles in the range 25-35 nm. SEM appears to show discrete nanoparticles; however, the particle sizes are 90-110 nm, indicating that 3-4 molecules are agglomerated. Further, DLS shows the particle size to be ~132 nm with a very low polydispersity index (PDI) of 0.045 (Figure 2.4). It is reported in literature that DLS and TEM analysis generally do not match accurately. In a recent paper on quasi-spherical

regenerated cellulose characterization, TEM showed particle sizes in the range 90-110nm, whereas the DLS measurements showed a range from 50-550nm, with the peak maximum at 200 nm and relatively high PDI 0.215. This difference generally arises from the fact that TEM studies are carried out in dry form (sample droplet evaporated on a TEM copper grid), while the DLS measurements are carried out in suspension and polymer molecules, have a good chance to agglomerate. It is interesting to note that our spherical shaped cellulosic nanoparticles have a much narrower PDI than the elongated cellulosic nanofibers. The DP of these particles was approximately 50 at 70^{o}C, or 70 at 50^{o}C (Table 2.1). These nanoparticles are highly efficient against *E. coli* (Figure 2.5, iv) and they stabilized single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT) in aqueous solution for several days (Figure 2.5, v).

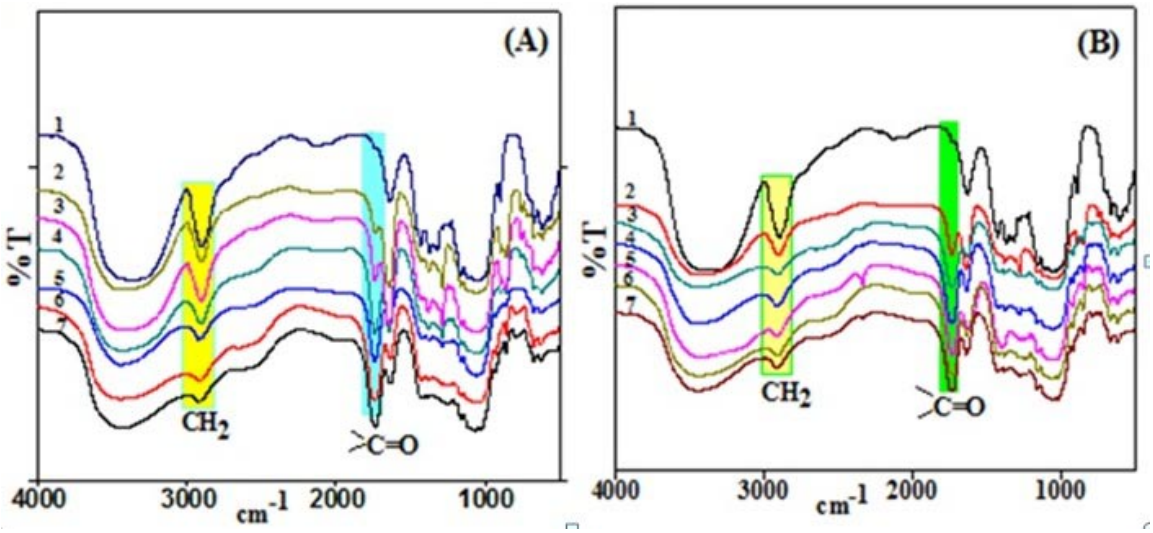

Figure 2.2: Overlapping of FTIR spectra of cellulose and 6-carboxycellulose. (A) Prepared at 25°C (1) Cellulose (2) 1.7 % (3) 3.0 % (4) 8.0 % (5) 14.0 % (6) 19.0 % (7) 22.0% carboxyl content.(B) Prepared at 40°C (1) Cellulose (2) 6.17 % (3) 13.2 % (4) 14.3 % (5) 14.0 % (6) 16.0 % (7) 17.0 % carboxyl content.

Table 2.1: Percent carboxyl content, yield, and degree of polymerization (DP) of 6-carboxy celluloses as a fibre and nanoparticles at different temperatures and time periods. *NP

Time (h)	-COOH Content (%) *				Yield (%)				DP			
	25°C	40°C	50°C	70°C	25°C	40°C	50°C	70°C	25°C	40°C	50°C	70°C
1h	1.7	6.17	-	-	84.0	83.0	-	-	87	86	-	-
3h	3.0	13.2	-	-	73.0	72.0	-	-	86		-	-
6h	8.6	14.3	-	-	71.0	68.0	-	-	84	81	-	-
8h	-	-	-	13.9	-	-	-	16.0*	-	-	-	50
12h	14.1	14.0	13.2	-	69.0	60.0	46.0*	-	82	78	70	-
24h (I crop) (II crop)*	19.7	16.0 18.0	-	-	63.0	25.0 5.0*	-	-	79	77 70	-	-
48h (I crop) (II crop)*	22.0	17.0 21.5	-	-	45.0	22.0 5.0*	-	-	77	76 70	-	-

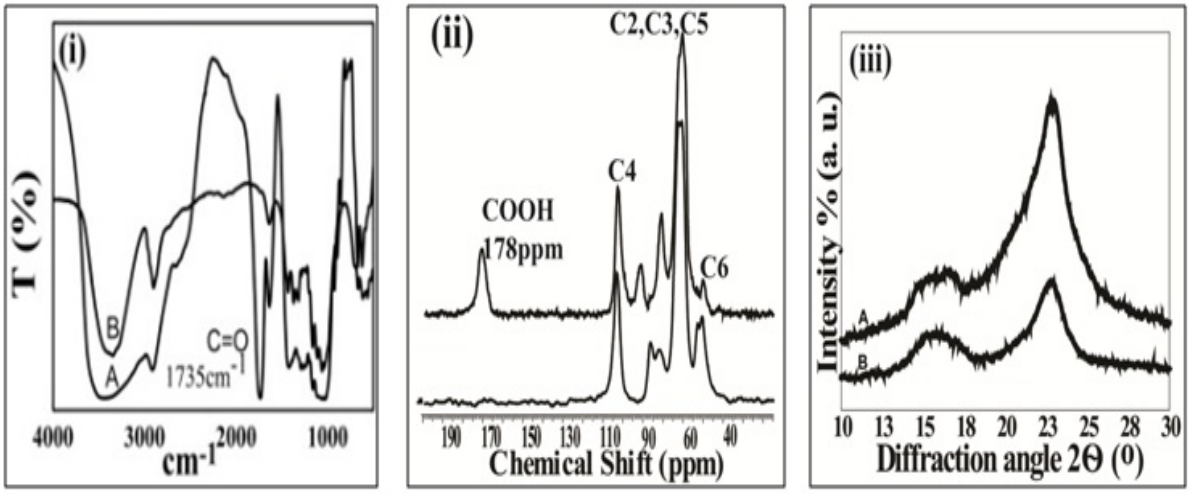

Figure 2.3: (i) FTIR spectra (A) 6CC-NP (B) Cellulose (ii) CP-MAS ^{13}C solid state NMR (A) 6CC-NP (B) Cellulose (iii) WAXRD of (A) 6CC-NP (B) Cellulose

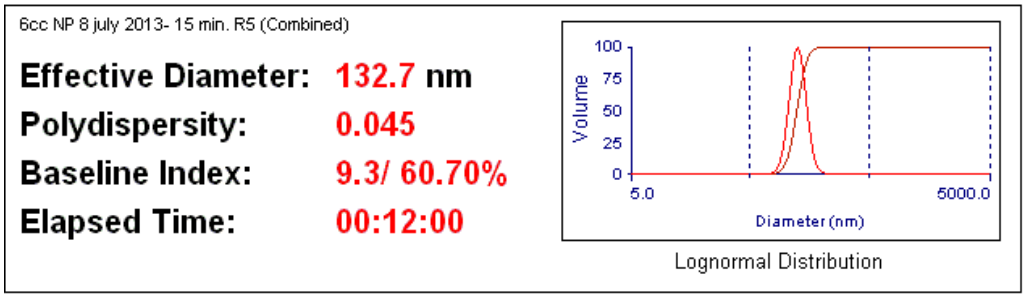

Figure 2.4: DLS of 6-carboxycellulose-NP prepared at 50°C.

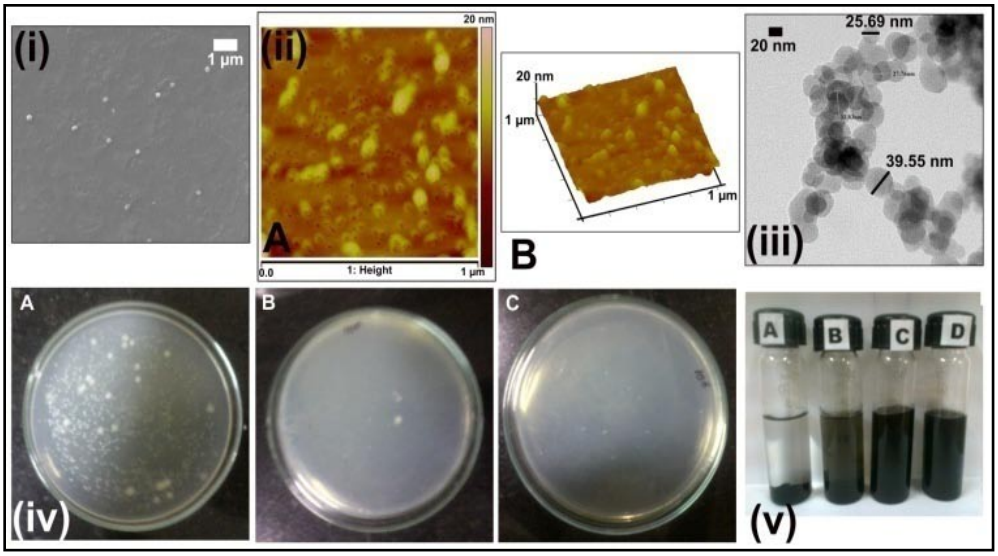

Figure 2.5: (i) SEM OF 6CC–NP (ii) non contact mode AFM images of 6CC–NP (A) corresponding height image (B) corresponding 3D image rotated at 45° (iii) TEM of 6CC-NP (iv) Inhibition of *E. coli* by (A) cellulose (B) 6CC (C) 6CC-NP (v) Dispersion study of CNT (A) cellulose + MWCNT (B) cellulose + SWCNT (C) MWCNT + 6CC (II crop; NP) (D) SWCNT + 6CC (II crop; NP)

2.5. Conclusion

In conclusion, the current study demonstrates for the first time that both low molecular weight agriculture residue derived cellulose, CP-100 (see Appendix I) as well as higher molecular weight cotton cellulose can be utlilized for producing quasi-spherical shaped nanoparticles of 6CC with low DP (~50 at 70^{o}C, or ~70 at 50^{o}C) (see Appendix II), that can extend the range of applications of fibril shaped nanoparticles of 6CC made by other oxidation systems. Preliminary studies have already shown the efficacy of these materials in stabilizing aqueous dispersions of single-walled and multi-walled carbon nanotubes for several days, and the isolated product of such stable dispersions gives rise to new hybrid materials which can have applications in

biocompatible semiconducting devices. The 6CC nanoparticles have also shown improved anti-microbial properties for *E. coli*. Many more biomedical applications for 6CC nanoparticles, fluorescent 6CC-NP and their role in bioimaging, 6CC-CNT hybrids, 6CC metal complexes, metal nanoparticles embedded in 6CC-NP, etc. are under exploration. These studies will add to the initial forays in this direction by innovative papers published on nanocellulose applications such as nanoporous cellulose gel as support for noble metal nanoparticles (Cai et al., 2009), preparation of stable high density silver nanoparticles templated on oxidized cellulose nanofibres (Ifuku et al., 2009), flexible magnetic aerogels and magnetic nanopaper using cellulose nanofibrils for potential applications in electronic actuators and microfluidics devices (Olsson et al., 2010) assembly of gold nanoparticles on a polysaccharide surface (Taajamaa et al., 2013), photoswitchable titanium dioxide nanocellulose aerogels which can open up new applications (Kettunen et al., 2011), and so on.

2.6. References

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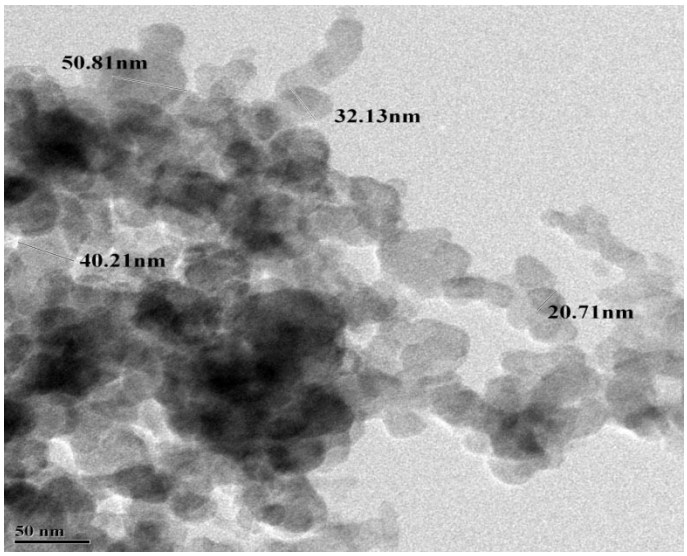
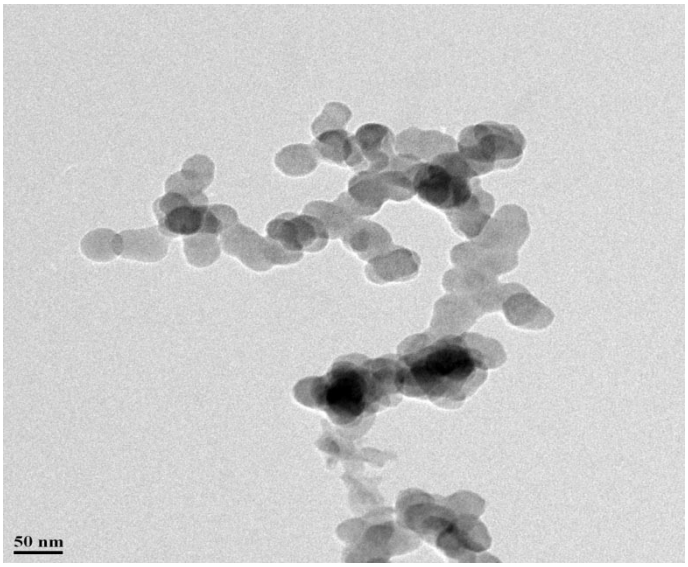
2.7. Appendix I: TEM image of 6-carboxycellulose nanopaticle obtained from cotton cellulose.



2.8. Appendix I: TEM image of 6-carboxycellulose nanopaticle obtained from CP-100.



Chapter 3

Synthesis of multi-oxidized celluloses and their nanoparticles: A biomedically important polymer

3.1 Introduction

Mainly due to sustainability and environmental issues, cellulose has come to occupy centre-stage amongst new materials that are being developed for a whole range of applications, from the traditional bulk applications in paper, textiles, industrial fibres, plastics, films, water soluble polymers, basic raw material for industrial fuels, solvents, and chemicals, to advanced biomedical and electronic applications, including solar cells. Particularly in the last two decades, developments in nanostructured celluloses have increased manifold the applications of cellulose in high-tech areas. While the major focus of nanocelluloses (popularly termed as nanocrystals, nanofibres, nanofibrils and nanowhiskers) (Okita, Saito & Isogai, 2010; Tanpichai et al.,2012) has been to develop new high-end applications in diverse fields such as nanocomposites, nanobiomaterials, cosmetics, and biomedical applications (Leung et al., 2011; Klemm et al., 2005; Klemm et al., 2011; Dong & Roman, 2007) recyclable organic solar cells (Zhou et al., 2013), fluorescent cellular bioimaging molecules (Lin, Huang & Dufresne, 2012), and drug delivery (Jager et al., 2012), recent researchers have found methods to obtain new spherical shaped nanoparticles of cellulose and functionalized celluloses, particularly amino cellulose (Nikolajaski et al., 2012), monocarboxy cellulose (Sharma & Varma, 2013) and cellulose acetate (Kulterer et al., 2012). These newly developed spherical shapes of nanoparticles of cellulose / functionalized cellulose can significantly expand the range of applications of these materials. For example, one of the major advantages of spherical cellulose nanoparticles is its stability in aqueous suspensions for several months (Liebert et al., 2011). Investigations on cellular uptake by such nanoparticles have revealed the profound effect of geometry of biocompatible nanomaterials on endocytosis (Liebert et al., 2011). Spherical nanoparticles (80-200 nm) of amino-functionalized celluloses have also been recently reported. Such materials have important

implications in biomedical applications, such as gene delivery. Amine functional polymers such as polyethyleneimine used in such studies possess toxicity, whereas polysaccharides are non-toxic (Nikolajaski et al., 2012). Spherical hydrophobic cellulose acetate composite nanoparticles with several polysaccharides have been prepared for potential applications in the pharmaceutical and food technology industries (Kulterer et al., 2012). Indeed, controlling the shape, size and functionality of nanoparticles has proved to be a important tool for expanding the properties and applications of nanoparticles. In the case of metal nanoparticles, intense research efforts have been directed towards controlling these parameters (Watt et al., 2013; Yi Wang et al., 2013; Xia et al., 2009; Niu & Xu, 2011; Quan, Wang, & Fang, 2013; Sun & Xia, 2002; Burda et al., 2005; Anker et al., 2008; Wang et al., 2008; Liu, Wang & Li, 2012). The dimensions of metal nanomaterials are often similar to several biomolecules, and the integration of metallic nanoparticles with biopolymers (such as nanocellulose and it's functionalized derivatives) can lead to new hybrid nanomaterials having a combination of properties of the two constituent materials.

We have just reported, for the first time in published literature, a methodology for preparing quasi-spherical shaped nanoparticles of 6CC, a biomedically important polymer (Sharma & Varma, 2013). We also demonstrated for the first time that these spherical functionalized nanoparticles are extremely efficient in stabilizing carbon nanotubes as well as possessing antimicrobial properties against bacteria like *E. coli*.

TEM and AFM images of these nanoparticles shows fairly uniform size of the particles in the range 20-50 nm and with low DP (50-70), that can extend the range of applications of TCC made by other oxidation systems and using either cellulose I or cellulose II as starting material. TCC, either alone or as a combination with other materials, has been reported to be an extremely

important biomedical polymer and is manufactured by several industries under different brand names, each referring to a different formulation for specific applications. Thus, perhaps due to their high industrial value, there are hardly any publications describing their detailed synthesis, properties, and morphology; however, most information is available only in patent literature (Kiyohiko et al., 1977; Montgomery et al., 1975; Fedor et al., 1992; Oridoroga & Medvedeva, 2000). A search of commercial literature shows that many products based on TCC are produced for high value medical applications, and are referred to a "oxidized regenerated cellulose" (ORC). ORC has been used clinically for well over 50 years (Bajerova et al., 2009).The role of ORC in surgery has been reported in several publications (Dineen, 1976; Hart et al., 2002; Cullen et al., 2002; Jeschke et al., 2005). An important TCC based medical product is commercially known as Surgicel® absorbablehaemostats (Spangler et al., 2003). Supercel is yet another important TCC based product used in blood-cellulosic interaction studies. Chemically these are oxidized regenerated celluloses, obtained by the nitrogen dioxide oxidation of 2,3-dialdehyde cellulose (Sinha & Vasudevan, 1985) Dialdehyde celluloses are produced by the action of sodium metaperiodate on cellulose I or cellulose II (Varma & Chavan, 1995). INTERCEED®, TC7, an oxidized regenerated cellulose has been tested for biodegradation. This polymer was observed to biodegrade readily to smaller fragments including glucuronic acid and glucose (Dimitrijevich, Tatarko & Gracy, 1990). Such (sterilized) oxidized cellulose is sometimes left intentionally at the site of surgical operation in cases where post-operative oozing of blood is anticipated, for example after splenectomise, transplantation, and vascular procedures (Young et al., 1993). BloodStop® iX is another surgical grade absorbent haemostat which is bioresorbable, and is biocompatible for long term implant use. It is an etherified oxidized regenerated cellulose (http://www.lifescienceplus.com/bloodstop/bloodstop-ix).Oxidized

regenerated cellulose is also a component of Promogran Prima A®, a product used for protection against infection whilst promoting cell growth (http://www.systagenix.net/our-products/lets-promote/promogran-prisma-187). A product known as Vocacit® consisting of TCC has been investigated as a substance for lowering the content of radioactive strontium in a body; (Razumovsky, Balabhukha & Torchinskaya, 1975; Ilyin,1976) another analogous product is Bioaktsellin® (Oridoroga & Medvedeva, 2000). TCC has also been investigated for removal of other toxic substances such as mercury, lead, antimony, and rare earth metals (Lejeune & Deprez, 2010). TCC has also been used in the production of meso tartaric acid by hydrolysis with sulphurous acid (Frank & Head, 1948; Hearon, 1977).

However, nanoparticles of TCC have never been reported. Considering the significant changes in properties of molecules when produced in the form of nanoparticles, we thought it would be important to develop methods to synthesize nanostructured TCC. Regenerated cellulose refers to cellulose II polymorph, whereas cotton linters and agricultural waste sugarcane bagasse cellulose, used in our study, is cellulose I. It may be more interesting and important to have nanoparticles of cellulose I as compared to cellulose II, since a lot of chemicals and energy are consumed to convert cellulose I to cellulose II by the regeneration process. We consider the use of cellulose I should be preferred to cellulose II, since the latter is produced by first dissolving cellulose I in corrosive solvents and then regenerating it as cellulose II (Veves et al., 2002). Therefore cellulose I is a more environment-friendly material, and it should displace cellulose II wherever possible. These nanoparticles can be expected to have enhanced anti-microbial activity as compared to their macro-sized analogs. Due to absorbency considerations, in some applications non-woven cellulose II fibres are preferred; (Dhanuraj et al., 2005) however, with the development of nanoparticles, there is scope to develop absorbent materials / formulations

from cellulose I as well. The carboxylic acid group attached to the cellulose chain provides active sites for immobilizing proteins as well as other molecules to TCC nanoparticles using simple peptide coupling methods (Ifuku et al., 2009). Under exploration are several biomedical applications for TCC nanoparticles, synthesis of fluorescent TCC-NP for exploring their role in bioimaging, TCC-Carbon nanotube hybrids, TCC metal complexes, etc. These studies will complement the initial forays in this direction by innovative papers published on nanocellulose applications such as nanoporous cellulose gel as support for noble metal nanoparticles (Cai et al., 2009), preparation of stable high density silver nanoparticles templated on oxidized cellulose nanofibres (Ifuku et al., 2009), flexible magnetic aerogels and magnetic nanopaper using cellulose nanofibrils for potential applications in electronic actuators and microfluidics devices (Olsson et al., 2010), assembly of gold nanoparticles on polysaccharide surfaces (Taajamaa, 2013), and so on.

3.2 Experimental

3.2.1 Materials

Sugarcane bagasse cellulose containing ~94% α-cellulose and 0.08% residual lignin was prepared by this method (Varma 2008).

3.2.2 Chemicals

Analytical grade nitric acid (65%), ortho-phosphoric acid (85.0%), sodium nitrite (98.0%), and calcium acetate (99.0%) were procured from Thomas Baker, Mumbai, India. Sodium metaperiodate (99.5%), sodium thiosulphate (99.5%), and sodium bicarbonate (99.5%) were obtained from S.D. Fine Chemicals, Mumbai, India. Soluble starch (Merck), sodium hydroxide,

methanol GR grade, potassium iodide and potassium dichromate were purchased from Rankem, Mumbai, India. All chemicals were used without further purification.

3.2.3 Preparation of 2, 3-dialdehyde celluloses (DAC) with different degrees of carbonyl content

The 2, 3-dialdehyde celluloses of 5, 15, 25 % dialdehyde content, were synthesized by using sodium periodate ($NaIO_4$), according to previously reported methods (Varma & Chavan, 1994; Varma & Jamdade, 1985). The 2,3-dicarboxy cellulose were synthesized from 2,3-dialdehyde (15%) by using sodium chlorite.

3.2.4 Preparation of 2, 3, 6-tricarboxycellulose (TCC) with different degrees of carboxyl content and TCC-NP's

2,3,6-Tricarboxycelluloses (5:15, 15:15, 25:15) (where the first number refers to the carboxyl content at C2-C3 position, and the second number refers to the C6 position carboxyl content of the glucose ring) were synthesized from 2,3-dialdehyde celluloses (5, 15, 25 % aldehydic carbonyl content). 10 g of 2, 3-dialdehyde cellulose was taken in a 2-neck round bottom flask equipped with a magnetic stirrer and guard tube. To this was added the acid mixture (2:1 v/v 65% HNO_3 and 85% H_3PO_4). The ratio of acid to the starting material was 1:14 (w/v). The reaction mixture was allowed to stir for 10 minutes, and then 1.96 g of $NaNO_2$ was added. The reaction was allowed to proceed at 25^oC for 16h. The reaction was quenched by adding distilled water (five times the volume of reaction mixture) and allowed to stand for half an hour. The solid

residue obtained was the I crop. The decanted portion was centrifuged at 12000 rpm to obtain a gel like material, which is referred to as the II crop. The I crop and II crop were both separately washed with 2:1 ratio of methanol and distilled water several times, until the pH of the filtrate was neutral. The final washing was done by acetone and the products were dried in a freeze drier. The I crop was in the form of micro fibrils (~6 μm) and the II crop was obtained as a white colour fine powder, which were the quasi-spherical nanoparticles (20-50 nm). The chemical structure of TCC is shown in Figure 3.1. The yield of varying carboxyl content TCC is given Table 3.1.

3.2.5 Preparation of 6-carboxy-2, 3- dialdehyde cellulose

The 6-carboxy-2, 3-dialdehyde cellulose (15:9) was synthesized from 6CC (15% carboxyl content). The 6-carboxy cellulose was further oxidized at C-2 and C-3, by using the well established procedure using sodium metaperiodate, described in the above section.

3.2.6 Screening test of oxidized celluloses with *Mycobacterium tuberculosis* strain: H37Ra (non-pathogenic bacteria)

The percent inhibition of 6CC, 6CC-NP, TCC, TCC-NP was determined for ***Mycobacteroum tuberculosis*** isolates from NCIM, Pune, India. The compounds 6CC, 6CC-NP, TCC, TCC-NP were weighed according to their concentration to make final volume 1 ml M.Phili (Defined media) prepared and added to all weighed compounds to make a stock of compound concentration and autoclaved. The inoculums of *Mycobacteroum tuberculosis* were prepared by incubating 1% inoculated M.Phili (Defined media) for 10-12 days with 150 rpm at 37^0C. After

incubation, log phase culture of Mycobacteroum tuberculosis showed OD 0.94 with wavelength 620nm.

A mass of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0 mg of each sample were re suspended in M.Phili (Defined media) to achieve a concentration of 1mg/ml and autoclaved at 121°C for 15 minutes. The 198µl of diluted samples were distributed to the first five well of a 96-well plate (the experiment was performed in triplicate). Next, three and two well of sterile 96-well plate were filled with inoculum and blank containing inhibitor Rifampicin 0.00192 ug/ml respectively. The 1% inoculation in all the samples was done with 2µl of 0.94 OD (620nm) freshly grown *Mycobacteroum tuberculosis* and allowed them to mix well. To check the inhibition effect of all the samples, parafilm sealed plate was incubated at 37 °C for 8 days. After 8 days, plate was taken out, and read the absorbance at 620 nm using SPECTRAMAX^{384} plate reader.

3.2.7. Screening Test of oxidized celluloses with Mycobacterium tuberculosis strain: H37Rv (pathogenic bacteria)

Media for the growth of culture was prepared by dissolving 7H9 broth in 900ml Mili-Q water

containing 2ml glycerol. This was autoclaved at 121°C for 10 minutes. Subsequently, 100 ml middlebrook ADC enrichment with 0.05 % Tween 80 was aseptically added to it. The pH of the medium observed was 6.4-6.8. After that, the culture of the H37Rv was grown in prepared medium till O.D (Optical Density) reached to 0.6-0.8.

In other side, the inhibitors (cellulose and oxidized cellulose samples) were dissolved in DMSO to a concentration of 10mg/ml to prepare stock solutions for all samples. The stock solutions

were then serially diluted to suitable concentration of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9 with assay media (middlebrook 7H9 brook + ADC+ 1g/mL Tryptone) µg/ml. Here, isoniazid was used as control. The inoculation was done by adding 2 X 10^5 cells to the wells of the 96-well plate. All the plates were sealed with paraffin and were incubated at 37°C for 7 days. After the period of incubation, mixure of 10% Tween 80 and alamar blue (1:1ratio), was added to each well. After overnight incubation, colour changes were observed to calculate MIC_{99}. The absence of color change suggests complete growth inhibition (MIC_{99}). (http://www.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/212352.pdf)

3.3 Methods

3.3.1 Determination of carboxyl content

The carboxyl content of oxidized cellulose was measured according to the method described by United state Pharmacopoeia (USP, 1995). A 0.5 g of sample was taken and emerged in 50 ml of 2% calcium acetate solution for 30 minutes. The mixture is then titrated with 0.1N NaOH (Standardized) by using Phenolphthalein as an indicator. The volume of NaOH used was corrected by blank titration. The % carboxyl content in sample was calculated by following formula:

$$\text{Carboxyl Content (\%)} = \frac{N \times V \times MW\ (COOH)}{\text{Wt. of Sample (mg)}} \times 100$$

Where N is the normality of NaOH, V is the volume of NaOH used in titration and MW is the molecular weight of COOH.

3.3.2 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 cupriethlenediamine 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 o.2500g o.d. of cellulose. The weighed cellulose was solvated with cupriethylenediamine and passed through SCHOTT (kapillar-Vis Kosimeter) at 25^{o}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 viscocity was converted to the degree of polymerization (see Morton ,J.H,; Viscocity /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.

3.3.3 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 resolution of 4 cm^{-1}.

3.3.4 Wide-angle X-ray Diffraction (WAXRD)

WAXRD spectra and patterns were collected on a PANalytical X'pert Pro dual goniometer diffractometer. A proportional counter detector was used for low angle experiments. The samples were put on sample holders made of aluminum alloy and flattened with a piece of glass. The Cu Kα radiation was generated at 40 KV and 40 mA (λ =0.15418 ˚nm) with a Ni filter. The data collection was carried out using a flat holder in Bragg-Brentano geometry (5-60^{o}; 4° min^{-1}).

3.3.5 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.

3.3.6 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) studies of cellulose nanoparticles were carried out by using FEI –Technai G^2-20 instrument. A 10µL 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)

3.3.7 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 10µl sample drop of 0.01% concentration was cast on silicon wafer.

3.3.8 Dynamic Light Scattering (DLS)

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

3.4 Results and Discussion

3.4.1 Discussion on the characterization of Fourier transforms infrared spectrometry (FTIR)

The prepared oxidized cellulose (TCC-I crop & TCC-NP) were characterized by FTIR spectroscopy. FTIR spectra of low molecular weight bagasse cellulose was overlapped with

tricarboxy cellulose (25:15) I crop and II crop (NP) in Figure 3.3(ii). The conversion of cellulose to tricarboxy cellulose was qualitatively analysed by some important changes appears in FTIR spectra. The -C=O stretching vibration at 1732cm^{-1} in tricarboxycelluloses, indicate the transformation of primary -OH into –COOH group. The –OH peak at 3360 cm-1 was found to be broaden in tricarboxy cellulose (I crop) and even more profound in tricarboxy cellulose (IIcrop; NP), which may be due to disruption in hydrogen bonding (Okita et al., 2011) and greater water absorption. The overlay FTIR spectra for different carboxy group content tricarboxy celluloses are presented in Figure 3.2(ii).

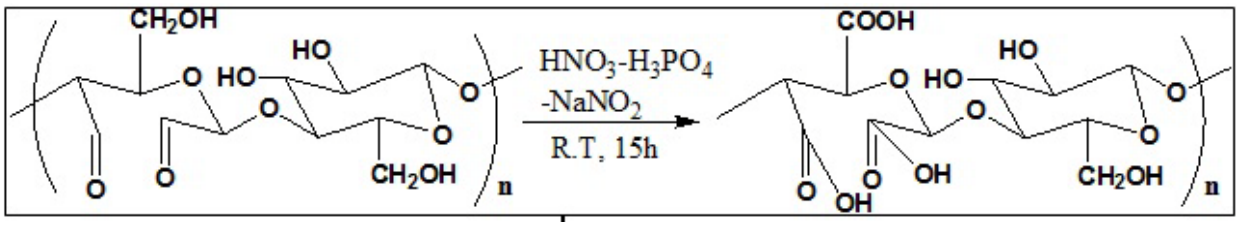


Figure 3.1: Synthesis of 2,3,6-Tricarboxycellulose.

3.4.2 Confirmation of oxidation of 2, 3-dialdehyde by $NaClO_2$

In order to further confirm the oxidation of 2,3-dialdehyde groups occurring simultaneously with C6 oxidation, the reaction products were subjected to further oxidation using $NaClO_2$ and work up was carried as reported for 2,3-DCC (Varma & Chavan, 1995). The FTIR of the product matched with the FTIR spectra of previous product (Figure 3.2 (i D)), thereby confirming that oxidation of 2,3-dialdehyde by $HNO_3/H_3PO_4/NaNO_2$ gives 2,3,6-tricarboxy cellulose.

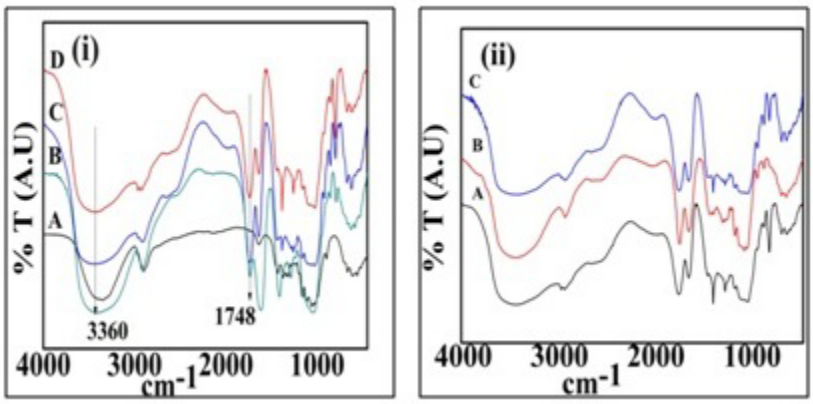


Figure 3.2: (i) Overlay of FTIR spectra of 2, 3, 6- tricarboxy cellulose (A) cellulose (B) 5:15 % (C) 15:15 % (D) 25:15 % (ii) Overlay of FTIR spectra of (A) 2,3,6-tricarboxy cellulose (25:15) (B) after further oxidation with $NaClO_2$ (sodium salt of acid) (C) after acidification of sodium salt of acid.

3.4.3 Solid state ^{13}C NMR spectra of 2,3,6-tricarboxycelluloses-nanoparticles

The solid state ^{13}C NMR spectra of cellulose and tricarboxycelluloses are presented in (Figure 3.3, ii). The region between 60 and 70 ppm is assigned to C6 of the primary alcohol group. The next cluster between 70 and 80 ppm is attributed to the C2, C3, and C5 carbons. The region between 80 and 95 ppm is associated with C4 and that between 100 and 110 ppm with the anomeric carbon C1. The signal at 178 ppm in TCC corresponds to the carboxy group. The peak intensity of carboxy is similar in TCC (I crop) and TCC-NP. However, the signal corresponding to the aldehydic carbonyl group in TCC is absent, proving that all the aldehydic carbonyls were converted to carboxyls.

Table 3.1: Yields of TCC prepared with different percent carboxyl contents.

	TCC	*Carboxyl (%) at C-6	Carboxyl (%) at C-2 and C-3	Yield (%)
1.	I Crop	15	5	78
	II Crop; NP	17	5	20
2.	I Crop	15	15	64
	II Crop; NP	18	15	20
3.	I Crop	15	25	63
	II Crop; NP	18	25	18

3.4.4 WAXRD spectra of 2,3,6-tricarboxycelluloses-nanoparticles

The diffraction pattern of the original cellulose in Figure 3.3 (iii) reveal the presence of two main peak at 2Θ=22.8° and 15.8°which corresponding to cellulose I. It was reported that peak intensity corresponding to 2θ =22.8° was found to decrease proportionally to the degree of oxidation . The diffractogram of TCC shows the broad peak (002) at (2θ) 22.8, and other peak get completely merged to single peak. The broadening of peak shows the significant loss of crystalline part, whereas the diffraction pattern of cellulose I remains maintained after oxidation. The Crystallanity index (CrI) measured for the cellulose (cotton linter) is 80%, and for TCC (I crop) 47 %.It is reported in literature, CrI decreases with increase in percent oxidation. However, such type of trend is not observed for the nanoparticle. On conversion from macro to nano size, cellulose become more crystalline due to loss of amorphous part present as disordered region. We observed the same trend for TCC (NP), the CrI incresase upto 66% as compared to TCC (I crop). Here, the peak signal for TCC (I & II crop) showing the major peak at (2θ) 22.8, which is corresponding to cellulose I.

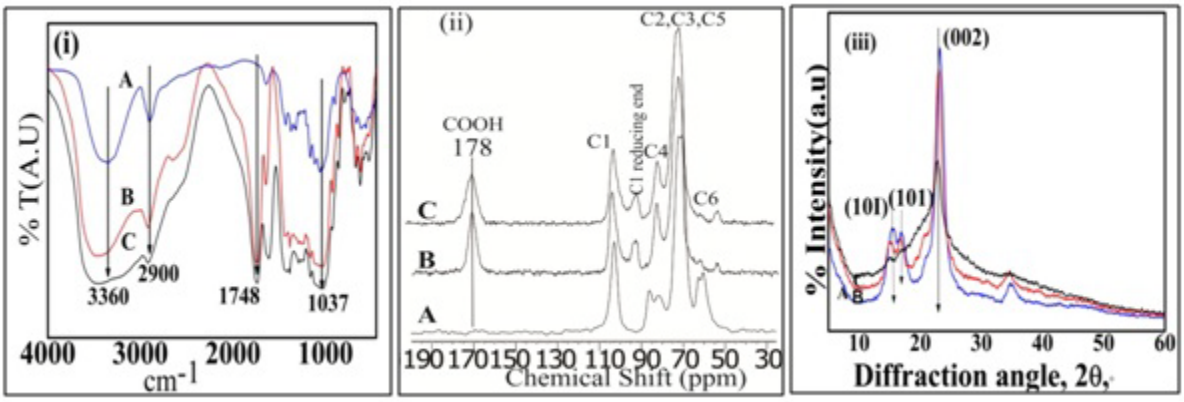


Figure 3.3: (i) FTIR spectra of (A) Cellulose (B) TCC (I crop) (C) TCC(II crop;NP) (ii) solid state 13 C-NMR of (A) Cellulose (B) TCC (I crop) (C) TCC(II crop;NP) (iii) WAXRD of (A) Cellulose (B) TCC (I crop) (C) TCC(II crop;NP)

3.4.4 Morphology of 2,3,6-Tricarboxycelluloses-nanoparticles

The atomic force microscopy (AFM) and transmission electron microscopy (TEM) micrographs confirm the preparation of spherical TCC-nanoparticles with uniform smaller size of 20-50nm (Figure 3.5). The DLS analysis further shows the particle size to be around 158 nm with a very narrow polydispersity index (PDI) of 0.169 (Figure 3.4)

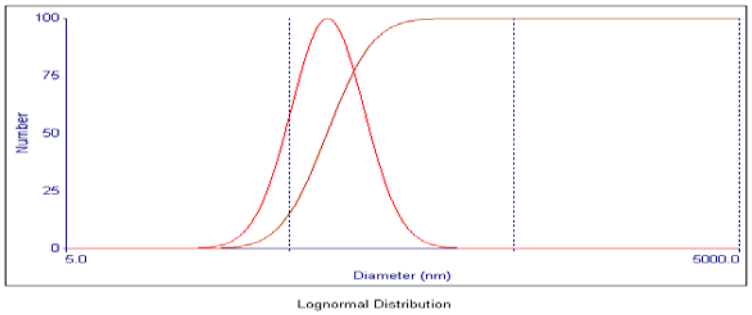


Figure 3.4: DLS of TCC-NP.

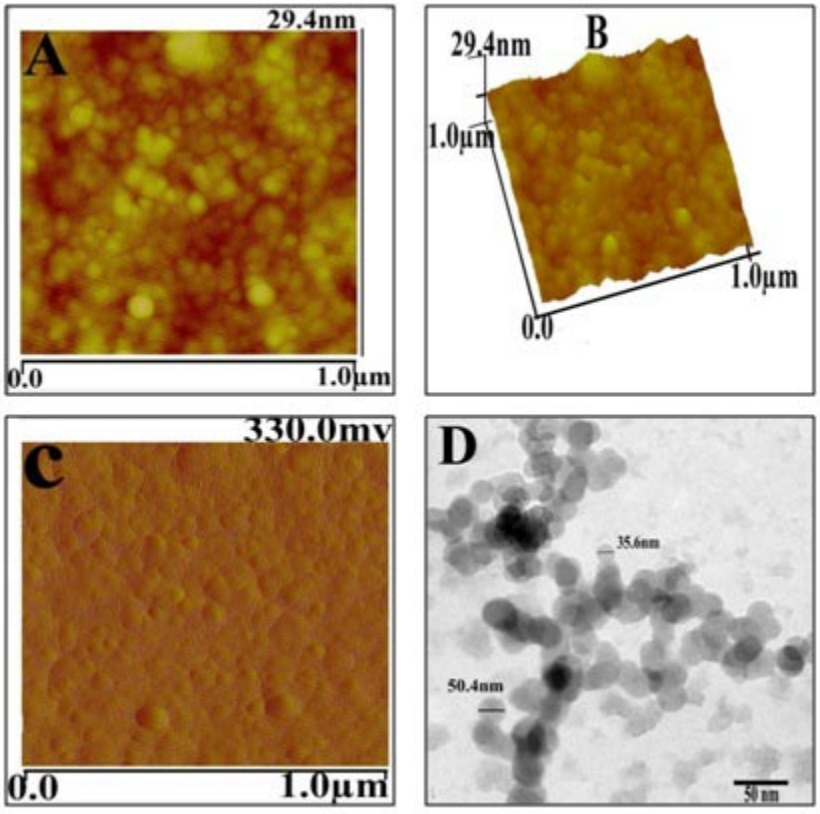


Figure 3.5: Non Contact mode AFM images of on silicon wafer TCC (bagasse cellulose) –NP (A) corresponding height image (B) corresponding 3D image rotated at 45° (c) Corresponding Amplitude image (D) TEM image of TCC (II crop;NP)

3.4.5 Application of 2,3,6-Tricarboxycelluloses-nanoparticles

The efficiency of these TCC quasi-spherical nanoparticles in anti-microbial applications was studied for *E. coli* (Figure 3.6, A-C). We also demonstrate for the first time that these spherical functionalized nanoparticles are extremely efficient in stabilizing carbon nanotubes (Figure 3.6, D) with minimal ultrasonication, thereby saving energy.

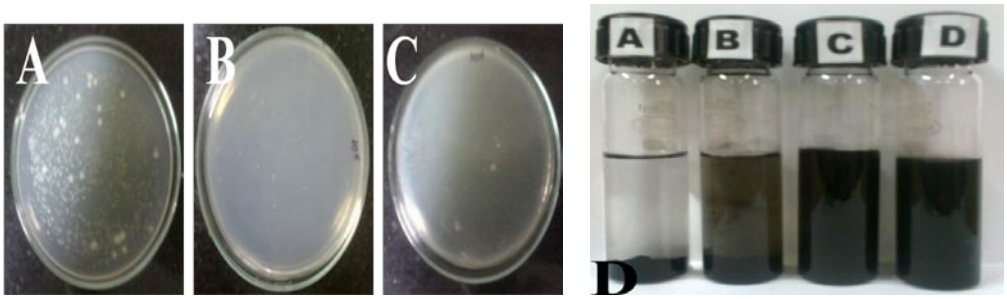


Figure 3.6: The inhibition of *E. coli* by (A) cellulose (B) TCC (C) TCC-NP after 6h incubation at 37^0C on LB-agar plate.(D) Dispersion study of CNT (A) cellulose+MWCNT (B) cellulose+SWCNT(C)MWCNT+TCC(II crop;NP)(D) SWCNT + TCC (II crop ;NP)

3.4.5 Anti-TB activity of carboxycelluloses-nanoparticles

Anti-tuberclosis activity of carboxycelluloses and their nanoparticles are tested against non-pathogenic (H37Ra). The MIC value of carboxy cellulose and their spherical nanoparticles are in the range of 3.0-5.0% (w/v), for 100% inhibition of bacterial growth (Figure 3.7). The minimum inhibitory concentration for controlling or inhibiting the growth of *Mycobacterium tuberculosis* of nanoparticle of 6CC and TCC is in between 4-5 % (w/v), within 6 to 8 h. The experimental data clearly shows the efficiency of carboxycelluloses and their spherical nanoparticles against the non-pathogenic species of mycobacterium tuberculosis. Further, testing of carboxycelluloses and their spherical nanoparticles against pathogenic bacteria will prove their application in pharmaceutics, particularly in the formulation of anti-TB medicines.

MIC_{99} value for *tuberculosis* drug isoniazid is 0.3µg/ml**.** Here, the MIC_{99} value calculated for 6-carboxycellulose (6CC) was 1000µg/ml, 6-carboxycellulose nanoparticles (6CC-NP) was 250 µg/ml. Similary for 2,3,6-tricarboxycellulse (TCC) was 500µg/ml , and for 2,3,6-tricarboxycellulse nanoparticles (TCC-NP) was 500µg/ml (Table 3.2). Although, the MIC_{99} values for oxidized cellulose are high, but these can be potentially used for anti-TB drug composition.

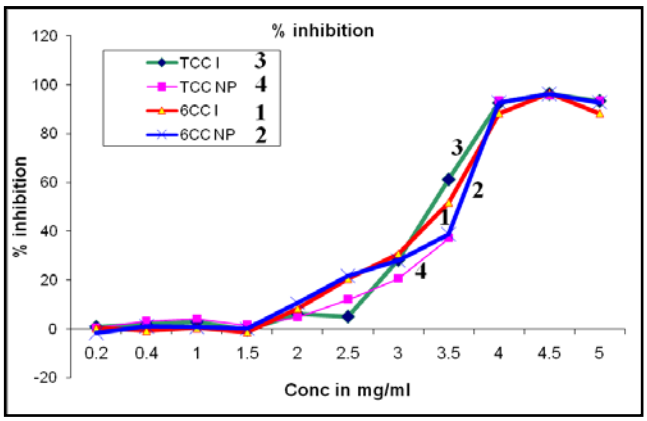


Figure 3.7: Activity of carboxy celluloses and their spherical nanoparticles against H37Ra.

Table 3.2: MIC_{99} values calculated for oxidized celluloses against H37Rv (pathogenic bacteria).

Compound Code	**MIC_{99} (µg/ml)**
Cellulose 6CC	1000
Cellulose 6CC-NP	250
Cellulose TCC	500
Cellulose TCC-NP	500

3.5 Conclusion

Herein we report for the first time the synthesis of quasi-spherical shaped TCC using cellulose I, having different contents of carboxy groups (high molecular weight cotton linters and low molecular weight sugarcane bagasse derived cellulose molecules). The spherical nanoparticles of TCC have never been reported. These new materials have been found to have sizes in the range 20-50 nm with narrow polydispersity (0.169). TCC molecules, due to their relatively high charge density as compared to 6CC, can expand the range of applications of 6CC. A previous report of

carboxylated cellulose nanocrystals (11-19% carboxyl content, similar to ours) prepared by a different procedure did not produce spherical nanopartiles (Leung et al., 2011). Thus, the shape of the nanoparticle is highly dependent on the synthetic method employed for oxidation.

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Chapter 4

Oxidized celluloses and their nanoparticles: Morphology, thermal properties and solubility studies

4.1. Introduction

In recent years, deforestation concerns have led to the development of non-wood sources of cellulose, especially cellulose extracted from agricultural wastes such as sugarcane bagasse, wheat straw, rice straw, etc. (Varma, 2013; Nuruddin et al., 2011). This source of cellulose is considered environment-friendly, as fewer forest trees have to be cut to produce cellulose. Another major advancement in the field of cellulose chemistry and technology is the development of nanoparticles of cellulose and cellulose derivatives (Eichhorn, 2011; Kulterer et al., 2012; Nikolajaski et al., 2012). Since most of these cellulose and nanocellulose molecules are biocompatible and biodegradable, their role in several biomedical applications, biosensors, diagnostic molecular probes, drug delivery vehicles, etc. are being vigorously pursued, along with other exciting applications in biocomposites, membranes, electronics, and solar cells (Klemm et al., 2005; Klemm et al., 2011; Zhou et al., 2013; Lin et al., 2012). As in metal nanoparticles, research on nanocelluloses has expanded to include shape-selective synthesis, such as nanofibres and nanospheres (Kulterer, Reichel et. al, 2012; Nikolajaski et al., 2012; Isogai et al., 2011; Sharma & Varma, 2013). Graphene-cellulose paper membranes have been used as electrodes for flexible super capacitors. Celluloses have also been used with carbon nanotubes and combined with conducting polymers for the fabrication of electroconductive composites; further, hybrid inorganic-organic nanocomposites are emerging as a new class of functional nanomaterials (Lin et al., 2012; Shi et al., 2013). 6-Carboxycelluloses, prepared by oxidation of cellulose, have been extensively investigated for over seventy years due to their applications in wound dressing gauzes and several other related biomedical applications (Houser, 1946; Anderson & McIntyre,1946*;* Kennedy, 1947; Scarff, Stookey & Garcia,1949**).** The earliest report on oxidation of cellulose to produce carboxylated cellulose was reported way back in 1883

(Cross & Bevan, 1883). Since then there have been regular streams of papers and patents on various methods of oxidation of cellulose and their applications (Yackel & Kenyon, 1942; Nooy et al.,1997; Kumar & Yang, 2002; Okita, Saito & Isogai, 2010**;** Shinoda et al., 2012). In recent years, nanofibres of 6CC have also been synthesized and their properties have been investigated (Okita, Saito & Isogai, 2010**;** Shinoda, et al., 2012; Nachtkamp et al., 2012; Crawford et al., 2012). Indeed, carboxy functionalized nanocelluloses can be expected to vastly expand the range of properties of electroconductive devices and biomedical devices, where currently un-functionalized nanocelluloses are used. This was the motivation for taking up the synthesis and characterization of both macro-sized carboxycelluloses and nano-sized carboxycelluloses, and to compare their thermal properties, morphological changes in the products, and solubility characteristics. These are all key properties for fabricating new devices based on these materials.

We recently patented our work based on extraction of cellulose from non-wood sugarcane bagasse (Varma, 2013). This cellulose was oxidized to 6CC, and the synthesis was fine-tuned so as to produce the usual macro-sized fibrils in addition to quasi-spherical shaped nanoparticles of narrow polydispersity, narrow size range (25-35 nm), and low degrees of polymerization (Sharma & Varma, 2013). We studied the solubility characteristics of this new series of 6CC (1.7 – 22% carboxyl content) and their nanoparticles, and also made a comparative study of the thermal properties of the materials. These results were used for deciphering gradual changes in the morphology of the cellulosic molecules caused during progressive carboxylation. Several properties of semi-crystalline polymeric molecules are guided by their morphology, thermal properties, and solubilities. Hence it is important to have a good knowledge of the thermal properties and morphology as a function of degree of carboxy substitution, molecular weights, molecular sizes, and molecular shapes of the different 6CC. Our previous work on aldehyde,

carboxy, and amine functionalized wood cellulose as reinforcements in epoxy composites had shown interesting advantages as compared to the use of un-functionalized cellulose (Varma & Chavan, 1994). Commercial availability of nano-carboxycellulose will most certainly lead to the development nano-biocomposites of such materials. Therefore we believe our current studies throw useful new light on the utilization of forest-free cellulose for preparing carboxy functionalized celluloses and their nanoparticles for developing their applications.

4.2. Experimental

4.2.1. Synthesis of 6-carboxycellulose in non-nano and nanoparticle forms

The detailed information has been given in Experiment 2.1

4.2.2. Preparation of 2, 3-dialdehyde cellulose (DAC) and 2, 3-dicarboxycellulose (DCC)

The 2, 3-dialdehyde celluloses of 5, 15, 25 % dialdehyde content, were synthesized by using sodium periodate ($NaIO_4$), according to previously reported methods (Varma & Chavan, 1994; Varma & Jamdade, 1985). The 2,3-dicarboxy cellulose were synthesized from 2,3-dialdehyde (15%) by using sodium chlorite.

4.2.3. Preparation of 2, 3, 6-Tricarboxycellulose (TCC)

The detailed information has been given in Experiment 3.1

4.2.4. Preparation of 6-carboxy-2, 3- dialdehyde cellulose (6C2,3DAC)

The 6-carboxy-2, 3-dialdehyde cellulose (15:9) was synthesized from 6-carboxy cellulose (15% carboxyl content). The 6-carboxy cellulose was further oxidized at C-2 and C-3, by using the well established procedure using sodium metaperiodate, described in the above section.

4.2.5. Preparation of amorphous cellulose

Sugarcane bagasse based cellulose I (1 g) was taken with o-phosphoric acid (85%) (5 ml) and stirred continuously for 14h at 25^{o}C, and then further stirred at 50^{o}C for 2h. The cellulose was completely dissolved in the acid and was re-precipitated by slowly pouring into distilled water under stirring. The washing was done initially with water, then with methanol/water (2:1, v/v), to obtain amorphous cellulose (Wei, Kumar & Banker, 1996).

4.3. Methods

4.3.1. Degree of Polymerization (DP)

The degrees of polymerization of oxidized celluloses were estimated by measuring the viscosity (cP) according to standard method TAPPI T 254 cm-10. The viscosity (cP) obtained for various oxidized celluloses were used to calculate the degree of polymerization using the graphical data taken from pg.99 of the standard book “Wood and Cellulose Science” by A.J. Stamm (Stamm, 1964). We were able to tailor the DP by controlling the reaction temperature. At 40^{o}C and 50^{o}C we obtained a DP of 70, while at 70^{o}C we obtained a DP of 50.

4.3.2. Determination of carboxyl content

The carboxyl content of oxidized cellulose was measured according to the method described by United state Pharmacopoeia (USP, 1995). A 0.5 g of sample was taken and emerged in 50 ml of 2% calcium acetate solution for 30 minutes. The mixture is then titrated with 0.1N NaOH (Standardized) by using Phenolphthalein as an indicator. The volume of NaOH used was corrected by blank titration. The % Carboxyl content in sample was calculated by following formula:

$$\text{Carboxyl Content (\%)} = \frac{N \times V \times MW\ (COOH)}{\text{Wt. of Sample (mg)}} \times 100$$

Where N is the normality of NaOH, V is the volume of NaOH used in titration and MW is the molecular weight of COOH.

4.3.3. Thermogravimetry (TGA) and Differential Thermogravimetry (DTG)

The thermal stability of partially oxidized cellulose was studied using Perkin Elmer STA -6000 (Simultaneous Thermal Analyzer) instrument. The samples were run at a heating rate of 10°C/min in the range 30-850°C, under nitrogen atmosphere.

4.3.4. Scanning electron microscopy (SEM)

The scanning electron micrograph (SEM) 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.

4.3.5. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) studies of 6-carboxycellulose nanoparticles were carried out by using FEI –Technai G^2-20 instrument. A 10µL aliquot sample of 1mg of 6CC in 10 ml distilled water was mounted on freshly glow discharged carbon coated Cu grids (200 mesh, ICON Analytical, India). TEM was observed without staining the samples.

4.3.6. 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 10µl sample drop of 0.01% concentration was cast on silicon wafer.

4.4. Results and discussion

4.4.1. Discussion on morphology of 6CC and 6CC-NP's

Cellulosic nanoparticles have long defied production on a large scale, which has affected their large scale industrial use. While 6CC celluloses have successfully proven their utilization in medical products such as wound gauzes and haemostatic material, most of the products have been based on the use of regenerated cellulose, i.e., cellulose II. The great disadvantage of cellulose II is that it requires severe chemicals and energy inputs for conversion from cellulose I (Sasaki, Adschiri & Arai, 2003**;** Cheng, et al., 2011). The morphology of 6CC is presented in Figure 4.1. The Figure 4.1 shows the SEM images of 6CC (1.7-21.5 %) carboxyl content. As the oxidation proceeds at C6 position, at 40^{o}C the fiber integrity undergoes continuous attrition with time, when finally at 24h and beyond, nanoparticles are seen adhering to each other (Figure 4.1

F,G). For the reaction, at 25^{o}C, nanoparticles begin to form after 48h (Figure 4.1 H). The SEM image (Figure 4.2 A) shows the quasi-spherical shape of nanoparticles and TEM images (4.2 B & C) confirms their shape and size (25-35 nm) of the nanoparticles.

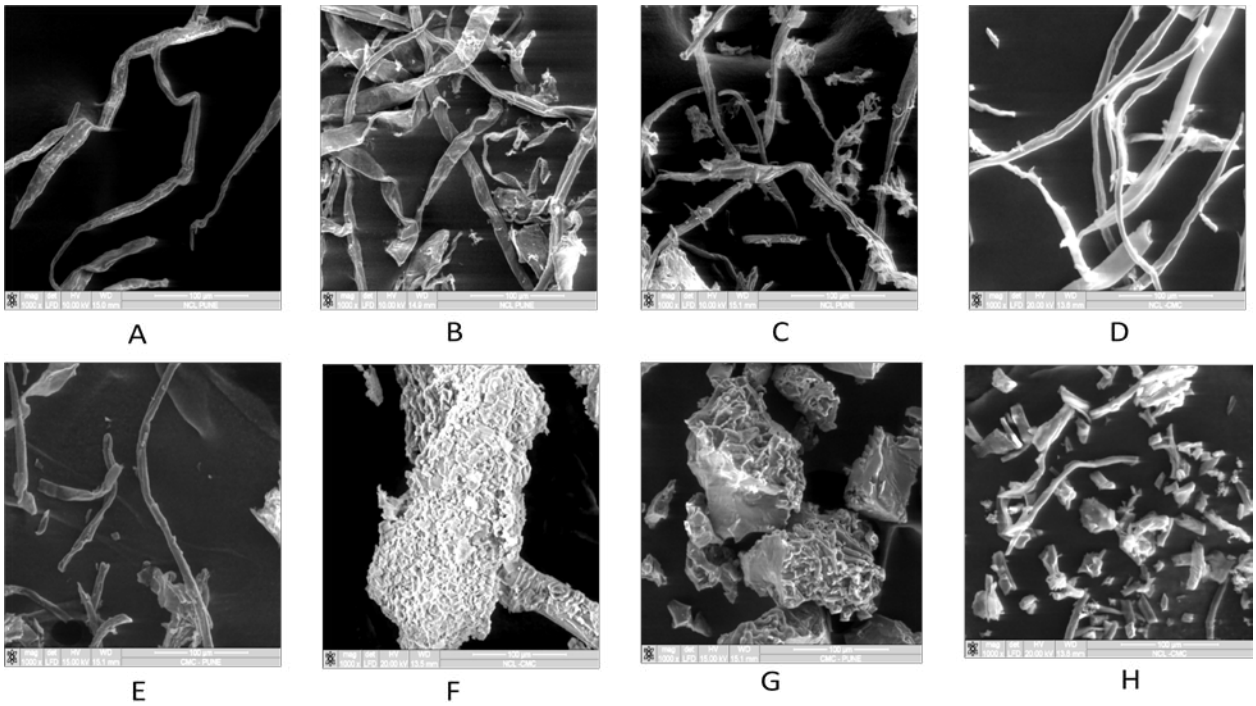

Figure 4.1: SEM images(1000X) of 6CC with varying carboxyl content (reaction performed at different time period at 40°C (A-G) and at 25^{o}C (H); (A) cellulose (B) 1h (6.2%) (C) 3h (13.2%) (D) 6h (14.0%) (E) 12h (14.3%) (F) 24h (16.0%) (G) 48h (17.0%) (H) 48h (21.5%)

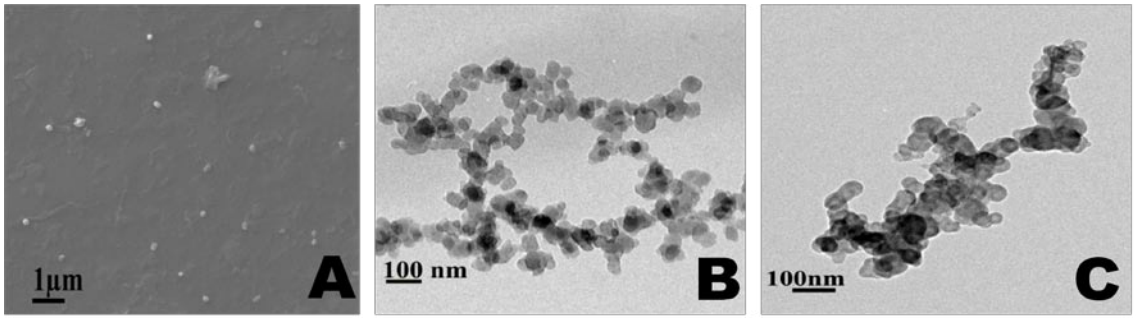

Figure 4.2: (A) SEM of 6CC (nanoparticles) prepared at 50°C (13.2%) (B) TEM of 6CC (nanoparticles) prepared at 50° C (13.2 %) (C) TEM of 6CC (nanoparticles) prepared at 40°C/48h (21.5 %).

4.4.2. Discussion on morphology of TCC and TCC-NP's

Figure 4.3 shows the SEM pictures of TCC (5:15, 15:15, 25:5) and 6C2,3DAC (15:9). One can see fiber breakage in 5:15; in the 15:15 fiber breakage is accompanied by clustering of fibers, and the 25:15 samples shows further fragmentation and clustering. However, sonication of these dilute solution did not yield clear indications of nanoparticle formation. TEM pictures of TCC (15:15) show the presence of some nanofibers, but further modification procedure is needed if TCC nanoparticles are to be obtained in entirety. The SEM of 6C2,3DAC (15:9) shows the molecules are present in sheet like morphology (Figure 4.3 D)

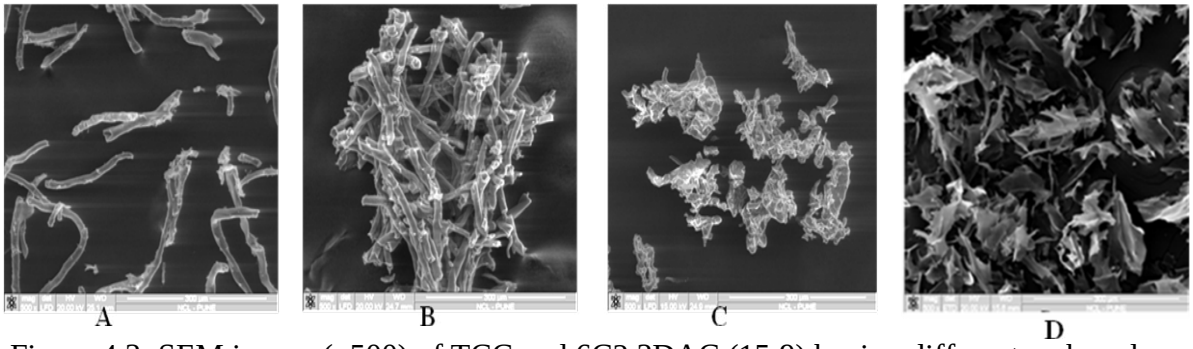

Figure 4.3: SEM images(x500) of TCC and 6C2,3DAC (15:9) having different carboxyl content (A) TCC (5:15) (B) TCC (15:15) (C) TCC (25:15) (D) 6C23DAC (15:9)

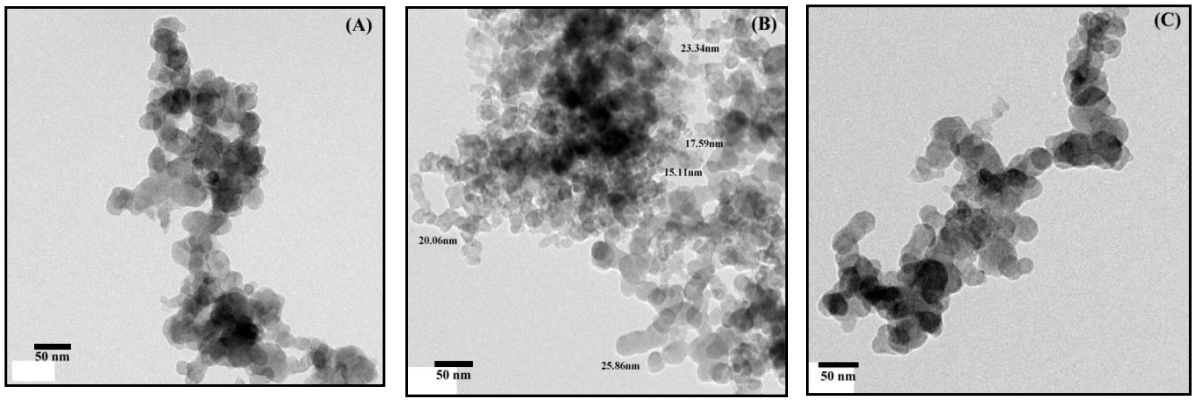

Figure 4.4: TEM images of TCC having different carboxyl content (A) TCC (5:15) (B) TCC (15:15) (C) TCC (25:15)

The TEM images of TCC-NP's with different carboxyl content obtained as II crop, are shown in Figure 4.4 (A, B &C). The TEM images shows no significant change in shape and size of nanoparticles on increasing the carboxyl content; Figure 4.4 (A) (B) & (C) represents nanoparticles containing 5:15, 15: 15, 25: 15 where first number denotes carboxyl at C2, C3 and second number to C6 carboxyl content. All the TCC-naoparticles are in the range of 25-50nm having quasi-spherical shape. Figure 4.5 shows the AFM image of TCC-naoparticles, which clearly represents the spherical shape of nanoparticles.

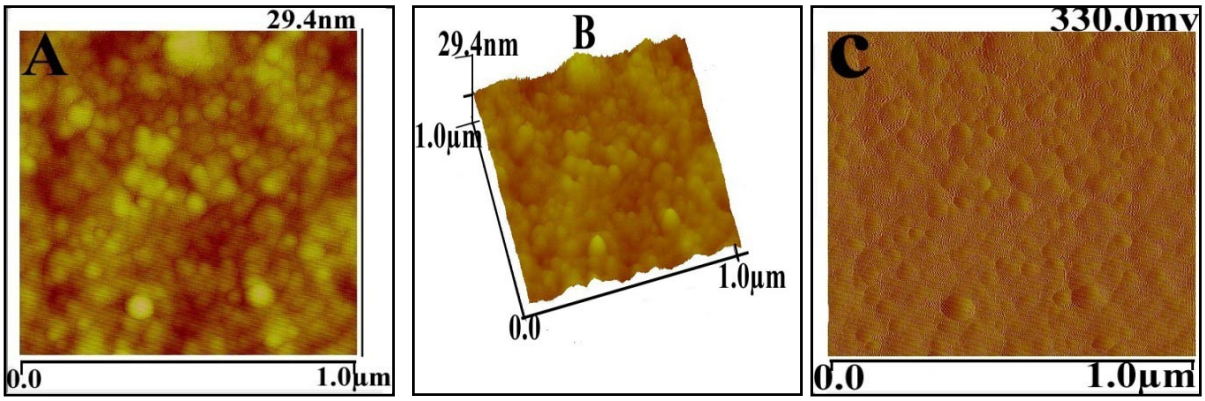

Figure 4.5: Non Contact mode AFM images of TCC –NP (A) corresponding height image (B) corresponding 3D image rotated at 45° (c) corresponding amplitude image (D) TEM image of TCC (II crop;NP)

4.4.3. Discussion on TGA (Thermogravimetry)

4.4.3.1. TGA of 6CC and 6CC-NP's

TGA of the samples (Table 4.1 and Figure 4.6) shows that even with very low degrees of carboxyl functionalization, 6CC exhibited significantly reduced thermal stability as compared to the original cellulose, and the onset of degradation temperature was a function of the degree of oxidation (i.e., carboxyl content). For the original cellulose, the maximum degradation occurs in the range of 320-377°C and the TGA curve showed a single step degradation curve and

maximum degradation in a narrow range (50% weight loss at 353^{o}C). Even for samples with a carboxyl content as low as 1.7%, the onset of degradation came down to 213^{o}C, and thereafter reduced incrementally for more extensive oxidations, finally reaching to 154^{o}C for 21.5% carboxyl content. When the reaction temperature was 25^{o}C, for the carboxyl content 22% (sample no. 8, Table 4.1) the T_{onset} was 178 ^{o}C, while at a reaction temperature of 40^{o}C for the same extent of carboxyl content, the T_{onset} was 154 ^{o}C (sample 14, Table 4.1). This was due to greater degradation of the molecular weight and reduced crystallinity of the product at the higher reaction temperature, keeping the reaction time constant (48 h). When the reaction temperature was further increased to 50 ^{o}C, the T_{onset} was 172^{o}C, even though the reaction was stopped after 12h (sample 15, Table 4.1). The T_{50} (temperature for 50% wt. loss) also decreased in a similar fashion with increasing carboxyl content, reaching 244^{o}C for the 21.5% carboxyl content sample. At 70^{o}C reaction temperature where the 6-carboxycellulose undergoes more severe degradation reactions, the T_{onset} was further reduced to 146^{o}C. Comparison with literature results of thermal analysis of partially C6-carboxylated cellulose nanofibrils also confirmed that the thermal decomposition is severely affected by the glucuronic acid moiety due to decarbonation during the heating process. The onset of degradation for the cellulose nanofibril films was about 200^{o}C, comparable to the samples nos. 4-8 (upto 19% carboxyl content) in Table 4.1 (Fukuzumi et al., 2010; Fukuzumi et al., 2009). Further comparing the thermal stability of nanoparticles of 6CC with the macro-sized 6-carboxycelluloses of the same carboxyl content, (samples 10 and 15 of Table 4.1 with 13.2% carboxyl content), we see that the nanoparticles had a T_{onset} of 172 ^{o}C while the non-nano particle had a T_{onset} of 182 ^{o}C. However, this decrease in T_{onset} for the nanoparticle is most likely due only to the higher reaction temperature of sample 15 causing greater degradation of the polymer molecule. Thus, from the TGA data it appears that nano and

non-nano particles have similar thermal stabilities. It should be noted from the TGA curves that while pure cellulose showed a single step degradation curve, their carboxy derivatives showed multi-step degradation curves. 6-Carboxycellulose can be considered to be a copolymer of glucose (G) and glucuronic acid (GA), with the latter decomposing at much lower temperature. As the carboxy content increases, the GA content of the 6-carboxycellulose increases, causing increased degradation at higher temperatures. These results indicated that presence of glucuronic acid was the major cause of thermal destabilization of cellulose, while molecular weight, and shape and size of the nanoparticle did not significantly affect thermal stability. This effect was seen more clearly in the differential thermogravimetry (DTG) curves, explained in the following paragraph.

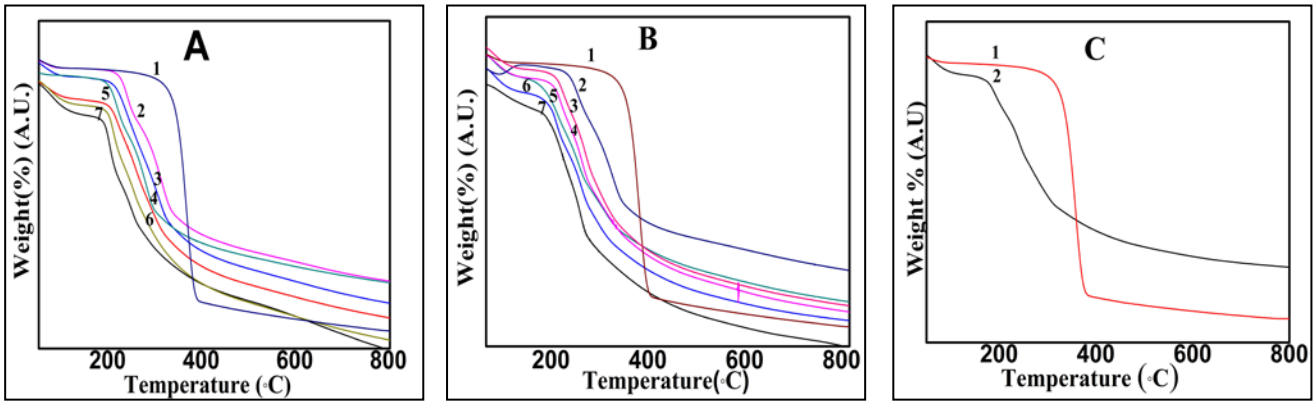

Figure 4.6: TGA of cellulose and 6CC of various COOH contents (%)

(A) prepared at 25°C (1) cellulose (2) 1.7% (3) 3.0% (4) 8.6% (5) 14.1 % (6) 19.7 % (7) 22.0 %.(B) prepared at 40°C (1) cellulose (2) 6.17 % (3) 13.2 % (4) 14.3 % (5) 14.0 % (6) 16.0 % (7) 17.0 % (C) TGA of (1) cellulose (2) 6CC-nanoparticles prepared at 50°C (13.2 % COOH content).

Table 4.1: TGA data of 6CC prepared at 25^{o}C, 40 ^{o}C, 50 ^{o}C and 70 ^{o}C.

Sr.No.	Sample Name (time/temp)	Oxidation (%)	T_{onset} (^{o}C)	$T_{final\ deg.}$ (^{o}C)	T_{50} (^{o}C)	Residue (Wt. %)
1	Cellulose†	0	320	377	353	5
2	Amorphous†† Cellulose	0	222	ND	326	26
3	**25°C** 1 h	1.7	213	ND	302	12
4	3h	3.0	206	ND	293	15
5	6h	8.6	204	ND	296	13
6	12h	14.1	188	ND	282	12
7	24h	19.7	184	ND	266	9
8	48h	22.0	178	ND	247	6
9	**40°C** 1h	6.17	213	ND	300	9
10	3h	13.2	182	ND	288	11
11	6h	14.3	177	ND	288	11
12	12h	14.0	169	ND	262	15
13	24h	16.0	166	ND	252	16
14	48h	17.0 I crop	164	ND	251	16
	48h	21.5 II crop; NP	154	ND	244	20
15	**50°C** 12h	13.2; NP	172	ND	282	17
16	**70°C** 8h	13.9; NP	146	ND	293	11

T_{onset} - onset degradation temperature; $T_{final\ deg.}$ - final degradation temperature; T_{50} – temperature at which 50% weight loss occurred; Residue (Wt.%) – weight remained at 850^{o}C.; ND- Not determined.; NP- Nano-particle† Sugarcane derived cellulose, †† Amorphous cellulose

4.4.3.2. TGA of multi-oxidized celluloses (DAC, 6C23DAC, TCC) and TCC-NP's

The thermal studies of all the oxidized cellulose derivatives are presented in the Table 4.2. The onset of degradation temperature (T_{onset}) for cellulose was 320ºC (sample no.1, Table 4.1; Figure 4.7 (A)). Slight decrease in thermal stability (349-357ºC) was observed for 2,3-dialdehyde cellulose (sample no.2-4, Table 4.2). On converting 2,3-DAC to 2,3-DCC, there is further decrease in stability (254ºC), due to decarboxylation of the carboxyl group. However, for 6CC's having carboxyl functionality at the C6 position, even 1.7% carboxyl content brings down the

T_{onset} to 213ºC, going down further to 184ºC for 19.7% carboxyl content. For the 2,3,6-tricarboxycellulose (TCC), the T_{onset} was 192ºC, due to the effect of the C6 carboxyl group in conjunction with the C2 and C3 carboxyls. In the firm of nanoparticles, it was seen that both 6CC (T_{onset} 172ºC) and TCC (T_{onset} 177-190ºC) were slightly more unstable than their macro-sized analogs. This could be due to lower molecular weight (Sharma & Varma, 2013), and consequently large number of reducing end groups which are more thermally unstable. The TCCs. Gave larger residues at 850ºC. This could be due to increased cross-linking reactions of TCC. 6C2,3DAC (sample 8, Table 4.2) shows similar thermal stability as 6CC (sample 7, Table 4.1) since it has a carboxyl group at C6.

Table 4.2: TGA data for multi-oxidized celluloses.

Sr.No.	Oxidation (%) **C2,3**	**C6**	Sample name	T onset (^oC)	T final deg. (^oC)	T_{50} (^oC)	Residue (Wt%)at 850(^oC)
1	0	0	Cellulose	320	377	360	1.7
2	5	0	DAC	308	357	339	5.6
3	15	0	DAC	306	351	333	7.5
4	25	0	DAC	303	349	333	14.6
5	5	15	TCC	192	ND	288	21.5
	5	17	TCC-NP	177	ND	242	20.8
6	15	15	TCC	191	ND	271	21.6
	15	18	TCC-NP	190	ND	256	21.2
7	25	15	TCC	192	ND	269	18.1
	25	18	TCC-NP	194	ND	265	25.4
8	15	9	6C23DAC	184	ND	294	22.9

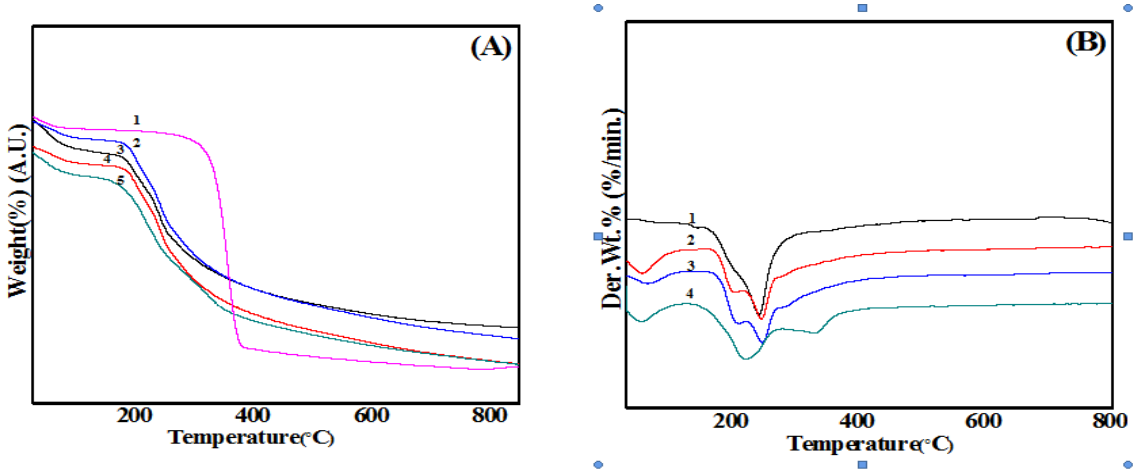

Figure 4.7: (A) TGA curves of (1) cellulose (2) TCC derivatives (25:15) (3) TCC (15:15) (4) TCC (5:15) (5) 6C2,3DAC
(B) DTG of (1) TCC (25:15) (2) TCC(15:15) (3) TCC(5:15) (4) 6C2,3DAC

4.4.4. Discussion on DTG (Differntial Thermogravimetry)

4.4.4.1. DTG of 6CC and 6CC-NP's

The DTG curve measures the maximum weight loss with respect to temperature. The DTG data for cellulose I, cellulose I + glucuronic acid, (80:20 w/w, approximately corresponding to the highest extent of carboxyl content in the 6CC samples), amorphous cellulose, and various 6CC samples is presented in Table 4.4 and Figures 4.8. A single sharp DTG curve is observed for cellulose at 355 ^{o}C, while the Cellulose+Glucuronic acid (GA) mixed sample showed a sharp peak at 351^{o}C corresponding to cellulose and another sharp peak at 176 ^{o}C corresponding to the GA added to the cellulose. Looking at the data for the 6-carboxycellulose samples, it is clear that they fall in three categories: Below 14% carboxyl content two well resolved peaks are observed, in which the major peak at higher temperature (291-302^{o}C) corresponds to the crystalline portion of cellulose I and the minor peak at lower temperature (around 228^{o}C) corresponded to the amorphous peak which we speculate included the glucuronic moeity. At around 14% carboxyl

content (sample nos.7, 11, 12, 13, 16, 17 in Table 4.4) there was a clear transition to three peaks, the lowest temperature peak at 211^{o}C corresponds to the GA, the middle peak at 246^{o}C corresponds to the amorphous cellulose peak, and the highest temperature peak (286^{o}C) was the residual crystalline cellulose peak. The crystalline peak had shifted to a lower value due to the carboxyl functionalization and lower DP. Now, as we go to 16-21.5% carboxyl content (samples 7, 8, 15, 16, in Table 4.4), a reverting back to two peaks was observed, as in samples with <8% carboxyl. Here the peak corresponding to the amorphous peak (merged with the glucuronic moiety) was the major peak (at lower temperature, 183^{o}C,) while the crystalline cellulose was the minor peak (at the higher temperature 233^{o}C). As the carboxyl content increased, all peaks shifted to lower values. These interpretations and transitions were more clearly seen in Figure 4.9, where the DTG curves of selected samples of 6CC are overlaid with the control samples of cellulose, amorphous cellulose, and cellulose+GA. It was observed that with the progress of oxidation, there were gradual changes in the morphological structure. Such a study of morphological changes occurring in a cellulosic polymer molecule with progressive oxidation, showing a continuous shift in the crystalline peak to lower values with degree of oxidation, and the merging of the glucuronic peak under the amorphous peak below and above a critical point (`14% oxidation) has not been reported.

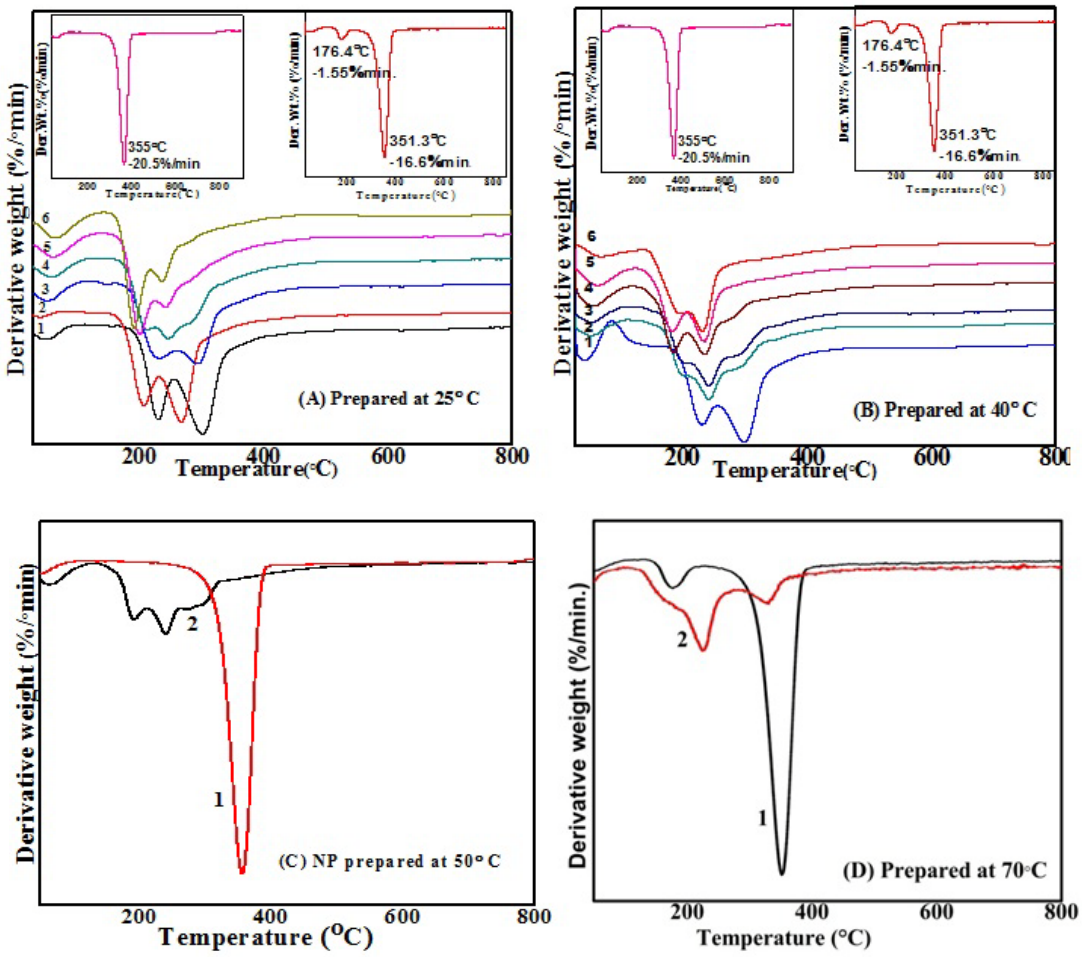

Figure 4.8: DTG of 6CC of various COOH contents (%)
(A) prepared at 25°C (1) 1.7% (2) 3.0% (3) 8.6% (4) 14.1 % (5) 19.7 % (6) 22.0%. (B) prepared at 40° (1) 6.17 % (2) 13.2% (3) 14.3% (4) 14.0 % (5) 16.0% (6) 17.0%, inset left : cellulose, inset right : physical mixture 20:80 w/w % glucuronic acid: cellulose I (in both graphs (A) and (B)), (C) DTG of (1) cellulose (2) 6CC-NP prepared at 50°C (13.2 %)
(D) DTG of (1) cellulose (2) 6CC-nanoparticles prepared at 70°C (13.9 %)

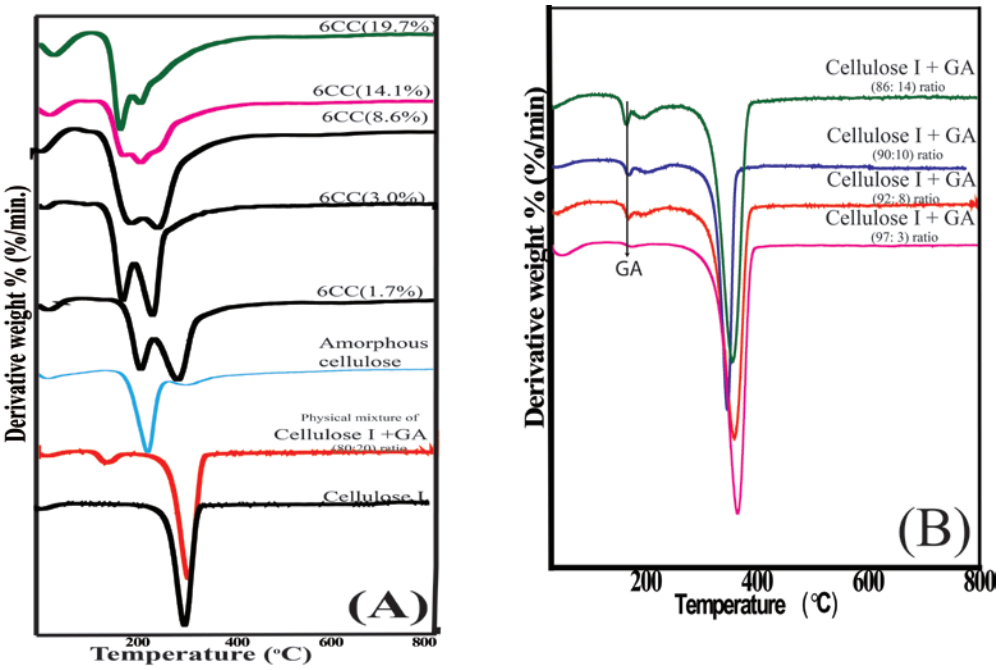

Figure 4.9: (A) Overlay DTG curves of cellulose, cellulose I +GA (physical mixture 20:80 ratio), amorphous cellulose, 6CC (1.7%), 6CC (3.0%), 6CC (8.6 %), 6CC (14.1%) and 6CC (19.7%).(B) Overlay DTG curves of cellulose I + GA -physical mixture in various ratios: (97:3); (92:8); (90:10);(86:14).

4.4.4.2. DTG multi-oxidized celluloses (DAC, 6C23DAC, TCC) andTCCNP's

Pure cellulose and DAC have similar TGA and DTG curves. The DTG of varying percent TCC and 6C23DAC is shown in Figure 4.7 (B) and Table 4.3. The DTG shows only one sharp major peak at 363ºC for cellulose and 333-339ºC for DAC. However, the DTG curve of 6CC has two peaks (<14% carboxyl, 235 ºC and 288 ºC) and (>14% carboxyl, 198ºC and 239ºC) while 6CC (~14%) has three peaks (211ºC, 246ºC, 296ºC). Similarly, DTG of TCC generally has three peaks for fibrils and two peaks for TCC-NP. This is due to the appearance of separate peak of amorphous region in 6CC (~ 14%) (Sharma &Varma, 2014), and analogously even for the TCC. The DTG curve for 6-Carboxy-2, 3-dialdehyde cellulose (15:9%), shows a two step degradation.

6C23DAC shows major peak at 330^{o}C, which is comparable to the peak in 5% DAC and minor peak at 221^{o}C, which is comparable to one of the minor peak of 6CC (14%), that is on 211^{o}C. Therefore these data distinctly shows the presence of both carboxyl and aldehydic carbonyl in the same oxidized cellulose.

Table 4.3**:** DTG of multi-oxidized celluloses.

Sr.No.	Oxidation (%) C2,3	C6	Sample Name (time/temp)	T $_{max.}$ (°C)	Wt. (%) loss	Nature of DTG graph
1	0	0	Cellulose (Single stage)	363	56	Only one major sharp peak at 363°C
2	5	0	DAC (Single stage)	339	51	Only one major sharp peak at 339°C
3	15	0	DAC (Single stage)	334	50	Only one major sharp peak at 334°C
4	25	0	DAC (One stage)	333	48	Only one major sharp peak at 333°C.
5 (i)	5	15	TCC (Three stage)	206 244 282	16 33 49	Three distinct peaks at 206°C,244 °C,282°C, tending to merge.
5 (ii)	5	17	TCC-NP (Two stage)	236 190	45 24	Two distinct peaks at 236 and 190°C
6 (i)	15	15	TCC (Three stage)	202 242 280	22 39 52	Three distinct peaks at 202°C, 242 °C, 280°C, tending to merge.
6 (ii)	15	18	TCC-NP (Two stage)	240 206	38 20	Two distinct peaks at 240 and 206°C
7 (i)	25	15	TCC (Three stage)	208 247 293	57 39 19	Major sharp peak at 239°C and minor at 195°C, distinctly resolved.
7 (ii)	25	18	TCC-NP (Two stage)	240 209	38 20	Two distinct peaks at 240 and 209°C
8	15	9	6C23DAC (Two stage)	222 330	43 58	Two separate peaks, major at 222^{o}C and minor at 331^{o}C.

Table 4.4: DTG data of cellulose I, cellulose II, amorphous cellulose, glucuronic acid(GA) + cellulose I (20:80, w/w%) and 6CC prepared at 25°C, 40 °C, 50 °C, and 70°C.

Sr.No	Oxidation (%)	Sample Name (time/temp)	$T_{max.}$ (°C)	Wt.% loss	Nature of DTG graph
1	0	Cellulose I†	355	54	Only one major sharp peak at 355°C.
2	0	Amorphous†† cellulose	247 328	30 51	Minor peak at 328°C (crystalline region), major (sharp) peak (amorphous region) at 247°C.
3	0	GA+cellulose I) (20:80%Mix) (Two stage)	176 351	7 56	Minor peak at 176°C, major (sharp) peak at 351°C.
4	1.7%	1h-25°C (Two stage)	228 302	15 50	Two well resolved sharp peaks, minor at 228°C, major peak at 302°C.
5	3.0%	3h-25°C (Two stage)	227 291	16 49	Two well resolved sharp peaks minor peak at 227°C, major at 291°C.
6	8.6%	6h-25°C (Two stage)	235 288	23 46	Two peaks, at 235°C and 288°C but not completely resolved, tending to merge.
7	14.1%	12h-25°C (Three stage)	211 246 286	20 34 52	Three peaks at 211°C, 246 °C, 286°C, tending to merge.
8	19.7%	24h-25°C (Two stage)	198 239	21 40	Major sharp peak at 198°C and minor at 239°C, distinctly resolved.
9	22.0%	48h-25°C (Two stage)	189 236	25	Major sharp peak at 189°C and minor at 236°C, distinctly resolved.
10	6.2%	1h-40°C (Two stage)	231 299	15 49	Two well resolved sharp peaks, minor at 231°C, major peak at 299°C.
11	13.2%	3h-40°C (Three stage)	194 242 270-322(b)	14 32 44-59	Three peaks with sharp middle one at 242°C with two sided peak left one at 194 and right broad peak at (270-321.8°C).
12	14.0 %	6h-40°C (Three stage)	197 242 270-327(b)	15 32 44-60	Three peaks with sharp middle one at 242°C with two sided peak left one at 197and right broad peak at (270-327 °C).
13	14.3%	12h-40°C (Three stage)	186 235 263-308(b)	11 29 42-56	Three peaks with sharp middle one at 235°C with two sided peak left one at 186and right broad peak at (263-3308 °C).
14	16.0%	24h-40°C (Two stage)	183 233	22 41	Two well resolved peaks, minor peak at 183C, major peak at 233°C.
15	17.0%	48h-40°C(I crop) (Two stage)	198 232	24 41	Minor peak at 198°C, major (sharp) peak at 232°C.
	21.5%	48h-40°C (II crop) (Two stage)	187 233	26 44	Minor peak at 187°C, major (sharp) peak at 233°C.
16	13.2%	12h-50°C (Three stage)	191 241 271-313(b)	17.0 35.0 45.0	Three peaks with sharp middle one at 241°C with two sided peak left one at 191and right broad peak at (271-313 °C).
17	13.9%	8h-70°C (Three stage)	129-199(b) 226 329	15 33 57	Three peaks with sharp middle one at 226°C with two sided peak left one broad at 129-199 and right broad peak at 329 °C.

4.4.5. Discussion on the Solubility

4.4.5.1. Solubility of oxidized celluloses in alkali

Another important property of these series of compounds is their solubililty. The solubility of all mono- and sequentially oxidized cellulose derivatives were studied in different concentrations of alkali (NaOH) from 0.2% to 10% (Table 4.5). In case of mono functionalized oxidized cellulose (6CC), the 22% oxidized sample was easily soluble even at 0.2% alkali solution. Recent literature shows the solubility of 21.29 % oxidized cellulose in 0.2 M NaOH (0.8 %) for 1% wt./vol. concentration at room temperature (Wu et al., 2012). The lower concentration of alkali needed in our case could be due to the lower molecular weight of our non-wood cellulose. Expectedly, as the oxidation percent decreases, the solubility of 6CC was found to continuously decrease in alkali solution. DCC (15%) was soluble in 0.4 % alkali solution while DAC (25 %) was soluble in 10 % alkali solution and 5% DAC was completely insoluble in 10% alkali solution. The multi-functionalized oxidized cellulose TCC and 6C2,3DAC were easily soluble in 0.2% alkali solution. Thus, alkaline solubility is a good indicator of the extent of oxidation of cellulose.

Table 4.5: Solubility of various carboxyl content oxidized celluloses in aqueous alkali solutions

Sr.No.	Sample Name C6:C2,C3%	NaOH(%)				
		10	5	2	0.4	0.2
1	(22:0)6CC	++	++	++	++	++
2	(14:0) 6CC	++	++	++	++	+
3	(8:0) 6CC	++	++	++	+	--
4	(3:0) 6CC	±	--	--	--	--
5	(0: 5) DCC	++	++	++	++	+
6	(0:25)DAC	++	±	--	--	--
7	(0:15)DAC	±	--	--	--	--
8	(15:5)TCC	++	++	++	++	+
9	(15:15)TCC	++	++	++	++	++
10	(15:25)TCC	++	++	++	++	++
11	(15:9) 6C23DAC	++	++	++	++	++

.

Where (++) complete soluble, (±) partially soluble, (--) complete insoluble, (+) soluble on heating.

4.4.5.2. Solubility of oxidized celluloses in organic solvents

Table 4.6 shows the solubility of all oxidized samples was checked in 1% w/v in several organic solvents. The higher oxidized 6CC (22%) was partially soluble in DMSO while all the TCC samples were completely soluble in DMSO. Further, TCC was also shows soluble in DMAc on heating. Similarly, 6C23DAC was soluble in acetonitrile on heating. TCC also swelled in several solvents like dioxane, acetone, ethanol and methanol. The solubility or swelling of oxidized celluloses in several organic solvents can lead to their facile transformation by a variety of organic chemical reactions and result in several new products. This can lead to the use of functionalized celluloses as platform molecules to produce series of new molecules. The fact that these oxidized celluloses can also be produced in the form of nanoparticles increases their applications many fold in high-tech areas. Applications of cellulose nanocrystals are enumerated several recent papers (Habibi et al., 2010; Johnson et al., 2011; Isogai et al., 2012).

Table 4.6: Solubility of various carboxyl content oxidized celluloses in organic solvents.

Sr.No.	Solvents (1 % Solution)	22% 6CC	15:15 TCC	25:15 TCC	15:5 TCC	15:9 6C23DAC
1	CH_3CN	--	--	--	--	+
2	DMAc	--	+	+	+	--
3	DMSO	±	++	++	++	--
4	Dioxane	--	(S)	(S)	(S)	--
5	Acetone	--	(S)	(S)	(S)	--
6	Methanol	--	(S)	(S)	(S)	--
7	Ethanol	--	(S)	(S)	(S)	--
8	$CHCl_3$	--	--	--	--	--
9	DCM	--	--	--	--	--
10	THF	--	--	--	--	--
11	Toluene	--	--	--	--	--
12	DMF	--	--	--	--	--
13	Pyridine	--	--	--	--	--
14	1,2-DCE	--	--	--	--	--

4.5. Conclusions

TGA/DTG studies of the new series of series of 6CC molecules were used to assess the thermal stability of low molecular weight spherical nanoparticles of 6CC's of different carboxyl contents, which have been synthesized and characterized for the first time. It was found that the thermal behaviour of the nanoparticles of 6CC were similar to that of the non-nano sized analogs, implying that the particle size had no effect on the thermal stability. This is important for applications in biocomposites, where curing at higher temperatures maybe required. These studies also threw light on the morphological changes occurring with the changes in the carboxyl content (continuous shift in the crystalline peak to lower values with degree of oxidation, and the merging of the glucuronic peak under the amorphous peak below and above a critical point of 14% oxidation). The solubility data shows that 6-carboxycelluloses (22% carboxyl) is easily soluble in low concentration of (0.2%) alkali. This sample also shows partial solubility in DMSO, and swells in some solvents like pyridine and DMF. These factors improve the versatility of the 6-carboxycelluloses and their nanoparticles for enabling further chemical modification through the carboxyl groups.

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Chapter 5

Structure-property relationship of 6-carboxycellulose and their spherical nanoparticles by using ^{13}C CPMAS-NMR and WAXRD

5.1. Introduction

Cellulose is the most sustainable and ubiquitous biopolymer molecule available on planet earth. It was the earliest polymer characterized, nearly 175 years ago, and since then has engaged generations of chemists, physicists and technologists in unraveling its multi-faceted structural features, conformational analysis, inter-conversion of polymorphs, reactions, as well as applications. Further, celluloses obtained from different sources (bacterial, fungal, cotton, hardwood, softwood, agricultural residues, etc.) have greatly different fibril sizes, aspect ratios, molecular weights, and purities, all of which affect their chemical and physical properties and hence their applications (Klemm et al., 2011; Roman & Winter, 2004). In nature, cellulose occurs as cellulose I, with two distinct crystalline forms Iα and Iβ, in which the two cellulose chains are arranged in parallel via hydrogen bonding (Atalla & Vanderhart, 1984; Kroon-Batenburg, Bouma & Kroon, 1996). Another polymorph, cellulose II, is generally obtained from cellulose I by mercerization and regeneration processes (Langan, Nishiyama & Chanzy, 1999, 2001). In addition to this, there are other methods to convert cellulose I to cellulose II. For example, treatment of cellulose I with super critical water, ball milling and dissolution and regeneration from ionic solvents (Sasaki, Adschiri & Arai, 2003; Zhao et al., 2006; Cheng et al., 2012). Cellulose II has antiparallel arrangement of cellulose chains through hydrogen bonding, and cannot revert back to cellulose I. Strong acids like H_2SO_4, under specific conditions, hydrolyzes cellulose in such a way that non-crystalline (amorphous) parts of the semi-crystalline cellulose molecule are removed, and a highly crystalline product (known as microcrystalline cellulose) is produced (Laka et al., 2000; Wang & Ding, 2004; Bondenson, Kvien & Oksman, 2006). Phosphoric acid treatment, on the other hand, leads to swelling of cellulose, and ultimately dissolution (Percival Zhang et al., 2006), and an amorphous cellulose results. Swelling

and dissolution depend on the concentration of acid, time and temperature (Sharrock, 1988; Wood & Bhat, 1988). A report shows that cellulose swells rapidly in 71-80% phosphoric acid, and at 81-85% concentration and temperature of 120^{o}C, dissolution occurs. Some studies indicate that the transformation of cellulose I to amorphous cellulose using phosphoric acid treatment occurs via a cellulose II transition state (Zhang et al., 2009; Percival Zhang et al., 2006). Sulphuric acid treatment of cellulose I under specific conditions can also lead to nano-fibril structures with high aspect ratios (Ranby, 1949; Ranby, 1951; Marchessault, Morehead & Walter, 1959). In 2007, using a novel method consisting of pretreatment with alkali solution followed by 8 hours of sonication under highly acidic conditions, spherical shaped nanoparticles of cellulose were synthesized (Zhang, Elder & Ragauskas, 2007). This appears to be the first instance of the cellulose molecule, a fibrous polymer consisting of bundles of fibrils entwined together, getting converted to a spherical shape. Since then, there have been some more examples of spherical shaped functionalized celluloses, such as aminocelluloses, cellulose acetate and 6-carboxycellulose (Nikolajaski, Wotschadlo, Clement & Heinze, 2012; Kulterer et al., 2012; Sharma & Varma, 2013). Most researches into applications of cellulose deal with either cellulose I or cellulose II. Amongst the derivatives of cellulose, 6-carboxycellulose has proved to be of immense interest for its biomedical properties. It was first investigated in 1883, and by the 1940's a variety of preparative methods had been investigated (Witz, 1883; Yackel & Kenyon, 1942; Maurer & Reiff, 1943, Unruh & Kenyon, 1942). Its outstanding antimicrobial properties were soon utilized in a variety of products like wound gauzes and haemostatic materials. Due to these important industrial applications, a review of the published literature shows several patents but relatively few papers on these materials (Nooy, Pagliaro, Bekkum & Besemer, 1997; Nachtkamp et al.; 2012, Jewell, Komen et al., 2001, Kenyon & Yackel, 1948). It

should be noted that most studies on oxidation of cellulose for biomedical applications are based on oxidation of cellulose II, due to its higher absorption capacity for physiological fluids. However, conversion of native cellulose I to cellulose II involves an additional step of treatment with chemicals (strong aqueous alkali, ionic liquids, cupriethylenediamine hydroxide, formation of cellulose xanthate, etc.), makes it a less environment-friendly material and also more expensive. It was our hypothesis that cellulose I may partially or fully convert to cellulose II and amorphous cellulose during oxidation using phosphoric acid as a reaction medium, since phosphoric acid is known to swell cellulose. This prompted us to explore oxidation of cellulose I using HNO_3-H_3PO_4-$NaNO_2$ oxidation system, and study the reaction products for their polymorph constitution by WAXRD and ^{13}C CP-MAS-NMR techniques. During our recently published studies on oxidation of cellulose I to produce 6CC's, we had succeeded in manipulating the reaction conditions so as to obtain 1.7% - 22% carboxy content celluloses with controlled shapes and sizes (spherical nanoparticles having particle sizes in the narrow range of 25-35 nm and macro-sized particles having lengths of several microns) (Sharma & Varma, 2013). These molecules were thoroughly characterized by SEM, TEM, AFM, DLS, TGA, DTG, and solubility studies, and preliminary studies on new and potential applications of the products were also discussed (Chapter 4, Sharma & Varma, 2013; Sharma & Varma, 2014) . Herein we present our results on wide-angle X-ray diffraction (WAXRD) and solid state ^{13}C CP-MAS NMR to demonstrate the systematic transformation of cellulose I to cellulose II and amorphous cellulose, and the unexpected difference in the crystallinities of nano-6CC and macro-sized 6CC's having the same carboxy contents, produced during the progressive oxidation of cellulose I in HNO_3-H_3PO_4-$NaNO_2$ system at different temperatures. Many electrical and biochemical properties of polymeric biomolecules are greatly dependent on the morphology of the molecules.

For example, hydrolysis of polymers like cellulose is greatly influenced by their crystallinity (Hall et al., 2010). Adhering of molecules, particularly biopolymers like proteins to surfaces is dictated by their surface morphology and surface area; knowledge of these features can aid in designing the most optimal performance in physiological applications (Kristian, 2006). Crystalline cellulosic surfaces are characterized by strong inter- and intra-molecular hydrogen bonding and therefore are more compact and more hydrophobic as compared to amorphous cellulose. Due to the added advantage of surface charges, spherical carboxylated cellulosic nanoparticles have the potential to be far superior to native unfunctionlized cellulose nanoparticles for several applications such as pharmaceutical excipients in cosmetics, drug delivery vehicles, etc (Wei, Kumar & Banker, 1996; Bellamy & Holub, 1977; Martina et al., 2009; Zhu, Kumar & Banker, 2001). Functionalization of cellulosic nanoparticles can extend the utility of 6CC in the fields of nanosensors, biocomposites, optics, electronics etc (Klemm, Heublin, Fink & Bohn, 2005; Klemm et al., 2011). Further, detailed microstructural studies on spherical nanoparticles of carboxy-functionalized cellulosic molecules have never been reported. Our study provides insights into the structural changes occurring during the progress of C6 oxidation of cellulose to produce a series of 6-carboxycelluloses and their nanoparticles. This information will be very useful for developing new biomedical applications of 6CC spherical nanoparticles, since carboxycellulose fibrils have already proven their utility in several biomedical applications.

5. 2. Experimental Section

5.2.1. Materials

Cellulose I was extracted from sugarcane bagasse using our patented process (Varma, 2013). Analytical grade nitric acid (65% conc.), ortho-phosphoric acid (85% conc.), sodium nitrite (98.0% purity), and calcium acetate (99.0% purity) were procured from Thomas Baker, Mumbai, India. All these chemicals were used without further purification.

5.2.2. Preparation of 6-Carboxycelluloses (6CC) and their nanoparticles

6CC's of varying carboxyl contents were prepared according to a procedure developed by us (Sharma & Varma, 2013). Briefly, the procedure consisted of taking finely powdered cellulose-I and adding an appropriate quantity of acid mixture (2:1 ratio, v/v) of 65% HNO_3 and 85% H_3PO_4 over a period of 5 minutes. The acid mixture was allowed to get absorbed in the cellulose for 10-15 minutes at the desired reaction temperature. This was followed by slowly adding powdered $NaNO_2$ (1.4 w/v %). The reaction was performed at four different temperatures: 25°C, 40°C, 50^{o}C and 70^{o}C. The reaction mixture was quenched by diluting with distilled water (5 times the volume of acid mixture), allowed to settle down for 30 minutes, then decanted off. The solid part was washed with water and water–methanol mixture (2:1 v/v), then centrifuged at 2000 rpm to remove the solid. The reaction products at 25°C were obtained as single crops, none of which were nanoparticles. For the reaction at 40^{o}C (24h and 48h), after centrifugation of the liquid-solid mixture at 5000 rpm for 5 minutes, the solid part was removed (first crop) and the supernatant liquid was separately centrifuged at 12000 rpm for 15 minutes to allow a second crop to separate out, which on analysis were found to be nanoparticles of narrow size distribution (25-35 nm). The first crop was of macro-sized fibrils of several microns. For the reactions at 50^{o}C and 70^{o}C, no solid part remained, and the entire hazy colloidal solution was centrifuged as above to allow the nanoparticles to be collected.

In both these cases the entire product consisted of nanoparticles.

5.2.3. ^{13}C CPMAS-NMR Spectroscopy

Solid state ^{13}C CPMAS-NMR spectra of 6CC's, 6CC nanoparticles (NP), and unmodified sample (cellulose) were obtained at room temperature on a Bruker AV-300 NMR spectrometer operating at a frequency of 75.47 MHz. The instrument was equipped with a 4mm CP- MAS probe. All spectra were acquired with a spinning speed of 8 KHz using 1 m sec contact time and 5 sec relaxation delay. About 1000 transients were collected and processed with a line broadening function of 25 Hz prior to Fourier transformation for sensitivity enhancement. The chemical shifts were referred to the CH_2 carbon of adamantane having a value of 38.48 δ.

5.2.4. Wide-angle X-ray Diffraction (WAXRD)

WAXRD spectra and patterns were collected on a PANalytical X'pert Pro dual goniometer diffractometer. A proportional counter detector was used for low angle experiments. The samples were put on sample holders made of aluminum alloy and flattened with a piece of glass. The Cu Kα radiation was generated at 40 KV and 40 mA (λ =0.15418 ˚nm) with a Ni filter. The data collection was carried out using a flat holder in Bragg-Brentano geometry (5-60^o; 4° min^{-1}). The 2D image was converted into 1D image by the standard software available with the instrument.

The Crystallinity index (CrI) of the samples were calculated by the following equation 1(Segal, Creely, Martin & Conrad, 1959).

$$CrI = \frac{I_{200} - I_{am}}{I_{200}}$$

Where I_{200} is the intensity of main peak for (200) lattice diffraction I_{am} is the amorphous region peak intensity evaluated as minimum peak arise between main and secondary peaks of lattice plane (200) and (101).

5.3. Result and Discussion

5.3.1. WAXRD Analysis

5.3.1.1. 6-Carboxycellulose (6CC)

The dried samples of cellulose, 6CC's and 6CC-NP were analyzed by WAXRD and the profiles are shown in Figure 5.1. The X-ray diffraction patterns for the various 6CC samples clearly shows that several changes have occurred in their crystallinity and crystallite sizes. For cellulose-I, the intensity of the main crystalline peak for (200) appears at 2Θ = 22.8°, the crystalline (101) peak appears at 15.8°, and the amorphous region peak appears at 18.7° (Segal, Creely, Martin & Conrad, 1959; Thygesen et al., 2005). In the case of cellulose II the main crystalline peak appears as doublet at 2Θ = 21.8° and 20.4° and secondary crystalline peak appears at 12.1°. Similarly, the amorphous peak of cellulose II is located at 2Θ = 16.3° (Park et al., 2010; Kumar, Gupta, Lee & Gupta, 2010). The main peak for (200) lattice diffraction at 2Θ = 22.8° is indicative of the distance between hydrogen-bonded sheets in cellulose I, while the secondary peaks for (101) and (10Í) lattice diffraction are related to intermolecular hydrogen bonding. To analyze the effect of acid treatment (H_3PO_4/ HNO_3) before adding the main oxidizing agent $NaNO_2$, the WAXRD of that sample was recorded and is referred to as "Control" in Figure 5.1 and Table 5.1. The peak intensity for control at 2Θ = 21.8°, 20.4°and 12.3°, was found to be at

different positions than the original cellulose (cellulose I). This observed transformation clearly shows the partial conversion of native cellulose I to cellulose II prior to oxidation, since the broad peak at 21.8 ends at a point beyond 22.8 (Ago, Endo & Hirotsu, 2004; Chukwuemeka, Rodriguez, Okhamafe, Rogers, 2012). The shift of the main peak of cellulose I from $2\Theta = 22.8°$ indicates an expansion of the lattice $2\Theta = 21.8°$; this may be due to the swelling of cellulose fiber, caused by HNO_3/H_3PO_4 treatment. The absence of peak intensity at $2\Theta = 15.6^o$ (and simultaneous appearance of 12.1^o peak) in the control sample corresponding to (101) plane of cellulose I indicates the conversion of cellulose I to cellulose II. However, the peak intensity corresponding to $2\Theta = 21.8°$ and 20.4° are broad as compared to peak intensity corresponding to (200) lattice plane of cellulose II, which may be possibly due to partially unchanged lattice plane of cellulose I, or it may be dependent on the particular characteristic of the species of cellulose. Furthermore, there is high probability of preliminary reaction occurring on the surface of cellulose. This is because on treatment with acid (HNO_3/ H_3PO_4), cellulose swells to some extent and the swollen part gets transformed to cellulose II polymorph and the un-reacted internal part remains as cellulose I (Sebe et al., 2012; Ranby, 1952). Similarly, the possibility of C6 oxidation on cellulose II fraction is higher. This was clearly demonstrated by WAXRD studies (and also by ^{13}C CP-MAS NMR analysis; see following section on NMR). Table 5.1 and Figure 5.1 illustrate that the peak intensity related to cellulose II ($2\Theta = 12.2°$) in 6CC of 1%, 3%, 8% carboxyl group content decreases and completely disappears for 6CC of 14% carboxyl content. These results probably indicate that initially on treatment of cellulose with the "solvent system" (H_3PO_4 + HNO_3) cellulose I is partially converted to cellulose II, which undergoes reactions on the surface; high oxidation levels are achieved on the surface, which get solubilized and are not recovered at the end of the reaction (therefore yields are continuously lower for successive higher degrees of

oxidation, up to 14% oxidation) Thereafter, it is noticed that at 14% 6CC and beyond, i.e., for the 19% and 22% 6CC, the peak intensity corresponding to 2Θ (200) plane again shifts to 2Θ = ~22.3° (cellulose I) with a small peak at 2Θ = ~15.6°, which clearly point out the disappearance of cellulose II polymorph and presence of mostly cellulose I polymorph. However, the broadening of the peaks from 14% 6CC indicates formation of amorphous cellulose.

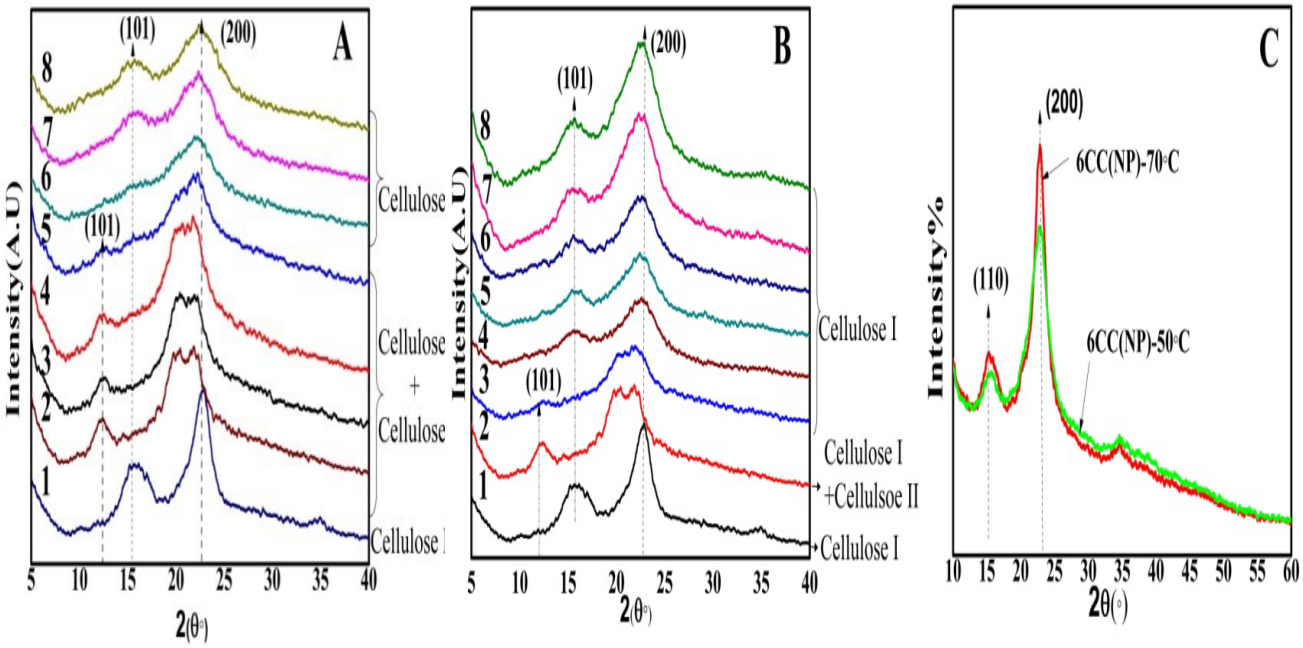

Figure 5.1: Overlapping of WAXRD spectra of cellulose and 6CC of various COOH contents (%) (A) Prepared at 25°C (1) cellulose (2) control (3) 1.7 % (4) 3.0 % (5) 8.6 % (6) 14.1 % (7) 19.7 % (8) 22.0% carboxyl content.(B) Prepared at 40°C (1) cellulose (2) controls (3) 6.17 % (4) 13.2 % (5) 14.3 % (6) 14.0 % (7) 16.0 % (8) 17.0 % carboxyl content. (C) 6CC-NP prepared at 50°C/12h and 70°C/8h.

Table 5.1: X-ray diffraction patterns and related peak intensity corresponding to different lattice plane for cellulose, control*, 6CC's, 6CC-NP and cellulose II.

Planes	2 (ɵ),^{0}										
	Cellulose I		Control * (H_3PO_4-HNO_3) Treated cellulose	6CC 1.7 %	6CC 3.0%	6CC 8.6%	6CC 14.1%	6CC 19.7%	6CC 22%	6CC-NP 13.2%	Cellulose II Maize Cob †
	Maize Cob †	SBC††									
(101)	15.0	15.8	12.3	12.3	12.25	12.53 Small	15.6 very Small	15.6	15.5	15.4	12.5
(10Ĺ)	16.6	16.9	20.4 (Two peaks)	20.4	20.45	20.27	-	-	-	16.5	20.4
(200)	22.4	22.8	21.8 (Two peaks)	22.0	22.08	22.24	22.13	22.3	22.4	22.9	21.9

† Data taken from *Cellulose*, 2012, 19,425-433. †† Sugarcane bagasse cellulose prepared by our proprietary method
* Cellulose treated with acid (HNO_3 +H_3PO_4 (1:2 ratio) prior to addition of oxidizing agent $NaNO_2$.

5.3.1.2. Nanoparticles of 6CC

3.1.2. Nanoparticles of 6CC

On conversion from macro- to nano-size, cellulose becomes more crystalline due to loss of disordered amorphous region. For 6CC-NP (Figure 5.1 C), the peak position corresponding to (101),(10Ĺ) and(200) lattice plane appeared at 15.4°, 16.5° and 22.9° respectively, which are similar to the peak position corresponding to the lattice plane of cellulose I (Fig. 5.1C). This evidence is consistent with the previously reported data that the nano-whiskers of cellulose prepared by sulfuric acid hydrolysis also show greater crystallinity as compared to the starting native cellulose-I (Cheng et al., 2012; Zhang et al., 2009).

5.3.1.3. Crystallinity Index

The Crystallinity Index (CrI) was calculated by equation 1, and the results are shown in Table 5.2. The CrI calculated for native cellulose I (extracted from sugarcane bagasse) and 'control' (by treatment of cellulose with HNO_3 + H_3PO_4) were 60% and 51% respectively. The lowest CrI

of 34% was obtained for 6CC- 48h/40^{o}C (Table 5.1). At 25^{o}C, there was not much change in the crystallinity up to 6h reaction time (8.6% -COOH) but sudden drop occurred from reaction times above 12 h. Thus, at 12h reaction time (14.1% -COOH) a CrI of 39%) and at 48h reaction time a CrI of 36% was obtained. At the higher reaction temperature of 40^{o}C, a sudden drop in crystallinity was noticed for 3h (13.2% -COOH) with CrI of 44%, which decreased steadily to 34% for a reaction time of 48h (crop I). Interestingly, the 6CC (14%-22% -COOH) showed almost similar crystallinity as amorphous cellulose (Ciolacu et al., 2011). This confirms the formation of amorphous oxidized cellulose 6CC's of high carboxyl content (14%-22% COOH). In the same reaction a crop II (40°C/48h) was obtained which consisted of nanoparticles of high crystallinity with cellulose I structure (6CC-NP, 13.2% carboxyl content, Table 5.2) . However, the crystallinity of nanoparticles increased when they were prepared at high temperature. At 50°C, 6CC-NP (13.2% COOH) showed CrI to 51 % and at 70°C, 6CC-NP (13.9% COOH) showed CrI of 63%, which is higher than the corresponding 6CC non-nanoparticle obtained at 25^{o}C and 40^{o}C (Table 5.2). The increase in crystallinity is further aided by the fact that the amorphous regions of the inner core also hydrolyze away as the reaction proceeds, thereby increasing the crystallinity over the starting cellulose I for the nanoparticles obtained at 70^{o}C (Fig. 5.1 C).

Table 5.2: Degree of crystallinity of cellulose, 6CC's and 6CC-NP of various COOH contents %

Reaction time (hr)	25° C	CrI	40° C	CrI	50° C (NP)	CrI	70°C (NP)	CrI
1h	1.7	55	6.17	55	-	-	-	-
3h	3.0	52.	13.2	44	-	-	-	-
6h	8.6	50	14.3	39	-	-	-	-
8h	-	-	-	-			13.9	63
12h	14.1	39	14.0	36	13.2	51	-	
24h (I crop) (II crop)	19.7	38	 16.0 18.0	 35 -	-	-	-	-
48h (I crop) (II crop)	22.0	36	 17.0 21.5	 34 41	-	-	-	-

5.3.1.4. X-ray diffraction patterns

The interpretation of data from measurements of WAXRD and crystallinity index were confirmed by the pattern of X-ray diffraction in Figure 5.2. Interestingly, partial conversion of cellulose-I to cellulose-II is clearly depicted in the Figure 5.2 (i, ii). The expansion of lattice plane of cellulose-I is clearly visible in the control sample (HNO_3 + H_3PO_4 treated) (Figure 5.2, ii). Subsequently, on oxidation almost the same crystallinity is maintained up to 6CC (3%) (Figure 5.2, iii**)** but as the oxidation proceeds the pattern of X-ray diffraction rings became faint (8-14%) (Figure 5.2, iv, v) and then completely dull for 6CC (22%) (Figure 5.2, vi). This clearly shows the conversion of most of the cellulose-I to amorphous cellulose. Furthermore, the pattern obtained for 6CC-NP, Figure 5.2 (vii) showed well defined rings, indicating highly crystalline molecules.

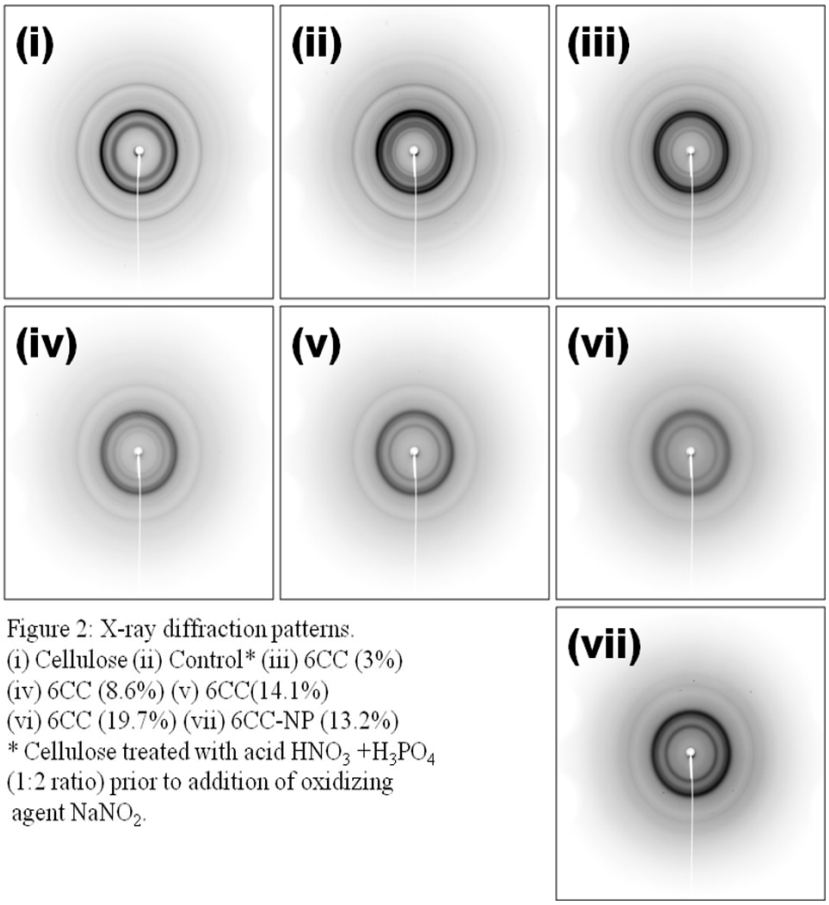

Figure 2: X-ray diffraction patterns.
(i) Cellulose (ii) Control* (iii) 6CC (3%)
(iv) 6CC (8.6%) (v) 6CC(14.1%)
(vi) 6CC (19.7%) (vii) 6CC-NP (13.2%)
* Cellulose treated with acid HNO_3 +H_3PO_4 (1:2 ratio) prior to addition of oxidizing agent $NaNO_2$.

Figure 5.2: X-ray diffraction pattern of cellulose, control, 6CC and 6CC-NP's.

The study conducted provides information on the transformation of cellulose polymorphs during oxidation of native cellulose I by $HNO_3/H_3PO_4/NaNO_2$. Due to the presence of phosphoric acid as a swelling agent, the progress of the oxidation reaction first led to cellulose II polymorph until conversion to 6CC with 14 % carboxyl, and from 14-22% carboxyl, the cellulose was largely amorphous. Thus, our results support the theory that cellulose I is the transition state during swelling and dissolution of cellulose I, finally giving rise to amorphous cellulose. Additionally, the prepared 6CC-NP (~ 14% carboxyl content) obtained as cellulose I with crystallinity greater than their macro-sized analogs.

5.3.2. Solid State NMR Studies

The solid state ^{13}C CPMAS-NMR spectra of cellulose and 6CC are presented in Table 5.3, and Figure 5.3. The region between 60 and 70 ppm is assigned to C6 of the primary alcohol group. The next cluster between 70 and 80 ppm is attributed to the C2, C3, and C5 carbons. The region between 80 and 95 ppm is associated with C4 and that between 100 and 110 ppm with the anomeric carbon C1 (Sebe et al., 2012). The signal at ~171 ppm corresponds to the carboxyl group of 6CC. The peak intensity of carboxyl increases simultaneously with increase in carboxyl content, as depicted in Figure 5.3.

The peak at about ~92 ppm, assigned to C1 anomeric carbon of reducing end of cellulose, starts increasing from 8% 6CC (peak no. 3 of Figure 5.3) and continuously increases with carboxyl content. This indicates lowering of DP with the progress of reaction. To confirm the reason of appearance of peak corresponding to ~94 ppm, the solid state ^{13}C CPMAS-NMR spectra was recorded for the 6CC (19.7%) oxidized sample (Fig. 5.4 A) and compared with 6CC (19.7%) after 0.01 NaOH treatment (Fig. 5.4 B), 6CC (19.7%) after $NaBH_4$ treatment (Figure 5.4 C)

under the same reaction conditions (pH=10.3 and with continuous stirring for 12hr at room temperature). The absence of ~94 ppm peak in the treated 6CC (19.7%) sample (Figure 5.4 B, C) clearly shows that the signal is due to oligomeric species. These oligomeric species are most probably soluble in alkali as well as with $NaBH_4$. It is also observed that there is no significant change in the intensity of carboxyl peak ~170 ppm. Only the carboxyl peak at ~172 ppm shifted to higher value ~176 ppm due to the formation of carboxylate anion due to NaOH or $NaBH_4$ treatment. The above experimental data ^{13}C CPMAS-NMR data clearly shows that the peak corresponding to (~92-96ppm) is due to the presence of oligomers. The solution state ^{13}C CPMAS-NMR study of 6CC (19.7%) and 6CC (22%) in d6-DMSO at 70°C clearly shows the absence of peak corresponding to ketonic groups (Appendix 5.2). The peak at 60 ppm corresponding to C6 decreased significantly for 6CC (22%), perhaps merging with the C2,C3,C5 peaks which get broadened, implying a drastic change in the structure of cellulose (from cellulose-I to amorphous cellulose). We have presented (Appendix 5.1 in supplementary data) the integral ratios measured, under identical spectral measurement conditions, ^{13}C CPMAS-NMR for cellulose and oxidized celluloses. It is observed that the integral ratios of peaks at ~105ppm and ~92 ppm are jointly taken as 1 for the cellulose sample. Similarly, the total integral ratios measured for peaks C2+C3+C4+C6 (90-50) ppm is 4.91 for cellulose. We found that C1 : (C2 + C3 + C4 + C5 + C6) ratio varies as the percent oxidation increases in cellulose (The data is shown in Appendix 5.1). In case of oxidized celluloses 6CC (8.6%, 14.1% and 19.7%), the total sum of integral area for 105ppm (original C1 carbon): 92 ppm (C1 reducing end) comes ~1. NMR data was collected under quantitative conditions (Ernst Angle of 30 deg flip angle, 20 sec relaxation delay. Maximum T1 observed was 110 sec). The CPMAS spectrum was collected with a contact time of 1Sec and relaxation delay of 5 Sec. Therefore, the calculated NMR

integration data clearly shows that the ~92 ppm peak arises due to the C1 reducing end. It is observed that there is not so much change in the integral ratios corresponding to C2+C3+C4+C5+C6 carbon peaks at (90-50) ppm. The calculated % oxidation from the solid state ^{13}C CPMAS-NMR analysis shows much higher value as compared to the previous calculated ratio by USP method. The calculated % degradation from these NMR values shows quantitative values of degradation of 6CC's on oxidation to be 15% in 6CC (8.6%), 24% in 6CC (14.1%) and 36% in 6CC (19.7%).

The crystalline peak intensity for C4 carbon atom of cellulose I appears at ~89 ppm. However, this peak was present in the control sample (Table 5.3) but gradually decreased for 6CC (8%) and became insignificant for 6CC (14%) and beyond 14%, this peak completely disappeared; now only the peak corresponding to the amorphous region of C4 at ~80-83ppm was present. Similarly, the crystalline region peak intensity for C6 carbon atom of cellulose I appeared at ~65 ppm . This peak decreased with increase in oxidation and became insignificant for 6CC (14%). For 6CC (>14% carboxyl), only the amorphous region of C6 carbon atom was seen, appearing at 63.35 and 60.85 ppm. Thus, solid state ^{13}C CPMAS-NMR analysis shows that for 6CC (>14% carboxyl) mainly the amorphous region of both C4 and C6 carbon exists, with almost no crystalline region. This correlates well with the WAXRD results discussed above. The solid state ^{13}C CPMAS-NMR peaks showed that the 6CC nanoparticles (13.2% carboxyl content) are generally very sharp, and even the carboxyl peak is more enhanced than for 6CC samples having 22% carboxyl groups. This could be due to the rounded shapes and smaller size (25-35 nm) of the molecule in nanoparticle sizes. All other samples of 6CC in Figure 5.3 are of macro-sized fibrils.

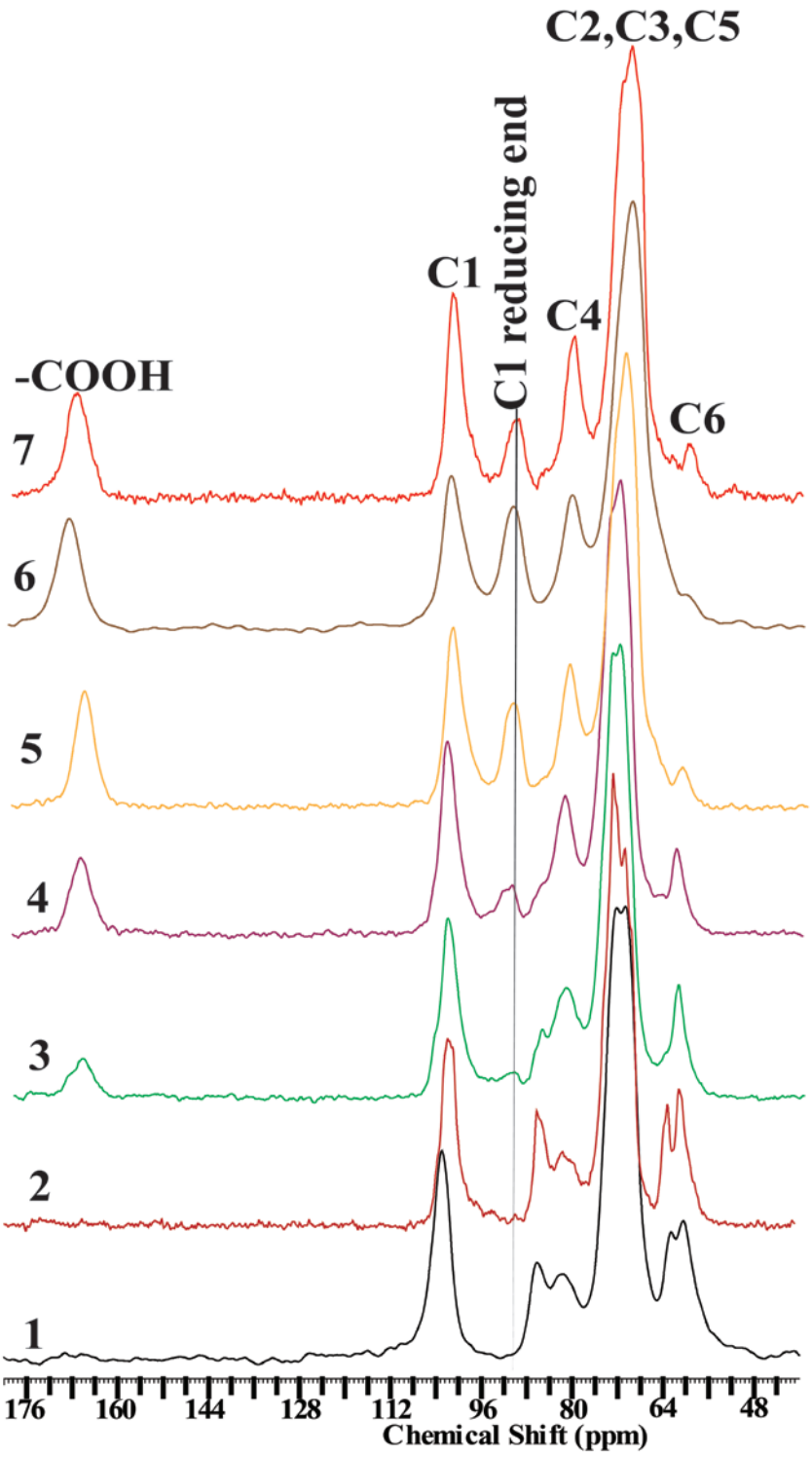

Figure 5.3: ^{13}C CPMAS-NMR spectra of (1) cellulose (2) Control * (Cellulose treated with acid (HNO_3 +H_3PO_4 (1:2 ratio) prior to addition of oxidizing agent $NaNO_2$) (3) 6CC (8.6%) (4) 6CC (14.1%) (5) 6CC (19.7%) (6) 6CC (22.0%) (7) 6CC (13.2%)-NP.

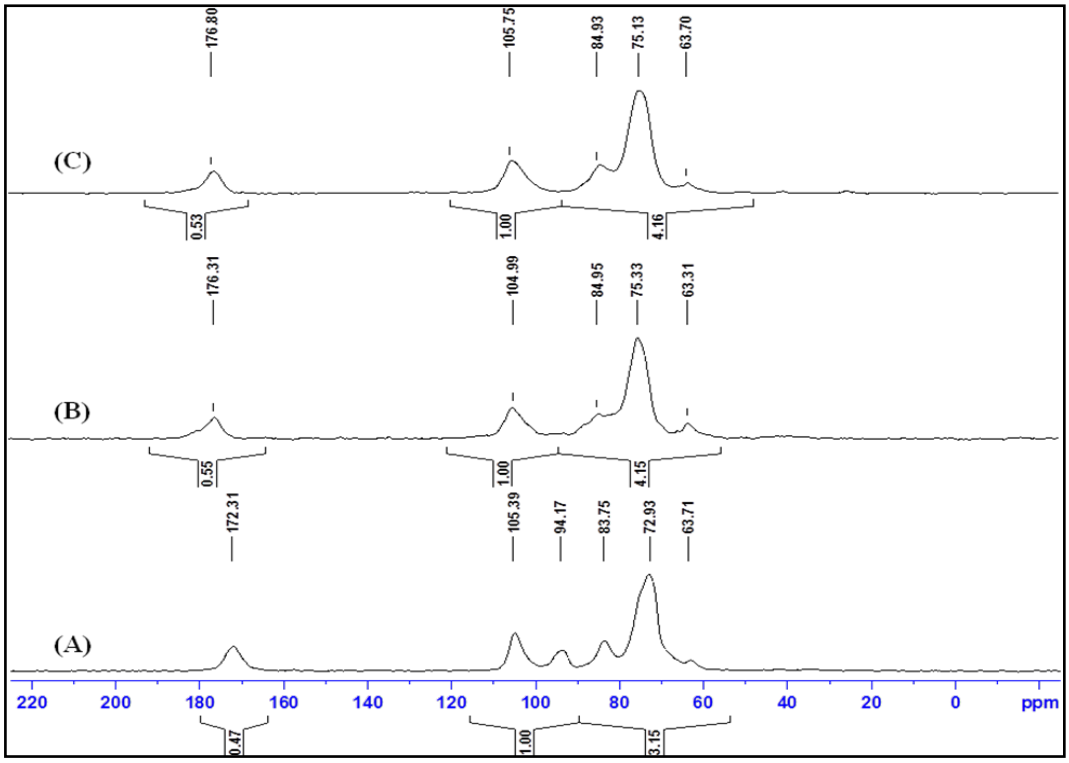

Figure 5.4 : ^{13}C CPMAS-NMR spectra's of (A) 6CC (19.7%) (B) 6CC (19.7%) after 0.01 NaOH treatment (C) 6CC (19.7%) after $NaBH_4$ treatment.

Table 5.3: The resonance assignment for the ^{13}C CPMAS-NMR spectra's of cellulose, amorphous cellulose, 6CC's and 6CC-NP of various COOH contents (%).

Carbon atom		Chemical Shift (ppm)							
	Cellulose	**Control***	**6CC (8.6%)**	**6CC (14.1%)**	**6CC (19.7%)**	**6CC (22.0%)**	**6CC-NP (13.2%)**	Cellulose II#	Amorphous cellulose
C1	105.37	103.07 102.21	104.98	104.98	104.98	102.10	102.88	~105	~105
CrystallineC4 AmorphousC4	89.07 84.21	86.79 84.09 82.11 80.33	87.96(s) 83.51	87.70(vs) 83.51	- 83.51	- 80.74 - -	- 80.87	~86	- ~83
C2,C3,C5	75.11 72.78	72.85 70.73	75.13 73.83	75.40(m) 73.30	- 73.04(m)	- - 70.13	- 72.12 70.55	~75 ~71	75
CrystallineC6 AmorphousC6	65.38 62.84	- 62.90 60.91	65.45 (s) 63.09	65.97(vs) 62.83	- 63.35(s)	- - 60.85	- 63.43(s) 60.53	~61ppm	- ~62
Extra Peaks -COOH	-	-	171.47 92.94	169.33 93.20	172.0 93.98	169.01 91.09	169.87 91.45	~94(C1 reducing end)	

* Cellulose treated with acid (HNO_3 +H_3PO_4 (1:2 ratio) prior to addition of oxidizing agent $NaNO_2$.

5.4. Conclusions

This work has shown that it is possible to use cellulose I for *in-situ* preparation of cellulose II type 6CC materials during the oxidation process using phosphoric acid in the reaction medium. The latter is known to swell cellulose and convert it to amorphous cellulose via cellulose II transition state. Previously most studies on oxidation of cellulose for biomedical applications are based on oxidation of cellulose II, due to its higher absorption capacity for physiological fluids. Further, it has been shown that the spherical nanoparticles produced by this synthesis procedure are highly crystalline materials, whereas macro-sized fibrils with the same carboxyl contents have low crystallinities.

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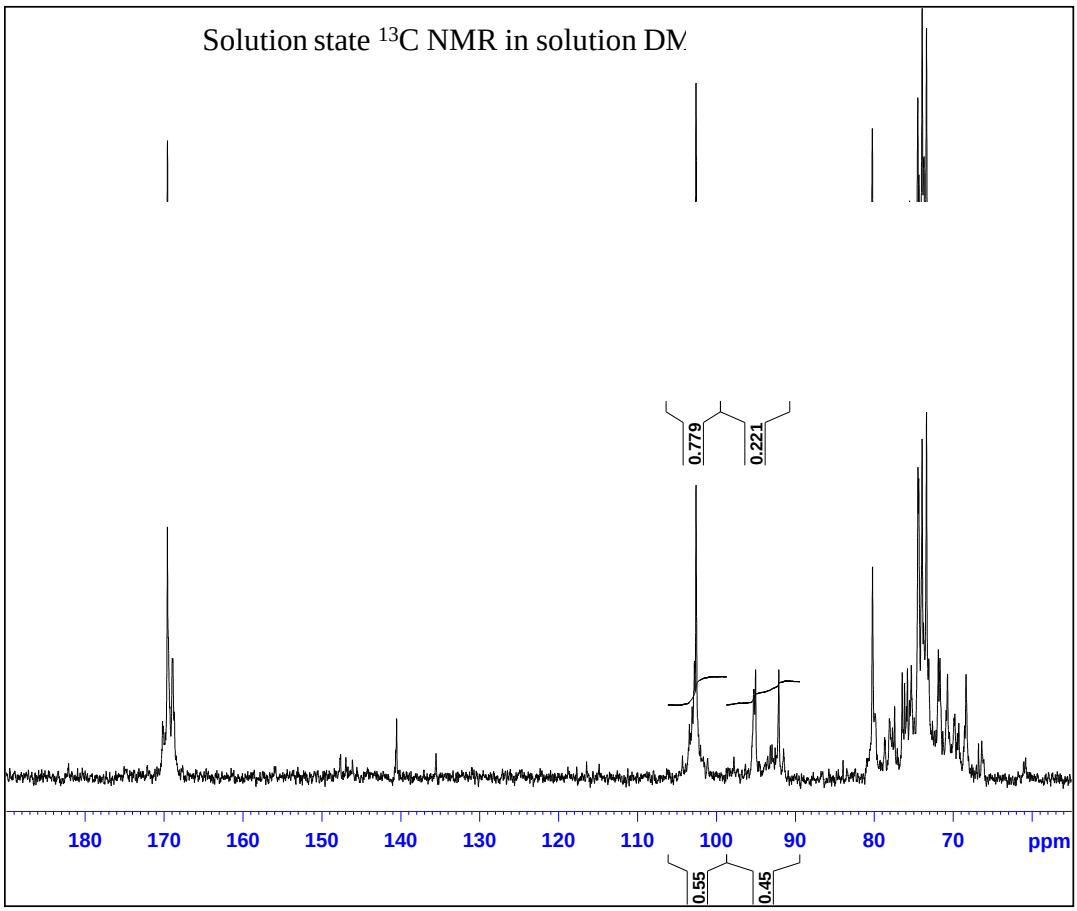
Appendix 5.1: The solid state CP-MAS ^{13}C NMR data showing the integral ratio for the carboxyl (A), C1 peak (B), C1 reducing peak (C) and combine C2+C3+C4+C6 peaks (D).

	COOH peak	B+C (C1 of original cellulose + C1 of reducing end peak)=1		C2,C3, C4,C5, C6			
Sample Name	Area of the peak at 172 ppm **A**	Area of the peak at 105 ppm **B**	Area of the peak at 92ppm **C**	Area of the peak at 90-50 ppm **D**	% COO from integral A/(B+C)*100	% oxidation from integral 100* (5-D)/(B+C)	% degradation from integral ratios 100* (5-D)/(B+C)
Cellulose	0	1	0	4.91	0	-	-
6CC (8%)	0.23	0.85	0.15	4.19	23	13.5	15
6CC (14.1%)	0.36	0.76	0.24	3.65	36	22.5	24
6CC (19.7%)	0.45	0.64	0.36	2.99	45	33.5	36

Appendix 5.2: Solution CP-MAS ^{13}C NMR spectra's of (A) 13 CPMAS of 6CC (19.7%) (B) 13C CPMAS 6CC (19.7%) after 0.01 NaOH treatment (C) 13C CPMAS 6CC (19.7%) after $NaBH_4$ treatment.



Chapter 6

Summery, conclusions and suggestions for future work

6.1. Conclusions

In this research we developed and experimentally proved a hypothesis for synthesizing shape and size tailored nanoparticles of carboxy celluloses using sugarcane bagasse derived cellulose as well as cotton cellulose. This was accomplished by first oxidizing the cellulose into derivatives such as 6-carboxycellulose, 6-carboxy-2,3-dialdehyde cellulose and 2,3,6-tricarboxycellulose, and then formulating work-up procedures for isolating nanoparticles or for designing experiments to synthesize only nanoparticles. Thus, by controlling parameters such as reaction temperature, time and ratios of reagents, we successfully prepared novel spherical shaped nanoparticles of carboxy celluloses having uniform quasi-spherical shapes, sizes (25-35 nm) and molecular weights (DP 50-70).

The new polymers were tested for their anti-bacterial properties against *E. coli*, *B. subtilis*, *S. aureus* and *Mycobacterium species*. These were found effective in the range 2.5-4.5 mg/ml concentration.

Currently TEMPO is a popular oxidizing system for celluloses. However, it is expensive, toxic in nature, and requires storage at low temperatures (~4°C). All these factors make TEMPO inappropriate for large scale production of carboxycellulose nanoparticles. Therefore, the system chosen by us (HNO_3-H_3PO_4-$NaNO_{2)}$ can prove to be cost effective, in addition to providing a new series of spherical nanoparticles. In general, spherical form is most stable shape, as it faces equal pressure from all sides in solvent media. This was proved by our studies on stability of CNT in aqueous media in the presence of these polymers. The 6CC spherical nanoparticles were found efficient in stabilizing single walled carbon nanotubes (SWCNT) as well as multi walled carbon naotubes (MWCNT) in aqueous dispersion.

The 6-carboxycellulose of varying carboxyl cellulose were further oxidized at C2, C3 position to prepare 6-carboxy-2,3-dialdehyd ecellulose (6C23DAC). The high carboxyl content carboxycelluloses with is prepared by the oxidation of 2,3-dialdehyde and 2,3,6-tricarboxycelluloses (TCC).

Study of supra-molecular transitions during oxidation by using DTG, ^{13}C CPMAS-NMR, and WAXRD has provided us with valuable data allowing for insights into the morphological changes that occur during the progress of reaction. A study of many more such studies with other cellulose reaction systems will enable one to understand how morphology can be controlled, since the latter is an important property in many applications.

Since temperature stability is an issue with most applications of cellulose derivatives in the solid state, we carried out detailed thermal analysis of the different types of carboxycelluloses: 6-caboxycelluloses, 2,3,6-tricarboxycelluloses, 6-carboxy 2,3-dialdehydecellulose and their spherical nanoparticles. The thermal stability of these derivatives was found to be less thermally stable as compared to cellulose. However, DTG data shows interesting transitions in the cellulose molecule with the progress of oxidation. The DTG curve of cellulose molecule shows single peak, while all other 6-carboxycelluloses shows two peaks for >14 % and <14% carboxyl content. The 6-carboxycellulose with ~14 % carboxyl content shows three DTG peaks. As the carboxyl content increases, the DTG peaks shifts to lower temperature. With the help of this data, we have shown that during oxidation with HNO_3-H_3PO_4-$NaNO_2$ oxidation, cellulose I converts to cellulose II for <14% carboxy content 6CC and >14 % carboxyl content 6CC to amorphous cellulose.

On further analysis of 6-carboxycellulose by using WAXRD and ^{13}C CPMAS- NMR techniques, the same transitions of cellulose I to cellulose II and then to amorphous cellulose was observed. WAXRD of cellulose I shows the main peak at 2Θ (22.8°) and 2Θ (15.6). However, on oxidation of cellulose the peak intensity at 2Θ (15.6) get disappeared and simultaneously other peak 12.1^{o} appeared. This clearly shows the appearance of cellulose II during oxidation. The peak intensity related to cellulose II 2Θ 12.2° in oxidized cellulose start decreasing for 6-carboxycellulose (1%, 3%, 8%) respectively. This corresponding peak completely disappear for 6-carboxycellulose ~14%. It is noticed that at 14% 6CC and beyond, i.e., for the 19% and 22% 6CC, the peak intensity corresponding to cellulose I again start appearing with the disappearance of cellulose II polymorph. The broadening of the peaks from 14% 6CC indicates formation of amorphous cellulose.

This way, we have successfully proved the appearance of different polymorphic forms (cellulose I, cellulose II and amorphous cellulose) at a particular degree of oxidation.

6.2. Suggestions for future work

(i) 6-carboxycelluloses and 2,3,6-tricarboxycelluloses spherical nanoparticles have potential applications in delivery of drugs and bioactive molecules, pharmaceutical compositions, skin ointments, bio-imaging, tumor tracing, nano-composites, electronics, optics and membrane technology. Therefore, more research should be done to develop the products of 6CC and TCC spherical nanoparticles.

(ii) The further studies on crystal structure of 6-carboxycellulose and 2,3,6-tricarboxycellulose in nanoform as well in macroforms has the potential to achieve new insights into the crystallography of cellulose and its derivatives.

(iii) More studies should be undertaken in the area of shape selective synthesis of nanoparticles of cellulose derivatives.

Publications and Provisional Patents

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Nanodigest- Jan.2014*, pg. no. 38.;* ***Sakal Newspaper (Pune) -28 February, 2014****; This paper came in top 10 articles in the domain of* ***BIOMEDLIB Article 23959448,*** *Since 2013.*

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Provisional Patent

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