DESIGN OF FUNCTIONALIZED NANOCRYSTALINE CELLULOSE-POLY(ACRYLIC ACID) BASED HYDROGEL WITH ENHANCED MECHANICAL PROPERTIES

A thesis submitted in partial fulfillment of the requirement for the degree of

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SUBMITTED BY

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Dedicated to
Beloved Parents
&
My Honorable Supervisor

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Abstract

Hydrogels are hydrophilic, cross-linked three-dimensional networks of insoluble polymer chains that can retain huge amounts of water and many other organic solvents. Various researchers and scientific communities are trying to explore such types of hydrogel which are ecofriendly and mechanically strong. That is why they have incorporated biopolymer such as cellulose and its derivatives into synthetic polymer to introduce hydrogels. However, most of this work using the cellulose was focused on the functionalization only at the C-6 position of the glucose moiety and they cannot be achieved desired goals. In this research, hydrogels are synthesized with improved mechanical properties using nanocrystalline cellulose functionalized at C-2 and C-3 positions of glucose moiety grafted at poly(acrylic acid) network. Here, microcrystalline cellulose was converted to 2, 3-dicarboxylated nano cellulose *via* nanocrystalline celluloses and 2, 3-dialdehyde nano cellulose. Finally, this dicarboxylated nanocrystalline cellulose incorporated into acrylic acid to prepare hydrogel. The hydrogel was characterized by Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM), Universal Testing Machine (UTM) and different chemical analysis. Surface morphology and particle size were investigated through FESEM. The analysis showed that all the cellulosic materials are in the form of nano fiber and the average fiber diameter was less than 20 nm with a length of 100 nm to few micrometers. FTIR spectra and hydrazine test confirmed the conversion of nanocrystalline cellulose (NCC) to different functional groups and grafting polymerization of modified nanocellulose onto acrylic acid. Chemical structure and crystallinity of carboxylated nanocrystalline cellulose and synthesized hydrogel were investigated by XRD analysis. The swelling capacity of hydrogel was observed by teabag method which showed prominent result with swelling ratio about 600 g/g. It was shown that water swelling can be tuned by the pH of the reaction media. Finally, mechanical properties of hydrogels including young's modulus, ultimate strength, toughness, ductility were analyzed by UTM machine. The test of mechanical properties suggested that functionalized nanocrystalline cellulose-poly(acrylic acid) based hydrogel showed higher mechanical properties in terms of Young's modulus (210.5 kPa), toughness (251.34 kJ/m^3) and tensile strength (160.0 kPa**)** than that compared with poly acrylic acid based hydrogel with Young's modulus (92.2 kPa), toughness (97.4 kJ/m^3), and tensile strength (53.0 kPa**)**. The improved compressive strength of the cellulose grafted hydrogel was also observed.

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CHAPTER ONE

INTRODUCTION

Hydrogels are three-dimensional network of hydrophilic cross-linked polymer that does not dissolve in water and different organic solvents but can swell in water and retain their original structure [1]. Generally, we can define a cross-linked polymer which can retain at least 10-20% water of its original weight [2]. Water up taking properties are due to the high thermodynamic affinity of this class of materials towards the solvent. The properties of hydrogels depend on various important parameters such as the hydrophilicity and the degree of cross-linking of the polymer chains [3]. Different functional groups like hydroxyl (–OH), carboxylic (–COOH), amidic (–CONH–), primary amidic ($–CONH_2$) and sulphonic ($–SO_3H$) are found within the polymer network of hydrogel [4]. The copolymerization and cross-linking of one or more functional monomers can be used to synthesize hydrogels. Generally, initiator, monomer and cross-linker are the main synthetic components of hydrogels. The characteristics of hydrogels can be regulated by changing the synthetic parameters such as initiator concentration, the concentration of monomer, temperature of reaction, reaction vessel, reaction time and the ratio of cross-linker and monomer.

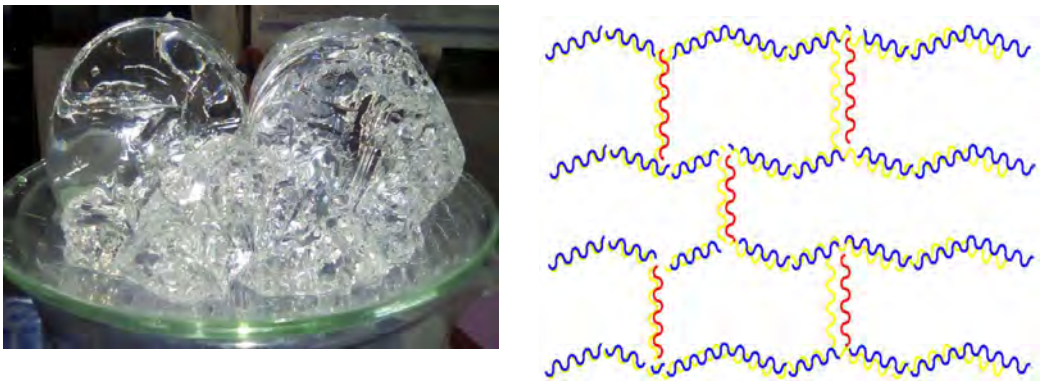

Fig. 1.1: Hydrogel – physical outlook (left), typical structure (right)

Along with swelling property, they show excellent mechanical, optical and electrical properties based on their monomer units and preparation processing. Again they contain hydrophobic and hydrophilic groups in their network which is very important to express their different properties at different conditions. Actually, these properties are prerequisites for their specific application. For example, the contact lens often requires a combination of different desirable properties, e.g. high optical transparency, good oxygen permeability, excellent biocompatibility, high water content, and strong mechanical properties. And hydrogel is an ideal candidate for combining these properties.

Hydrogels are of different types based on source, cross-linking method, composition, charge etc. According to the source, hydrogels can be divided into natural and synthetic polymers based hydrogel while on the basis of the cross-linking method, the hydrogels can be divided into chemical gels and physical gels. Physical gels are formed by molecular self-assembly through ionic or hydrogen bonds but chemical gels are formed by covalent bonds [5]. Hydrogels can be designed to exhibit significant volume changes in response to small changes

in their environment such as pH, ionic strength, temperature, electric field, solvent, or magnetic field [6]. These types of hydrogels are called smart hydrogel. Micro- and nano-sized hydrogels are faster in responding to changes in their environment than their macroscopic or bulk counterparts.

Although hydrogels were first reported by Wichterle and Lim in 1960 [7], which was based on poly(hydroxyethylmethacrylate) (PHEMA) and used in the biomaterial field. The area of hydrogel research has expanded dramatically in the last 10 years, primarily because hydrogels perform well for biomedical applications. Recently, scientists have devoted much energy to developing novel hydrogels for applications such as biodegradable materials for drug delivery [8], tissue engineering [9], sensors [10], contact lenses [11], water purification [12] etc. This is true for both the synthetic and natural hydrogels. Synthetic polymer-based hydrogels have been reported such those formed by cross-linking poly(ethylene glycol) [13], poly(vinyl alcohol) [14], poly(amido-amine) [15], poly(N-isopropylacrylamide) [16], poly(acrylamide) [17], and poly(acrylic acid) [18] and their copolymers [19].

Synthetic hydrogels like PEG-based hydrogels have advantages over natural hydrogels, such as the ability for photo-polymerization, adjustable mechanical properties, and easy control of scaffold architecture and chemical compositions, but PEG hydrogels alone cannot provide an ideal environment to support cell adhesion and tissue formation due to their bio-inert nature [20]. A number of natural polymers have similar properties to PEG in terms of biocompatibility and low protein and cell adhesion, and they can be biodegraded to nontoxic products that are easily assimilated by the body [21]. Natural polymers include polynucleotides, polypeptides, and polysaccharides are derived from a variety of naturally occurring sources such as plants, animals, and humans, or are synthesized. The polymer collagen, for example, is obtained from cows, pigs, and humans, depending on the type of collagen required. Polypeptides can be synthesized by a protection/solid support scheme or through recombinant DNA techniques. Hydrogels of naturally occurring polymers are prepared by the chemical or physical cross-linking of these polymers. The chemical crosslinking reaction of polysaccharides (alginate, chitin, chitosan, cellulose, oligopeptides, and hyaluronic acid [22]) and proteins (albumin, gelatin) leads to a variety of well-defined hydrogels [22]. Hydrogels prepared from these polymers exhibit excellent biocompatibility, primarily because they mimic the structural components of the body. In humans, glyco-aminoglycans are hydrogels that exist in the connective tissue, such as skin, tendon, and bone [22].

Among these natural polymers, cellulose is the most abundant naturally occurring polymer of glucose which is found in plants and natural fibers such as cotton and linen [23]. Cellulose having abundant hydroxyl groups can be used to prepare hydrogels easily with fascinating structures and properties. Hydrogels based on natural cellulose can be prepared from a pure cellulose solution through physical cross-linking due to the presence of numerous hydroxyl groups, which can link polymer network through hydrogen bonding [24]. Moreover, numerous new functional materials from cellulose are being developed over a broad range of applications, because of the increasing demand for environmentally friendly and biocompatible products [25].

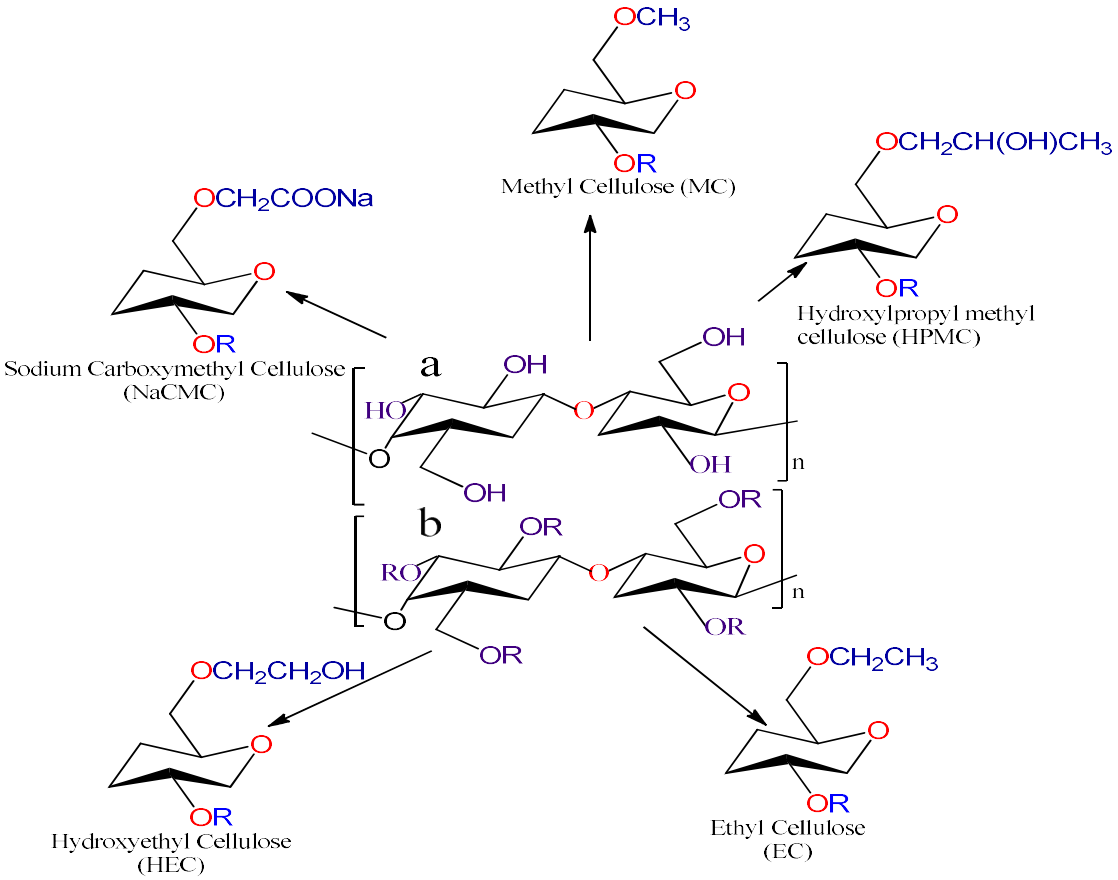

Fig. 1.2 (a) Repeating unit of cellulose "cellobiose." (b) Repeating unit of cellulose derivatives. The substituent group "R" is indicated for MC, HPMC, EC, HEC, and NaCMC

Most of the water-soluble cellulose derivatives are obtained by etherification of cellulose, where the active hydroxyl groups of cellulose react with organic species, such as methyl and ethyl units [26] to give MC, EC, HEMC, HPC and CMCNa [27] and by esterification of cellulosic hydroxyl and various organic acids in the presence of a strong acid as a catalyst to get cellulose acetate, acetate trimellitate, acetatephthalate, hydroxypropylmethyl-phthalate and hydroxypropylmethyl-phthalate acetate succinate [28]. Cellulose and its derivatives are blended with natural and biodegradable polymers or synthetic polymers [29] to achieve a new structural design and functional properties [30]. For instance, phase-separated composite hydrogels have been prepared by blending cellulose and chitin in thio-urea aqueous solution in the presence of H_2SO_4 as coagulant [31].Cellulose powder and chitosan solution are mixed together to form cellulose/chitosan hydrogel beads, which are cross-linked with ethylene glycol diglycidyl ether to make them considerably denser and chemically stable for Cu adsorption applications [32]. In addition, cellulose/starch composite gel can be prepared by keeping their homogeneous mixture for several days [33]. Another special type of water-soluble hydrogel called polyelectrolyte complexes can be constructed by introducing other positively charged polyelectrolytes in the presence of CMC [34]. Chitosan and CMC solutions are blended to form an amphoteric hydrogel membrane followed by crosslinking with glutaraldehyde. This hydrogel is flexible and can bend toward either anode or cathode, depending on the pH of the solution [35]. Cellulose-inorganic hybrid hydrogels are designed

by incorporating inorganic contents into carbon black (CB) hydrogel system to improve functional performance [36].

It is important to note that almost all high water content hydrogels possess poor mechanical properties. Again, due to the random nature of the crosslinking reactions during the polymerization, many polymer gels have poor mechanical properties. This is a significant drawback of the hydrogels restricting their applications specifically where high stress is required [37]. In order to overcome the poor mechanical properties of these materials, it is necessary to copolymerize these "super absorbent" monomers with monomers that are capable of improving the overall tear strength of the material. Incorporating different materials such as ceramics, metals, silicates, magnetic and natural or synthetic particles into the hydrogel matrix usually lead to improved properties, enhanced mechanical strength and structural stability.

Nanocrystalline cellulose (NCC) has been incorporated into synthetic polymer units to prepare hybrid hydrogel which shows improved mechanical properties in terms of good compressive stiffness together with high porosity, interconnected pore structure, high degradation stability and thermal stability making them suitable for bone tissue engineering applications [38]. But NCC is insoluble in water and many other organic solvents. That's why it is hard to dissolve them in water and to make homogenous mixture which limits the mechanical properties of hydrogels. Again the special type of solvents is needed to make it soluble. This process is time-consuming and not cost-effective. But carboxylate functionalized NCC is soluble in water which is miscible in the polymer matrix and form a homogenous mixture with other polymer unit[39] Carboxylate groups reinforce the polymer network and enhance mechanical properties. Again, the carboxylate group of polymer networks can form more intra and inter molecular hydrogen bond which also strengthens the polymer network of hydrogel.

Carboxylic groups can be introduced in the cellulose network in different positions. The most of research on the functionalization of cellulose network by carboxylic group has been carried out at the C-6 position of the glucose moiety. The scope of incorporation of carboxylic group in the glucose moiety has been explored by other researchers. Selective oxidation by periodate has been carried out for the cleavage of C-2 and C-3 bond of the glucose moiety to introduce aldehyde group in the glucose unit [40]. Consequently, the present research aims to synthesis hydrogel based on the poly(acrylic) acid (PAAc) a carboxylate functionalized NCC at C2 and C3 position of glucose moiety through free radical polymerization.

The main objective of the present was

I. Preparation of carboxylated functionalized nanocrystalline cellulose *via* nanocrystalline cellulose and dialdehyde nanocrystalline cellulose from microcrystalline cellulose as the starting material.
II. Polymerization and crosslinking of the functionalized cellulose nanocrystals with acrylic acid in the presence of suitable crosslinker and initiator.
III. Characterization of synthesized hydrogel *viz.* chemical analysis, crystallinity, surface morphology, water swelling and mechanical properties
IV. Investigation of mechanical behavior of hydrogel when varies the conditions such as pH of reaction medium, DCNC content and carboxylate group content.

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CHAPTER TWO

BACKGROUND

2.1 Hydrogels

Today we define hydrogel as three dimensional crosslinked polymers that have water uptake capacity but the word "hydrogel", first introduced in 1894 to explain a colloidal gel made with inorganic salts. The first water absorbent was synthesized in 1938 using thermal polymerization process of acrylic acid and divinyl benzene. Later on, 1950, Otto Wichterle and Lim introduced hydrogel to use in contact lenses. But now hydrogels are used in various sectors such as pharmaceutical, agriculture, water purification, tissue engineering, and optoelectronics. That's why, hydrogels are pampered by scientific and commercial communities and huge amounts of hydrogels are produced around the world for their commercial application. These huge amounts of hydrogel can be classified by different angles.

2.2 Classification of Hydrogels

In the present world, hydrogels are used in different fields. A huge number of hydrogels are produced around the world. We can classify these hydrogels into several categories on the basis of origin, composition, ionic charge, physical structure and cross-linking.

2.2.1 Classification Based on Origin

Natural Hydrogels

This type of hydrogels is nature originated and are prepared using natural polymers such as proteins and polysaccharides like alginate, chitosan and dextran [1].

Synthetic Hydrogels

Synthetic originated polymeric substances that are formed from synthetic products or man-made products are called synthetic hydrogel and these types of hydrogels are synthesized via chemical polymerization. These hydrogels can be homo polymeric, co-polymeric and multi-polymeric [2].

Hybrid Hydrogels

The hydrogels which are formed by a combination of natural polymers with synthetic polymers are called Hybrid Hydrogels. Wang et al. synthesized a protein cross-linked 2-hydroxypropyl meth acrylamide hybrid hydrogel [3].

2.2.2 Classification Based on Composition

Homopolymer Hydrogels

This type of hydrogels is cross-linked polymer networks derived from only one type of monomer. The structural framework of homopolymer hydrogels is dependent on the nature of the monomer, polymerization technique and cross-linker [4].

Copolymer Hydrogels

Copolymer hydrogels are produced from two different types of monomer in which one monomer contains hydrophilic group and responsible for the swelling property of the hydrogel [5].

Multiplier Hydrogels

An example of multipolymer hydrogel is poly (acrylic acid-2-hydroxy ethyl methacrylate)/gelatin hydrogel [6]. These types of hydrogels are produced from three or more monomers using polymerization and cross-linking reactions.

Interpenetrating Network (IPN)

The hydrogels which are made up of two intertwined polymer networks without any chemical bond between the polymers are called interpenetrating networks. Although the network of the first polymer is linear but the second polymer has a cross-linked network. The linear network of the first polymer diffuses into the second polymer [7].

2.2.3 Classification Based on Ionic Charge

Hydrogels are classified into three groups on the basis of nature of electric charge on cross-linked chains [8].

Neutral (Non-Ionic) Hydrogels

The hydrogels which have no charge on their backbone or side groups are called neutral hydrogels. Poly (Acrylamide) is used to prepare neutral hydrogel [9].

Ionic Hydrogels

Ionic hydrogels can be cationic or anionic. Cationic hydrogels containing positively charged groups (e.g. amines and sulphonic acid) and exhibit an increase in the swelling at low pH, whereas anionic hydrogels containing negatively charged groups (carboxylic acid, sulphonic acid) and show an increase in swelling at high pH [10].

Ampholytic Hydrogels

Ampholytic hydrogels carry negative as well as positive charge on the same polymer chain, which balances at the isoelectric point. PAC is an ampholytic hydrogel which was prepared from acrylamide (AM) and 4 (2 ((carboxylatomethyl)dimethylammonio)ethoxy)−4-oxobut-2-enoate (CMD) through free-radical polymerization by using ammonium persulfate (APS) as an initiator and methylenebisacrylamide (MBA) as a crosslinker.

2.2.4 Classification Based on Pore Size

Hydrogels are classified into three types on the basis of porosity [11] namely

a. nonporous
b. microporous and
c. super porous

2.2.5 Classification Based on Physical Appearance

On the basis of physical appearance, hydrogels can be a matrix, film or microsphere, depending on the polymerization method [12].

2.2.6 Classification Based on Configuration

Depending on the physical structure and chemical composition, hydrogels can be classified as follows [12].

Amorphous (Non-Crystalline)

In amorphous hydrogels, polymeric network contains randomly arranged macromolecular chains.

Semi-Crystalline

Semi-crystalline is a complex mixture of amorphous and crystalline phases and characterized by dense regions of ordered macromolecular chains.

2.2.7 Classification Based on Cross-Linking

On the basis of nature of cross-linking, hydrogels are of two types:

a. Physical hydrogels
b. Chemical hydrogels.

Physical hydrogels are cross-linked by various physical processes such as crystallization, hydrogen bonding and hydrophobic interactions, whereas covalent cross-linking is used to prepare chemical hydrogels [13].

2.3 Monomers used in hydrogels

2.3.1. Synthetic Materials used in hydrogels

Synthetic polymers offer engineers highly versatile materials with physical and chemical properties that can be easily controlled and altered. One such property is degradability, which can be altered by creating copolymers or polymer blends. These polymers and blends are generally easier to process than natural polymers and have more predictable results. Conversely, synthetic polymers are less biocompatible than naturally derived polymers and not as bioactive.

2.3.1.1 Poly(acrylic acid) (PAAc)

The synthetic polymers allow for a higher degree swelling, and impart superior mechanical properties and adhesion strength to the hydrogels as compared to those in the case of natural polymers. PAAc is a hydrophilic and a highly absorbent polymer that has been widely applied to biomaterials due to its high solubility and biodegradability [14]. PAAc hydrogels have been widely used as drug carriers because of their good bio-adhesive properties and enhanced drug penetration.

2.3.1.2 Polyethylene glycol (PEG)

PEG is a water-soluble synthetic polymer [15]. PEG used in hydrogels due to its biocompatible, nontoxic and water-soluble properties. PEG based hydrogels are called “smart polymers” or “intelligent gels” because they are stimuli based hydrogels. The stimuli may be physical (temperature, solvent, light, radiation, pressure) or chemical (PH, specific ions). These hydrogels are also used for controlled release of drugs [16].

2.3.1.3. Poly(acrylamide) (PAAm)

Poly(acrylamide) (PAAm) is an important and hydrophilic polymer for preparation of hydrogels [17]. PAAm hydrogels and their derivatives are the subject of many studies [18]. PAAm hydrogels have proven capability of water absorption and biocompatibility with physiologic body fluids. The application of PAAm hydrogels in controlled release of agrochemicals and bioactive have been investigated.

2.3.1.4 Polyvinyl alcohol (PVA)

PVA is also another synthetic polymer which extensively is used to prepare hydrogel. Due to its water retain ability and biocompatibility it is used as scaffold for tissue cultures, contact lenses [15], cartilage reconstitution [15] and wound dressing [16]. PVA based hydrogels are obtained by freezing and thawing process.

2.3.1.5 Polyvinyl pyrollidone (PVP)

PVP is soluble in water as well as in polar solvents [15]. PVP is used in wound dressing because of storing large quantities of water, low production cost and good elasticity properties. PVP in combination with other polymers like CMC are also used to enhance mechanical properties and biocompatibility. Retaining high quantity of water PVP are also used in making contact lenses [16].

2.3.2. Natural Materials used in Hydrogels

Nature is the vast source of polymeric materials which can be used to synthesis polymeric materials. Naturally derived scaffolds materials such as alginate carrageenan, collagen, chitosan, gelatin, alginates, hyaluronic acid etc. have been processed from nature and possess good biocompatibility properties with low cytotoxicity. Due to their biocompatibility and biodegradability these naturally derived polymers are used to synthesis hydrogel which have vastly used in different sectors specially in biomedical applications. Here some of them are illustrated.

2.3.2.1 Alginate

Alginate is a linear polysaccharide extracted from brown, sea weed and algae [19]. Alginate is made up of (1-4)-b-Dmannauronic acid and (1-4) –a-L-gluronic acid [19]. In presence of ions they undergo complexation reaction due to presence of carboxylic group [20]. Gelling property of alginate is due to the presence of G block [20]. Hydrogels containing famotidine for stomach targeting are formed by incorporating sodium alginate and polyacrylamide by grafting technique [19].

2.3.2.2 Chitosan

Chitosan is a natural polymer can be obtained from shrimp, crab and lobster shell [20]. It is a cationic polysaccharide [20]. Mostly chitosan is obtained by deacetylation of chitin [21]. Chitosan is made up of glucosamine and N-acetyl glucosamine units [21]. A thermo and PH

sensitive based hydrogel of chitosan were prepared containing doxorubicin hydrochloride as a model drug.

2.3.2.4 Hyaluronic acid

Hyaluronic acid is also called hyaluronan and hyaluronate (HA) or sodium hyaluronate [21]. It is a N-acetyl-D- glucosamine and beta gluronic acid [20]. HA is a naturally occurring polymer and found in tissues of higher animals [21], as well as in vitreous of eye and in synovial fluid [21]. HA based hydrogels are used for delivery of therapeutic agents for tissue repair.

2.3.2.5 Gelatin

Gelatin is a water-soluble natural polymer [21]. Gelatin is produced by the hydrolysis of collagen obtained from connective tissues and bones of animals [20]. Hydrogels based wound dressings consist of sodium alginate and gelatin were prepared by crosslinking of sodium chloride / glutaraldehyde.

2.3.2.6 Dextran

Dextran is a natural polymer synthesized from sucrose. Due to its water solubility, biocompatibility and unique properties it is used for sustaining the effects of proteins, interleukin-2 and other drugs. Paclitaxel an anti-cancer drug is used to make dextran-based hydrogels. These hydrogels are pH sensitive and are utilized for colon targeting.

2.3.2.7 Xanthan gum

Xanthan gum is produced by fermentation of carbohydrate with xanthomonascampestris. It is a cream-colored powder soluble in hot as well as cold water. Xanthan gum is stable over a range of pH 4-10 [22]. Super porous hydrogels were prepared by using xanthan gum, acrylic acid and 2- hydroxyethyl methacrylate (HEMA) by graft copolymerization technique.

2.3.2.7 Starch

Starch is synthesized and stored by all plants for energy purposes. Available in the form of small granules ranging in size from 1-100 micro meters. Starch is a biodegradable polymer due to which it has pharmaceutical applications [22]. pH sensitive based hydrogels were prepared by using starch and methacrylic acid by copolymer method loaded with ketoprofen as a model drug.

2.3.2.8 Cellulose

Cellulose is the most abundant natural biopolymer available on the earth and it is a structural constituent of the cell wall of various plants. It is important not only for plant growth and development but also for industrial use. Apart from plants, cellulose is also present in a wide variety of living species, such as algae, fungi, bacteria, and even in some sea animals such as tunicates [23]. Cellulose is a fibrous, tough, and water-insoluble polymer. The chemical structure of cellulose is shown in figure 2.1.

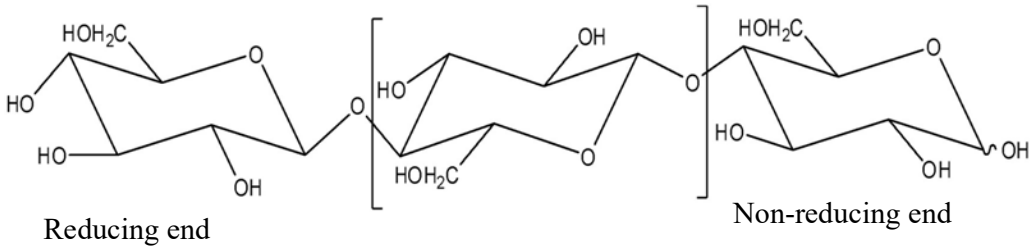

Figure 2.1 Typical structure of cellulose

It contains three different parts-a reducing end group with a free hemiacetal at the C_1 position, a non-reducing end group with a free hydroxyl group at the C_4 position, and internal anhydroglucopyranose rings joined at the C_1 and the C_4 positions through β 1-4 glycosidic bonds. The internal anhydroglucopyranose units are predominant due to the long chain lengths and contain three hydroxyl groups. The hydroxyl group at the C_6 position is a primary alcohol, while the hydroxyl groups at the C_2 and C_3 positions are secondary alcohols and these are corkscrewed at 180° with respect to its neighbors [24]. Due to the linear and quite regular structure of cellulose and having many hydroxyl groups, cellulose polymers can form ordered crystalline structures. These crystalline regions give important mechanical properties to the cellulose fibers. The hydroxyl groups in the cellulose polymer can form hydrogen bonds between different cellulose polymers (intermolecular hydrogen bonds) or within the polymer itself (intramolecular hydrogen bonds). The intramolecular hydrogen bonds give stiffness to the polymer chain, while the intermolecular bonds allow the linear polymers to form sheet structures. With the van der Waal force, hydrogen bond helps to aggregate polymer chain together side-by-side and promotes parallel stacking of cellulose microfibrils into crystalline cellulose [25]. The high crystallinity and the many hydrogen bonds in the cellulose fibers make cellulose insoluble in water and in most conventional organic solvents.

2.3.2.9. Cellulose nanocrystals (CNCs)

Naturally occurring bulk cellulose consists of highly ordered, crystalline regions along with some disordered (amorphous) regions in varying proportions, depending on its source [26]. CNCs have been extracted from different cellulose sources. Different types of cellulose sources give some different structures of the nanocrystals and the aspect ratio will differ for the different sources. By removing amorphous regions applying mechanical, chemical or enzyme treatments we get highly ordered crystalline regions. These crystalline regions are referred to as cellulose nanocrystals (CNCs). Generally, CNCs are extracted by acid hydrolysis of cellulose. Acid hydrolysis performed by using different acids like sulfuric acid, hydrochloric acid, phosphoric acid, nitric acid, acetic acid and some mixture of acids . Among these acids sulfuric acid gives the best crystallinity index and hence it is used in a large scale. During sulfuric acid treatment, the hydronium ions migrate to the amorphous regions since they have lower density compared to the crystalline regions. The hydronium ions cleave the glycosidic linkages hydrolytically and thereby releasing the individual crystallites. Sulfuric acid treatments produce negatively surface charge which is very important for the dispersiveness of CNCs. CNCs are spherical or stiff rod-like particles consisting of cellulose chain segments in a nearly perfect crystalline structure. Compare to native cellulose, which

has greater amorphous fractions, these nanocrystals exhibit high specific strength, modulus, high surface area, and unique crystalline properties.

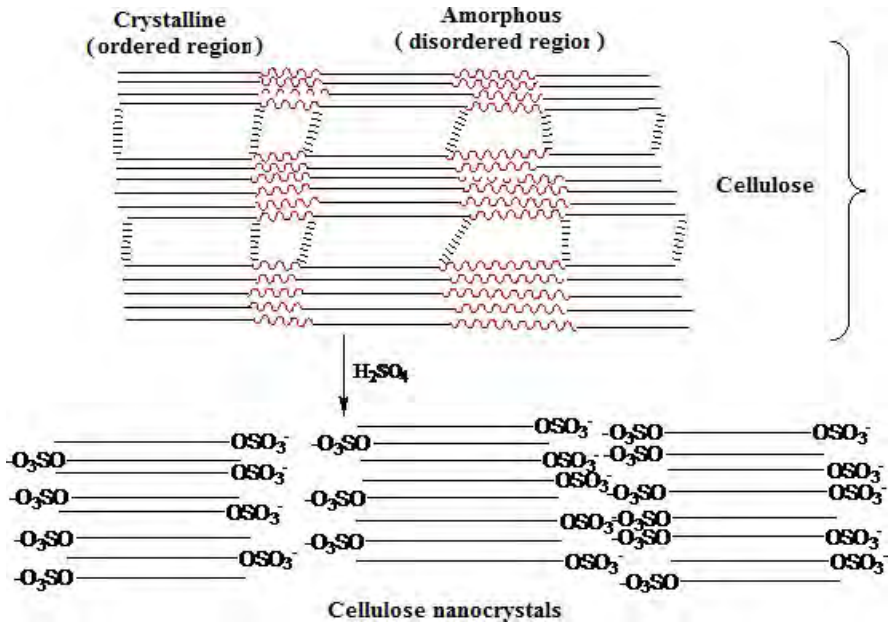

Fig.-2.2: Hydrolysis process to prepare nanocrystalline cellulose.

2.3.2.10. Chemical Modification of Cellulose

Cellulose is a distinctive natural polymer that possesses several attributes such as a fine cross section, the ability to absorb moisture, high strength and durability, high thermal stability, good biocompatibility, relatively low cost, low density, and good mechanical properties [27]. Yet, there are some drawbacks for cellulose. These include poor solubility in common solvents, poor dimensional stability, lack of thermo-plasticity and lack of antimicrobial properties. Thus, controlled physical and/or chemical modification of the cellulose structure is necessary to prevail over such drawbacks [28].Introducing functional groups into cellulose molecules through chemical modification is one of the key ways of adding new properties to the cellulose without destroying its many attractive intrinsic properties. For instance, the formation of cellulose nitrate involves the esterification of cellulose with nitric acid in the presence of sulfuric acid, phosphoric acid, or acetic acid. Currently, other commercially important cellulose derivatives include hydroxyl ethyl cellulose, carboxymethyl cellulose, etc.

This chemical modification can provide polymeric materials with valuable properties and different chemical structures. It can also permit one to combine the best properties of two or more polymers in one physical unit. This can be achieved by controlling some parameters such as the polymer types, the degree of polymerization and the polydispersities of the main chain and the side chains, the graft density, and the distribution of the grafts (graft uniformity) [29]. The creation of cellulose graft copolymers is one of the key ways of modifying the

physical properties and chemical properties of cellulose [30]. These grafts can be linked together via their functional groups to form a three-dimensional network structure.

2.3.2.11. Dicarboxynano-crystalline cellulose (DCNC)

As CNC has high crystallinity, dispersive property and great mechanical strength, it exhibits multidimensional application in different fields. But in case of wastewater management, the application of CNCs is much less explored because it aggregates due to the formation of tightly hydrogen bonded networks as the surface of CNC has numerous hydroxyl groups. The aggregation of CNC can be reduced by surface modification. Generally, the surface of CNCs is modified by esterification, etherification, oxidation, amidation, carbamation, nucleophilic substitution, silylation, polymer grafting, etc. by introducing negative or positive charge on the surface of CNC. By oxidation reaction surface hydroxyl group converted to carboxyl group which ultimate produce negative charge on the surface. Carboxyl group containing CNC is called Carboxylated cellulose Nano crystals *(*CCN*),* which acts as efficient adsorbent for removal of dye and heavy metals. Recently, 2, 2, 6, 6-tetramethylpiperidine-1-oxyl radical (TEMPO) mediated oxidation [31], periodate-chlorite oxidation [32] and ammonium persulfate (APS) oxidation [33] have been used to produce CCN. In case of periodate-chlorite treatment of CNC, periodate selectively oxidizes C_2 and C_3 hydroxyl groups to two aldehyde groups and then to carboxyl groups called dicarboxylic nano-crystalline cellulose (DCNC). Introduction of carboxyl group into the matrix, cellulose become soluble in water. Therefore, the application sectors increased in a wide range for cellulosic material. As it is biocompatible and biodegradable substances that's why it can be incorporated into polymer matrix to prepare biomaterial for drug delivery and medical purposes. Again, these DCNC can be further functionalized introducing double bond and amino-group which alter its properties and increase applicability.

2.3.2.12. Cellulose-Based Hydrogels

Manufacture of hydrogels from cellulose and its derivatives generally is accomplished in two steps [34], (i) dissolving of cellulose fibers or powder and (ii) cross-linking (chemical and/or physical) of the chains, in order to obtain a three-dimensional network of hydrophilic polymer chains, which is able to absorb and retain a significant amount of water.

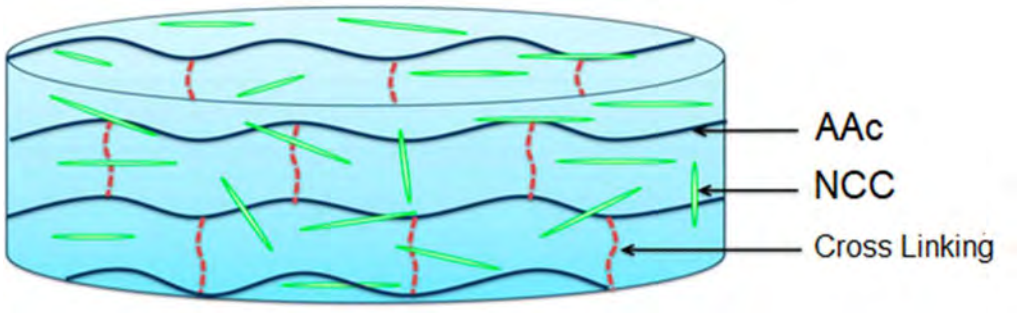

Fig.-2.3: Cellulose based hydrogel

Additional natural and/or synthetic polymers might be combined with cellulose to obtain composite hydrogels with specific properties. These properties can be changed under different environmental conditions. This hydrogel is known as stimuli sensitive hydrogel. Cellulose

and its derivatives can be used to prepare stimuli sensitive hydrogels. Some common derivatives of cellulose with their common applications are given below which are being used to prepare stimuli sensitive hydrogel.

Table-2.1: List of cellulose derivatives and their applications

Cellulose derivatives	Applications	Ref.
Carboxy methyl cellulose	Biomedical and agriculture	[35]
Methyl cellulose	Releasing fertilizers	[36]
Hydroxy ethyl cellulose	Smart materials	[37]
Hydroxypropyl methyl cellulose	Controlled release	[38]
Cellulose acetate	Drug carrier system	[39]

However, still very limited works are performed by using native cellulose for the preparation of hydrogels. This is due to the difficulty of dissoluble property of cellulose in several solvents for the regeneration process [40]. Although cellulose derivatives are some extent soluble in water and other liquids but their applications are limited due to lower mechanical properties. The present study will describe the fabrication such type of cellulose based hydrogel which is biocompatible and less cytotoxic materials for a new type of environmentally friendly with enhanced mechanical properties.

2.4. Synthesis of Hydrogels

Hydrogels are polymer networks prepared from natural or synthetic polymers using various polymerization techniques such as bulk, solution and suspension by physical and chemical cross-linking routes [12]. Physical cross-linking involves hydrogen bonds, stereo-complexation and soft assembly, whereas chemical cross-linking involves cross-linking in the presence of different cross-linkers. There are others some methods to synthesis hydrogel which are given below including physical and chemical method.

- Physical method
- Chemical method
- High energy irradiation
- Using enzymes

Of all the above-mentioned cross-linking methods, chemical methods and physical method are the most widely used.

2.4.1. Physical method

In this method hydrogels are crosslinked via non-covalent bonding or various physical interactions including hydrogen bonding, hydrophobic interaction, aggregation, association, complexation, crystallization, and ionic interactions [41]. On the basis of this method, hydrogels may be grouped into weak or strong physical hydrogels. In addition, they are reversible in nature due to conformational changes. Structures of few strong physical hydrogels and weak physical hydrogels are shown in Figure 2.4(A) and 2.4(B).

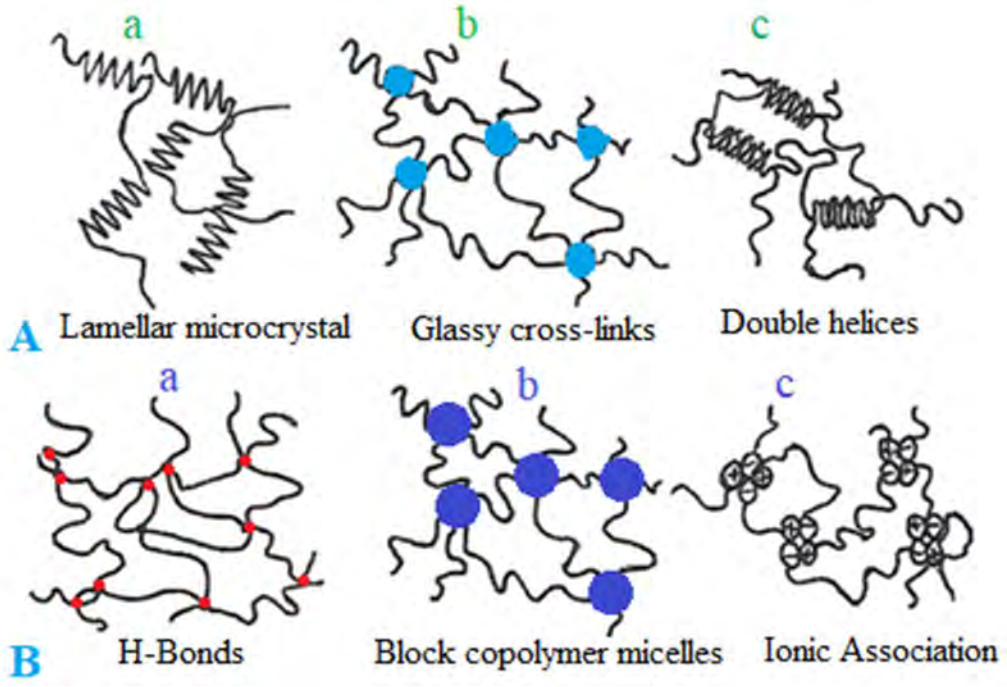

Fig.2.4: (A) strong physical hydrogels and (B) weak physical hydrogels

A list of few well-known physical hydrogels and their applications is presented in Table-2.2.

Table- 2.2: List of physical hydrogels and their applications

S.N.	Name	Application	Ref.
1	Polyelectrolyte complex hydrogel	Tissue engineering matrices	42
2	Blend hydrogels	Drug delivery, wound dressing	43
3	Block copolymer gels	Drug delivery	44
4	Thermally induced hydrogel	Drug and gene delivery	45
5	Counter ion induced hydrogel	Dentistry and drug delivery	46
6	Interpenetrating polymer hydrogels or network (IPNS)	Drug delivery and tissue scaffold	47

2.4.2. Chemical method

In contrast to physical network-based hydrogels, chemical cross-linking-based hydrogels involve chemical covalent cross-linking in their formation. Monomers with vinyl groups like acrylic acid, AAm, hydroxyl ethyl methacrylate, and so on or some macromolecules modified with vinyl groups such as albumin, dextrin, starch, and so on can be polymerized through chemical initiation to prepare chemical gels.

Similarly, photo-ionization or gamma irradiation processes may be utilized to synthesize chemical hydrogels. The phenomenon of chemical hydrogel synthesis takes place in the presence of molecules having divinyl groups, which further act as covalent cross-linkers [41]. Polymers having other functional groups can also be cross-linked to produce chemical hydrogels. Gelatin and albumin form inter molecular Cross-link through dialdehyde group; cysteine, and polypeptides through cross-linking of cysteine bonds are good examples of chemical hydrogels [48]. Chemical hydrogels may be formed with degradable polymer backbone or degradable cross-linking agent prepared by using chemical cross-linking [41]. This type of hydrogel is considered irreversible or permanent due to the involvement of configurational changes.

2.4.3. Radical cross-linking method

Chemical hydrogel can convincingly form by Irradiation method including irradiation of solid polymer, monomer (in bulk or in solution) or polymer aqueous solution [49]. The advantage of radical cross-linking is that cross-linkers are not required in the fabrication process here which limited the applications of hydrogels in the food, drug, and pharmaceutical industries due to their toxicity. The concentrated aqueous solutions of cellulose derivatives such as CMC, HPC, and MC, can be cross-linked under ionizing radiation to prepare cellulose based hydrogels [50]. Bin et al. have found that carboxymethyl cellulose with high degree of substitution (DS) and high concentration can be effectively cross-linked to form CMC hydrogels through irradiation [50]. The effects of the aging time, concentration, and dose rate on the cross-linking of CMC in aqueous solutions under ionizing radiation are also examined.

2.4.4. Ionic Interaction method

Ionic interaction method can be employed to prepare hydrogel using alginate (a polysaccharide with mannuronic and glucuronic acid residues) is a well-known polymer that can be cross-linked with calcium ions [51]. This type of cross-linking can be done under ambient conditions of temperature and physiological pH; hence these hydrogels are commonly employed as templates for the encapsulation of living cells and for the release of proteins [52]. Similarly, a synthetic polymer, poly-di(carboxylatophenoxy) phosphazene (PCPP), can also be cross-linked with Ca ions like as alginate ions. Such hydrogels are called ionotropicand can degrade under physiological conditions.

2.5. Properties of Hydrogels

2.5.1 Swelling properties of hydrogel

A crosslinked polymer hydrogel swells but not dissolve when water or a solvent enters it. The imbibed liquid serves as a selective filter to allow free diffusion of some solute molecules but

the polymer network serves as a matrix to hold the liquid together. Hydrogels may absorb from 10-20% (an arbitrary lower limit) up to thousands of times their dry weight in water [53].

The swelling properties depend on many factors such as network density, solvent nature, polymer solvent interaction parameter. Water acts as a plasticizer in a hydrophilic polymer network system. The swelling process of the hydrogel can be considered under rubbery state and can be described by the free energy of mixing ΔG_{mix} from the polymer and solvent interaction and the elastic free energy $\Delta G_{elastic}$ from the crosslinked network:

$$\Delta G_{system} = \Delta G_{mix} + \Delta G_{elastic} \cdots\cdots\cdots\cdots\cdots\cdots (2.1)$$

At the beginning of swelling, the $\Delta G_{mix} << 0$, $\Delta G_{elastic} > 0$, $\Delta G_{mix} + \Delta G_{elastic} < 0$, so the swelling is favored and the solvent diffuses into the network. During the processing of swelling, the ΔG_{mix} and $\Delta G_{elastic}$ both increased until $|\Delta G_{mix}| = |\Delta G_{elastic}|$ and $\Delta G_{system} = \Delta G_{mix} + \Delta G_{elastic} = 0$, so that the driving force for swelling is gone: equilibrium swelling is reached and swelling stops.

When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, leading to primary bound water. As the polar groups are hydrated, the network swells and exposes hydrophobic groups, which also interact with water molecules, leading to hydrophobically-bound water, or secondary bound water. Primary and secondary bound water are often combined and simply called the total bound water. After the polar and hydrophobic sites have interacted with and bound water molecules, the network will imbibe additional water, due to the osmotic driving force of the network chains towards infinite dilution.

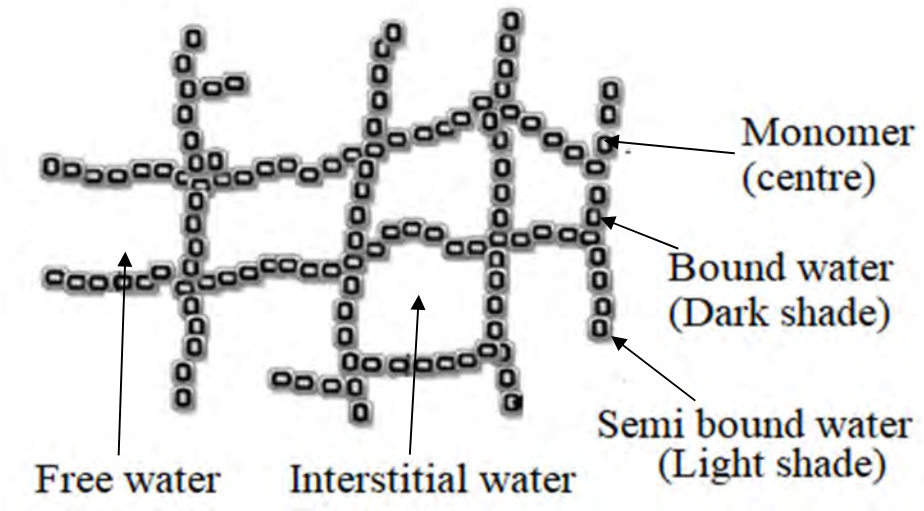

Fig.-2.5: Water swelling process by hydrogel

This additional swelling is opposed by the covalent or physical crosslink, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level. The additional swelling water that is imbibed after the ionic, polar and hydrophobic groups become saturated with bound water is called free water or bulk water, and is assumed to fill the space between the network chains, and/or the center of larger pores, macropores or voids.

As the network swells, if the network chains or crosslink are degradable, the gel will begin to disintegrate and dissolve, at a rate depending on its composition.

2.5.1.1 Swelling ratio measurement

2.5.1.1.1. Free-absorbency Capacity

Generally, when we have been used the terms swelling or absorbency without specifying its conditions; it implies uptake of distilled water without any load putting on the testing sample. On the basis of sample viz. the amount of the available sample, the sample absorbency level, and the method's precision and accuracy there are several simple methods for the free absorbency testing.

2.5.1.1.2. Tea-bag Method

Tea bag method is fast and more suitable among all the conventional method for limited amounts of samples (W_0= 0.1-0.3 g) [54]. In this method sample is placed into a tea-bag (acrylic/polyester gauze with fine meshes) and the bag is dipped in an excess amount of water or saline solution for a definite time to reach the equilibrium swelling. Then excess solution is removed by hanging the bag until no liquid is dropped off. The tea bag is weighed (W1) and the swelling capacity is calculated by equation (1.2). The method's precision has been determined to be around ±3.5%.

$$Se = (W_1-W_0)/W_0 \cdots\cdots\cdots\cdots (2.2)$$

2.5.1.1.3. Centrifuge Method

The centrifugal data are more reliable than the tea bag method and are occasionally reported in patents and data sheets [55]. Thus, 0.2 g (W_1) of sample is kept in a bag (60×60 mm) made of non-woven fabric. This bag is dipped in 100 mL of saline solution for half an hour at room temperature. Then the bag is taken out and excess solution is removed with a centrifugal separator (3 min at 250 g). After that, weight of bag (W_2) is measured. This experiment is repeated without sample and the weight of empty bag (W_0) is measured. The swelling capacity is calculated by the equation (2.3).

$$Se = (W_2-W_0-W_1)/W_1 \cdots\cdots\cdots\cdots (2.3)$$

Since the inter-particle liquid is noticeably removed by this method, the measured values are often more accurate and lower than those obtained from the tea-bag method values.

2.5.2. Mechanical properties

The mechanical properties can vary and be tuned depending on the purpose of the material. It is possible to obtain a gel with higher stiffness increasing the crosslinking degree or lowering it by heating the material. There are different causes and variables are responsible for changing the mechanical properties of polymeric materials. That’s why different analysis must be made according to the material, the conditions and the aim of the study. For example, if we want to know elasticity of a hydrogel we can use UTM machine but if we want to know viscoelastic properties then we should use a Dynamic Mechanical Analysis (DMA) device or a rheometer. It’s important to note that in a hydrogel, the Young Modulus is the result of the

union between water and gel matrix. If we have to seeds osteoblast cells, we will need a stiffer material than if we culture adipocyte, the same rationale is valid for the development of a heterogeneous prosthetic device, for example, substitute for the intervertebral disc.

2.5.2.1. Measurement of mechanical properties of hydrogels.

Common methods for measuring mechanical properties of hydrogels include tensile and compression testing methods.

Most tensile tests are run at constant extension rates with varying loads until the sample reaches ultimate failure. From tensile testing at various loads, one obtains information about several sample properties. At present, the most commonly used method to determine the mechanical properties of hydrogels is tensile testing. These methods have been extensively used to study the mechanical behavior of various hydrogels [56]. For most uniaxial tensile testing, dumbbell-shaped samples of the polymer are placed between two clamps and one end of the material is pulled away from the other at varying loads and rates of extension. Dies are available to cut samples to the appropriate shape. A dumbbell shape is desirable because it prevents samples from breaking at the clamps where the stress concentration would be high in a uniform strip. In materials without structural defects, a dumbbell-shaped sample should exhibit ultimate fracture at the Centre of the sample. The technique involves applying a tensile force to strips of material held between two grips (Figure 1.9a). Alternatively, the force can be applied to a ring instead of a single strip (Figure 1.9b). Applied force and the elongation of the material are used to obtain a stress-strain chart. This chart can be used to derive several mechanical properties of the hydrogel including Young's modulus, yield strength and ultimate tensile strength. This method can also be used to examine the viscoelastic characteristics of a hydrogel material by elongating the material strip to a particular length and examining the stress relaxation response over time at a constant strain. Figure-2.10 shows the typical mechanical behavior in uniaxial tensile test.

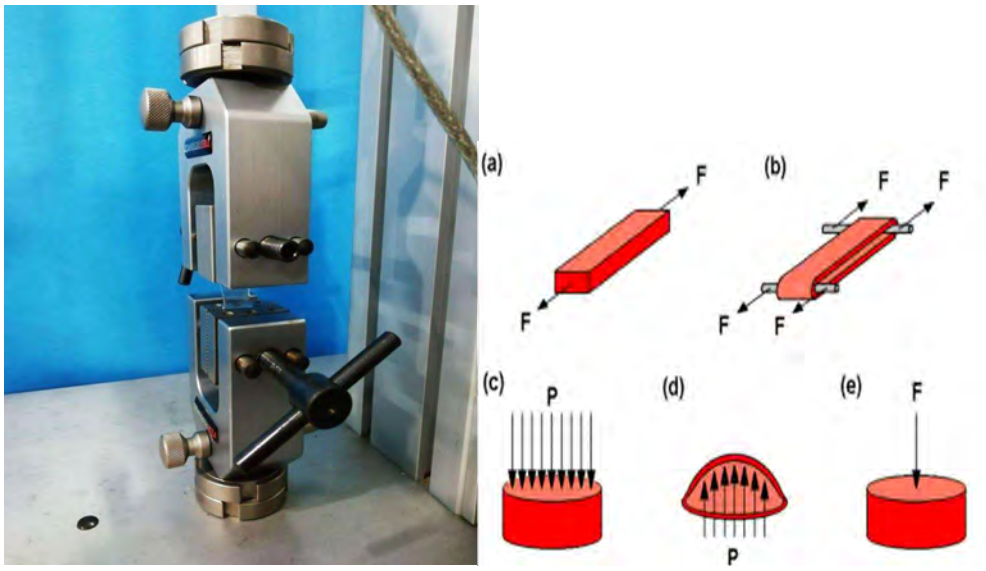

Fig.-2.6: Determination of mechanical properties by UTM machine

Compression test is another technique that has previously been used to examine the mechanical properties of several different types of hydrogels [57]. This technique involves placing the material between two plates and compressing it (Figure 1.9c). The pressure applied to the surface of the hydrogel and distance the hydrogel is compressed, can be used to calculate the mechanical properties of the hydrogels using a theoretical model. One of the advantages of the compression test over extensimeter is that it does not limit the hydrogel geometry to strips or rings although it does require a flat surface. This approach has several limitations including bulging of the hydrogel under compression and difficulty in applying pressure evenly. Bulging can be overcome by confining the hydrogel around its outer edge although this changes the nature of the measurements. A number of studies have used the compression test to examine the mechanical properties of cell-seeded hydrogels.

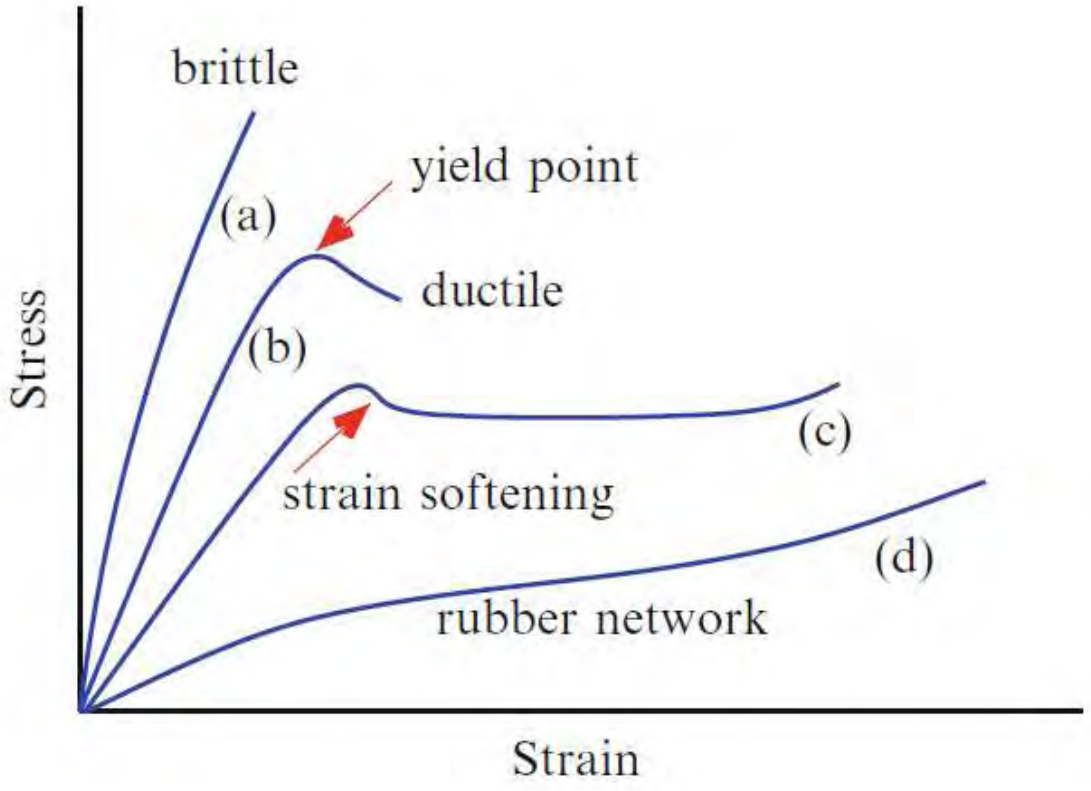

Figure-2.7: Typical stress–strain curves of polymers: curves (a)–(c) represent glassy polymers. The curve (d) corresponds to the rubber state. Ends of the curves indicate the points of material failure: (a) brittle and (b)–(d) ductile

(Fig. curtsey: V. G. Rostiashvili* and T. A. Vilgis. Encyclopedia of Polymeric Nanomaterials-2014)

2.5.2.2. Control of mechanical properties

Once the mechanical characteristics of material have been determined, it is often necessary to improve them in some manner to make the material suitable for the desired application. In this section, we will explore three different ways of controlling the mechanical properties:

altering the co-monomer composition, increasing or decreasing the cross-linking density, and changing the conditions under which the polymer is formed. We will also address the relationship between the hydrogel's mechanical properties and its degree of swelling. It must be remembered that these changes in the polymer will affect not only the mechanical properties but also other behavior of the material.

2.5.2.2.1. Effects of comonomer composition

One of the first and simplest changes that can be performed is altering the composition of the comonomers used in preparing the hydrogel. If the hydrogel is not a homopolymer, then increasing the relative amount of physically stronger components will lead to an increase in the mechanical strength of the final product. Such a change may alter the mechanical strength by increasing the stiffness of the backbone polymer or it may alter the hydrophilicity of the polymer. Additional changes to the comonomer composition can include varying the amount and type of cross-linking agent. For example, the addition of NVP to the HEMA copolymer [58], reduced the Young's modulus, a direct measure of the material's strength of the swollen polymer by an order of magnitude as the NVP content was increased from 5 to 60%. These results are to be expected since NVP is an extremely hydrophilic moiety which significantly increases swelling when compared to HEMA. Increasing the relative content of more hydrophilic monomers will lead to an increased degree of swelling for the resulting hydrogel. This, in turn, will lead to a decrease in the mechanical strength of the polymer.

2.5.2.2.2. Effects of cross-linking density

The mechanical strength of a hydrogel is often derived almost entirely from the cross-links in the system. The strength of the material increases dramatically with increasing cross-linking density. The cross-linking density can easily be increased by the addition of a larger amount of cross-linking agent. But larger number of cross-linking agents create heterogeneities in polymer networks. Generally, this highly cross-linked heterogeneities exist when more than 5% cross-linking agent is present during the radical polymerization. A wide range of studies on the dependence of mechanical properties on the concentration of cross- linking agent have been performed on hydrogels [59]. Poly(acrylic acid) (PAAc) based copolymers hydrogel have been shown to have small-scale heterogeneities [60] which were found to increase as the amount of cross-linking agent was increased. Though the material strength increases as the amount of cross-linking agent increases in these polymerizations, the heterogeneities do result in a reduction in the number of cross-links which are formed. Within these heterogeneities, the cross-linking agent forms a large number of cycles which do not contribute significantly to the mechanical strength of the polymer. Different studies have demonstrated that it is possible to significantly enhance material strength with increases in cross-linking agent concentration [61]. However, when the cross-linking density is altered, changes to properties other than the strength are likely to occur. Diffusivities, and hence release and swelling rates, are likely to be reduced and the maximum degree of swelling is also likely to decrease. These changes would likely affect the desired properties of the material and should be re- examined as the cross-link density is changed.

2.5.2.2.3. Effects of polymerization conditions

The reaction conditions will dramatically affect the final polymer product that is formed. Considerations with regard to the polymerization reaction are the reaction time, temperature and amount and type of solvent. Of particular importance are the type and amount of solvent used during the polymerization. If a large amount of solvent is used during the polymerization, the cross-linking agent will tend to form cycles rather than cross-links. This change will reduce the effective cross-link density, thus lowering the material strength. When the type of solvent or the nature of the solvent (e.g. pH or ionic strength of aqueous solutions) is altered, the copolymer structure may be changed. Since ionic strength and pH affect the reactivity of monomers differently. It is possible that changes in these conditions would convert the copolymer from random to block or cause significant copolymer compositional changes. In general, monomers will be more reactive in their unionized state or when the solution ionic strength is high and shields ion-ion interactions. Recent studies on copolymerization of HEMA and 2- dimethylaminoethylmethacrylate have clearly shown that the relationships between the polymerization conditions and the hydrogel properties exist [62]. Clearly, the structure and nature of the polymer formed in the presence of a large amount of solvent is significantly different from the polymer formed in a bulk polymerization. Other reaction conditions which can be varied include the reaction time and temperature. In the case of photo-polymerizations, the light intensity can be varied as well. With all of these parameters, the functional group conversion can be controlled. Increased double bond conversion lowers the amount of residual soluble fraction and may increase the cross- link density. If hydrogels are to be used without any post-reaction treatments, increasing the double bond conversion can lead to greatly enhanced mechanical strength [62]. Post-reaction treatments can also be quite affective in changing the network structure and the material strength. One method of altering the polymer structure is to add a compound which complexes with the monomer/polymer [63]. Another method of altering the structure is to thermally cycle the polymer [64]. This technique involves the successive freezing and thawing of the polymer. By cycling the polymer in this manner, the Young's modulus of the polymer is significantly increased [65], while many of the other properties of the gel remain relatively unchanged.

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CHAPTER THREE

EXPERIMENTAL

3.1 Materials and Instrument

3.1.1. Chemicals and Reagents

For this research, most of the chemicals were purchased from Sigma Aldrich and Merck, Germany and others are from BDH Chemicals Ltd, UK. The chemicals and reagents used in this research were analytical grade and used without further purification. Deionized water was used as solvent to prepare most of the solutions of this work. The chemicals and reagents which were used in this research are given below:

i. Microcrystalline Cellulose (Sigma Aldrich, Germany)
ii. Sodium metaperiodate (Sigma Aldrich)
iii. Ethylene Glycol
iv. Sodium chloride
v. Sodium chlorite
vi. Hydrogen Per Oxide
vii. Ethanol (Merck, Germany)
viii. Sodium hydroxide (Merck, Germany)
ix. Ammonium per sulfate (BDH)
x. Sulfuric acid (Sigma Aldrich, Germany)
xi. Hydrochloric acid (analytical grade)
xii. Hydrazine (analytical grade)
xiii. Ethanol
xiv. Acrylic acid
xv. N,N'-methelynebisacrylamide (Sigma Aldrich)

3.1.2 Instruments

Analysis of the samples was performed using the following instruments:

i. Fourier Transform Infrared Spectrophotometer
ii. X-ray Diffractometer
iii. Centrifuge machine
iv. pH meter
v. Digital Balance
vi. Freeze dryer
vii. Oven dryer
viii. Digital Balance
ix. Universal testing machine

3.2. Preparation 63.5% H_2SO_4 from 98.0% H_2SO_4

Here 63.5% (W/W) H_2SO_4 was prepared from 98.0 % H_2SO_4-with a density of 1.84 g/mL. So, 100 mL H_2SO_4 was contained 100 × 1.84 = 184.0 g H_2SO_4. Hence, to prepare 100 mL 63.5 % (W/W) H_2SO_4, 48.6 mL 98.0% H_2SO_4 was taken and 51.4 mL water was added to satisfy 100 mL.

3.3. Preparation of NCC through H_2SO_4 hydrolysis

The preparation procedure of NCC through H_2SO_4 was as follows: A round bottom flask with a volume of 500 ml containing 5.0 g Microcrystalline cellulose (MCC) was placed on an oil bath at 60ºC temperature. Then 100 mL 63.5% (w/w) H_2SO_4 was added to the round bottom flask at the ratio of 1 to 20 (MCC to acid). The hydrolysis was carried out for 90 minutes with continuous stirring with a magnetic stirrer. After hydrolysis, the suspension of the product was tenfold diluted to stop further reaction and ultrasonic sound was applied. Then the reaction mixture was allowed to settle for several hours until two-layer is formed-one is for suspension and another for clear water. The clear top layer was decanted off for washing with distilled water until the products were dispersed in the solution. The dispersed suspension was then taken to centrifuge tubes and repeated centrifugation was performed at 2000-4000 rpm for 10 minutes to remove excess acid and water-soluble fragments. The fine cellulose particles became dispersed in the aqueous solution approximately at pH 4. The turbid supernatant containing the polydisperse cellulose particles was then collected for further centrifugation at 4000 rpm for 45 minutes to separate the NCC suspension. The suspension was then washed with distilled water for four to five times which reduced the acid content. Then the suspension was put into regenerated cellulose dialysis tubes having a molecular weight cutoff of 12,000-14,000 to dialyze against deionized (DI) water. The dialysis was stopped when the pH did not change. At 5 ºC the purified NCC was kept in a refrigerator for further characterization.

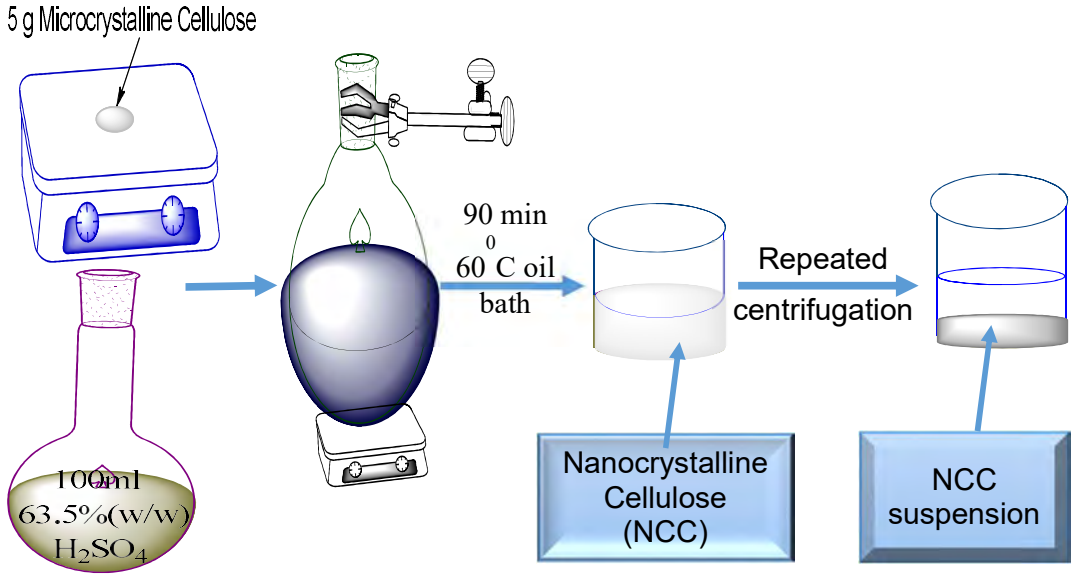


Figure-3.1: Synthesis of nanocrystaline cellulose from microcrystalline cellulose

3.4. Functionalization of NCC

3.4.1 Di-aldehyde preparation by selective oxidation

For selective oxidation NCC to Di-aldehydenanocellulose (DANC) 500 mL 2% (w/v) NCC and 13.38 g sodium periodate was taken. The mixture was stirred with a magnetic stirrer for 20 hours in the absence of light at 48 °C temperature in an oil bath. Excess ethylene glycol was added to stop the reaction after 20 hours. The products were washed with distilled water and separated out from the mixture by repeated centrifugation. Some fraction of the product suspension with aldehyde group was dried either in oven or freeze drier to subsequent characterization. The remaining portion of the product suspension was stored in refrigerator at 5 °C.

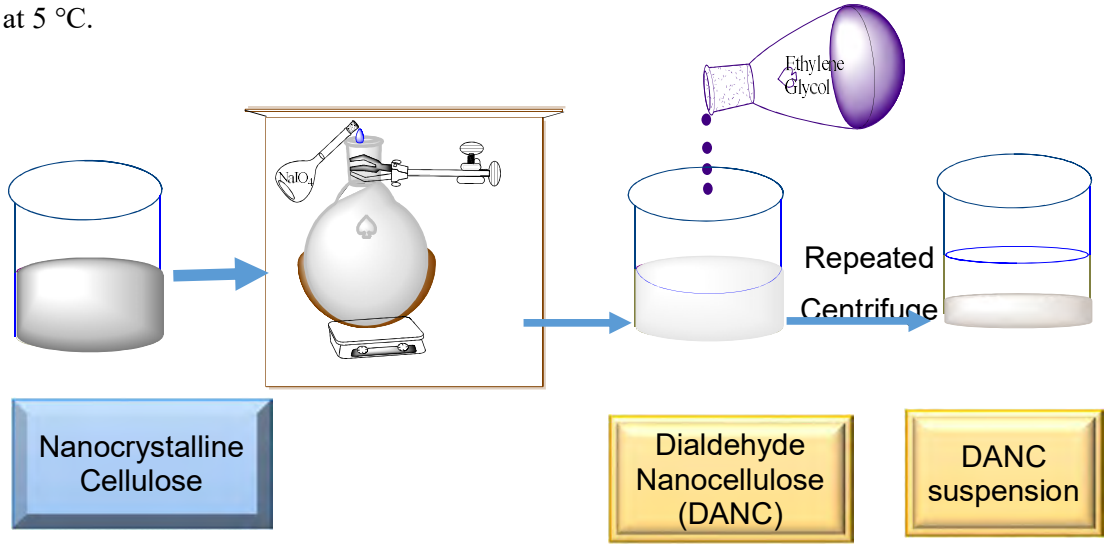


Figure-3.2: Synthesis of dialdehyde nanocrystalline cellulose from nanocrystalline cellulose

3.4.2.a. Determination of carbonyl groups

Determination of aldehyde group content (AGC) (Oxime formation method):

AGC was determined according to the method of Veelaert et al [7]. The pH of DANC suspension (0.50 g oven-dried) was adjusted to 5.0, and then hydroxylamine hydrochloride solution (20 mL, 0.05 g/mL) was added to the system.

$$-CHO + NH_2ON{\cdot}HCl \rightarrow -CHNOH + HCl + H_2O;$$

$$HCl + NaOH \rightarrow NaCl + H_2O;$$

$$H = \frac{30V}{m}(\text{mmol g}^{-1});$$

After stirring for 4 h at 40°C, the suspensions were titrated against 0.01 M NaOH standard solution until the pH value was 5.0. The control experiment of NCC was carried out under the same conditions. The aldehyde group content was calculated using the following equation

$$\text{AC } (\%) = \frac{MNaOH \times (Vc \quad)}{m/160} \text{.....(3.1)}$$

Here,

Vc = The volume consumption of NaOH solution in liter.

V_b = The same concentration of NCC suspension at pH 3.5 was used as a blank and its volume consumption of the alkali solution in liter was recorded.

M = Dry weight of DANC sample in g and

160 = Approximate the molecular weight of repeating unit in DANC.

Each set of the test was done in triplicate.

3.4.2. Dicarboxylated nanocellulose preparation

In a round bottom flask 150 mL (1.223 g) DANC was taken and 4.0 g NaCl, 2.0 g $NaClO_2$ and 5.0 mL H_2O_2 were added into the suspension. The mixture was kept for 24 hours at a speed of 400 rpm with a magnetic stirrer to complete the reaction. The reaction mixture turned into yellow after that regio-selective oxidation. Then ethanol was slowly added to the reaction mixture which formed transparent gelatinous precipitate. To separate these precipitate centrifugation was done. Further ethanol was added until precipitation was not observed.

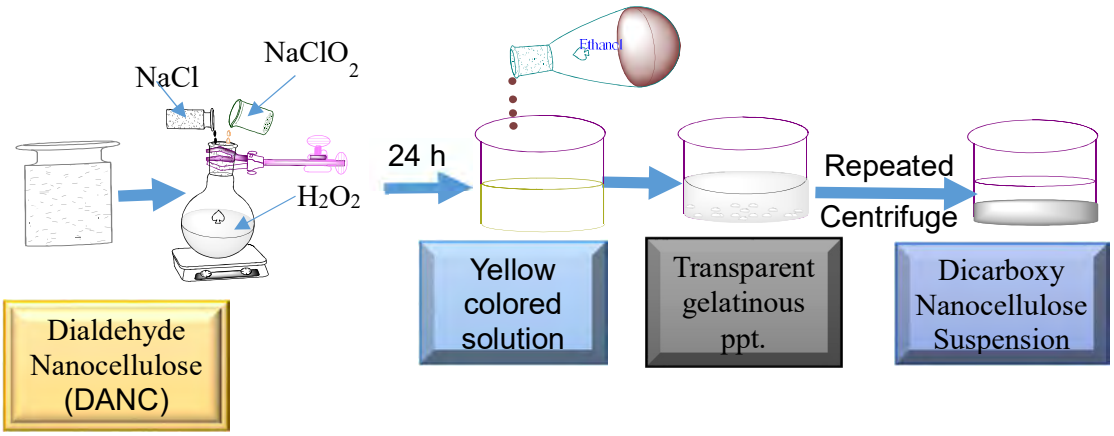


Figure-3.3: Chlorite oxidation of dialdehyde nanocrystalline cellulose to dicarboxy nanocrystalline cellulose

3.5. Preparation of hydrogel

Hydrogels were prepared by a free radical polymerization using DCNC and acrylic acid monomer in the presence of ammonium persulfate as initiator and N,N-methylenebisacrylamide (BIS) as a cross-linker. 1.2 mg (0.058 wt. %) DCNC and 8 mg (0.12 mol %) initiator were bubbled with N_2 gas to make the solution free from oxygen. In another container the aqueous solution of 2.058 g (.029 mol) monomer and 4 mg (0.089 mol %) cross-linker was also bubbled with N_2 gas. Then two solutions were mixed immediately and transferred to a glass cell separated with a Teflon spacer of desired shape and size. The polymerization reactions were carried out at 60-70 °C temperature for 24 hours. Then hydrogels were washed by distilled water several times for purification and further

characterization. This reaction procedure was optimized by changing different factors such as amount of monomer, amount of solvent, varying the carboxylate group of DCNC, pH of the reaction media etc. with the respect to swelling ratio and mechanical strength. The different types of hydrogels were prepared here are (a) poly(acrylic acid) hydrogel / PAAc hydrogel (b) poly(acrylic acid –graft- Nanocrystalline cellulose) hydrogel / (PAAc-NCC) hydrogel (c) poly(acrylic acid) -graft- Dialdehydenanocellulose hydrogel / (PAAc-DANC) hydrogel following same procedure, In all four types of hydrogel common monomer was acrylic acid..

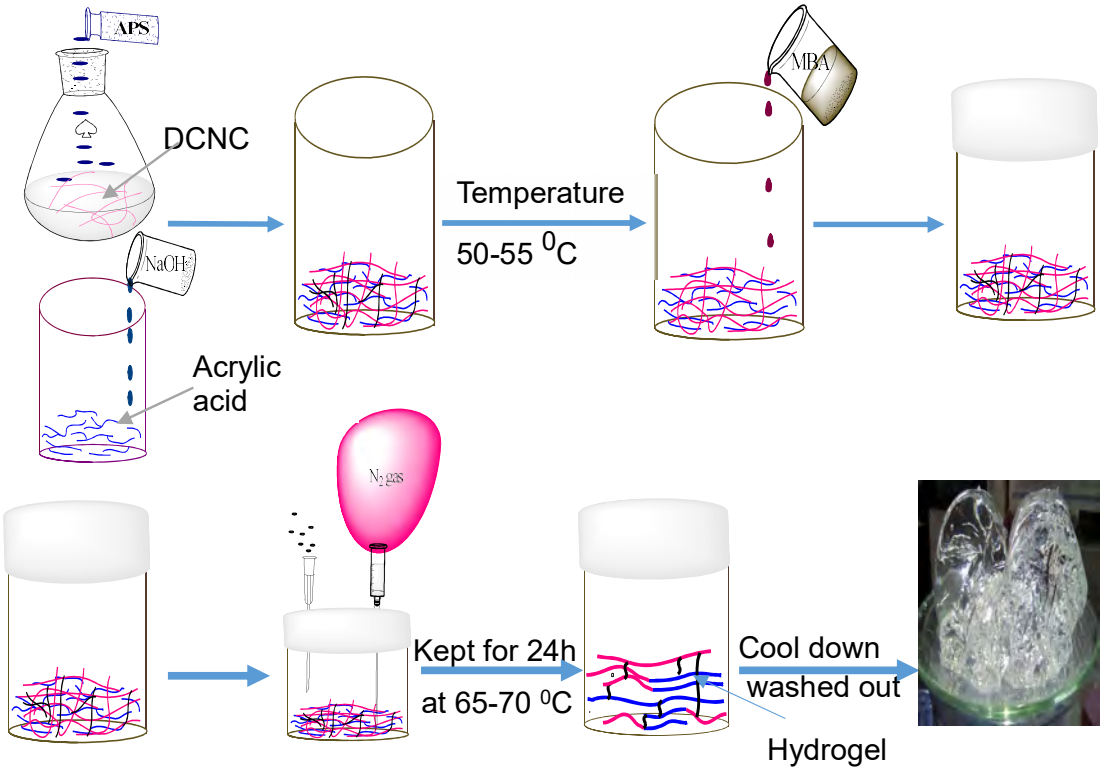


Figure- 3.4: Synthesis schemes of hydrogel

3.6. Characterization:

3.6.1. Fourier transform infrared measurement

The synthesized NCC or modified NCC and samples were dried in a vacuum oven at 50°C for 24 hours and then cooled to room temperature for Fourier transform infrared (FTIR) analysis. The oven dried film of NCC and its modified films were analyzed by single bounce diamond ATR (Attenuated Total Reflectance) accessory and the freeze dried samples were pressed into KBr pellets (1:200). Transmission mode of FTIR spectra were collected with Shimadzu IR spectrometer. Spectra were obtained in 400-4000 cm^{-1} range and for each sample 90 scans were taken at a resolution of 4 cm^{-1}.

3.6.2. Hydrazine test

The functionalization of glucose moiety was confirmed by 2,4-dinitro phenyl hydrazine test. In three different test tubes much diluted amounts of NCC, DANC and DCNC were taken to test functional groups present in the glucose moiety of the cellulose.

3.6.3. Scanning electron microscopy

Morphological analysis was performed using Field Emission Scanning Electron Microscopy (FESEM). A drop of dilute suspension was deposited on small piece of carbon strip and allowed to dry at vacuum drier at 40 °C. The sample surface was coated with a thin layer of gold to provide electrical conductivity. The sample was then placed in the main SEM chamber to view its surface. The system was computer interfaced and thus provides recording of the surface images in the computer file for its use as hard copy.

3.6.4. Grafting percentage

When the grafting reaction was complete, the produced hydrogel was removed from the spacer and washed several times with distilled water and was kept it in methanol for 24 h to extract the residuals monomer and homopolymer such as acrylic acid in the hydrogel. After 24 h that grafted hydrogel was dried in a vacuum oven at 50°C for 24 h and weighed. The percentage of the grafting was determined by using the following equation:

$$GP(\%)=\frac{Wg-Wo}{Wo}\times 100 \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3.2)$$

Where Wg and Wo are the weights of the grafted and un-grafted hydrogel respectively

3.6.5. X-ray diffraction (XRD)

It is well known that the mechanical properties of hydrogel were strongly dependent on the monomer crystallinity and crystal structure. To determine the crystal structure and crystallinity, XRD patterns of the hydrogel were measured by automated powder X-ray diffractometer. Before testing, freeze dried powder samples were dried in a vacuum oven at 50°C for 24 h to remove moisture. The powder samples were pressed in a square aluminum sample holder (40mm X 40mm) with a 1 mm deep rectangular hole (20mm x 15mm) and pressed against an optical smooth glass plate. The upper surface of the sample was labeled in the plane with its sample holder. The sample holder was then placed in the diffractometer. The WXRD data were generated by a diffractometer with Cu-Ka radiation (λ = 1.542˚ A) at 40 kV and 30 mA over the range 2θ = 10°–50°, a size step of 0.02°, and a time step of 2.0 s, (1.0 h per scan)

The degree of crystallization was determined using the method of X-ray Crystallinity index. The formula below was used in calculating degree of crystallization.

$$\text{Crystallinity index (\%)} = \frac{Area\ of\ all\ crystalline\ \ peaks}{Area\ all\ peaks\ (Amorphous+Crystalline)} \times 100 \ldots\ldots\ldots\ldots (3.3)$$

3.6.6. Determination of swelling ratio

Here, swelling ratio was determined by the combination of tea bag method and filtration method using deionized water. To measure water uptake capacity a tea bag was made with filter net. A certain amount of hydrogel was inserted, which represents the exact mass m_1. To ensure the reliability of the results, three individual tea-bags were prepared per sample. The tea-bag containing the sample was hung in a beaker filled with the deionized water (about 200 mL). The beaker covered with aluminum foil to avoid evaporation of the water. After 24, 48, 72, 96, 120 and 144 h the contact of the tea-bag (with the hydrogel inside) was removed and weighed (mass m_2). The tea-bag was placed on a dry cloth and gently wiped with another dry cloth for a short time of approximately 30 s to remove surplus and weakly bound liquid. However, in order not to disturb the sorption degree, the sample should neither be squeezed nor come into contact with the cloths longer than necessary. After weighing, the tea-bag containing the hydrogel was returned into the refilled stock solution until the next time step of mass recording. Equation-1 provides the formula to calculate the absorption capacity (**W**) at each time of readin**g.**

$$W = \frac{m_2 - m_1}{m_1} \quad \text{..... (3.4)}$$

3.6.7. Determination of mechanical properties.

Here two different shapes of hydrogels viz. cylindrical and film shapes were prepared for the investigation of mechanical behavior of the hydrogels. Both compressive and tensile test were conducted on a universal testing machine (UTM). For compressive test, cylindrical shape samples were cut with initial length of l_0 (= 6 mm) and original radius of r (=5 mm) The stress (from the initial cross–section A ($A=\pi r^2$) and strain curve were recorded. The stress (σ) was calculated according to the equation $\sigma = F/\pi r^2$, where F was the recorded load. The compression tests were performed at room temperature with a compression rate of 10 mm/min. The strain (ε) was calculated from the change of the fracture length (l) to the initial gauge length (l_0) of the measured sample and was calculated by the equation $\varepsilon = l/l_0 \times 100$. For tensile tests, the samples were cut from equilibrated gel sheets into dumbbell shapes with initial gauge length of 10 mm and width of 6 mm. The tensile tests were performed at room temperature with a stretch rate of 100 mm/min. The young modulus was calculated from the initial slope of the stress−strain curve. The fracture toughness and ductility were calculated by integrating the area underneath the stress−strain curve of each sample.

CHAPTER FOUR

RESULTS & DISCUSSION

4.1. Preparation and characterization of NCC from MCC

4.1.1 Preparation of NCC through H_2SO_4 hydrolysis

There are different factors for acid hydrolysis including reaction time and temperature, acid concentration, the ratio of MCC to acid and the effect of applied external energy (ultrasonic). All these factors were examined during the hydrolysis process. The main focus was to optimize the conditions needed for the production of transparent gel of NCCs. The ultrasonic energy was applied after the reaction to break down the aggregates which increase the transparency of nanocrystals and promote the efficiency of acid hydrolysis. To prepare transparent NCC gel without complete hydrolysis a set of experiments were performed. The white powder form of NCC like MCC were formed when sulfuric acid concentration was less than 60%. But blackish products were found when acid concentration was more than 70% gave blackish products due to the carbonization of cellulose. These findings gave us an indication to choose 64% sulfuric acid concentration for hydrolysis purpose and at this concentration, the product formed to look like almost transparent gel [1].

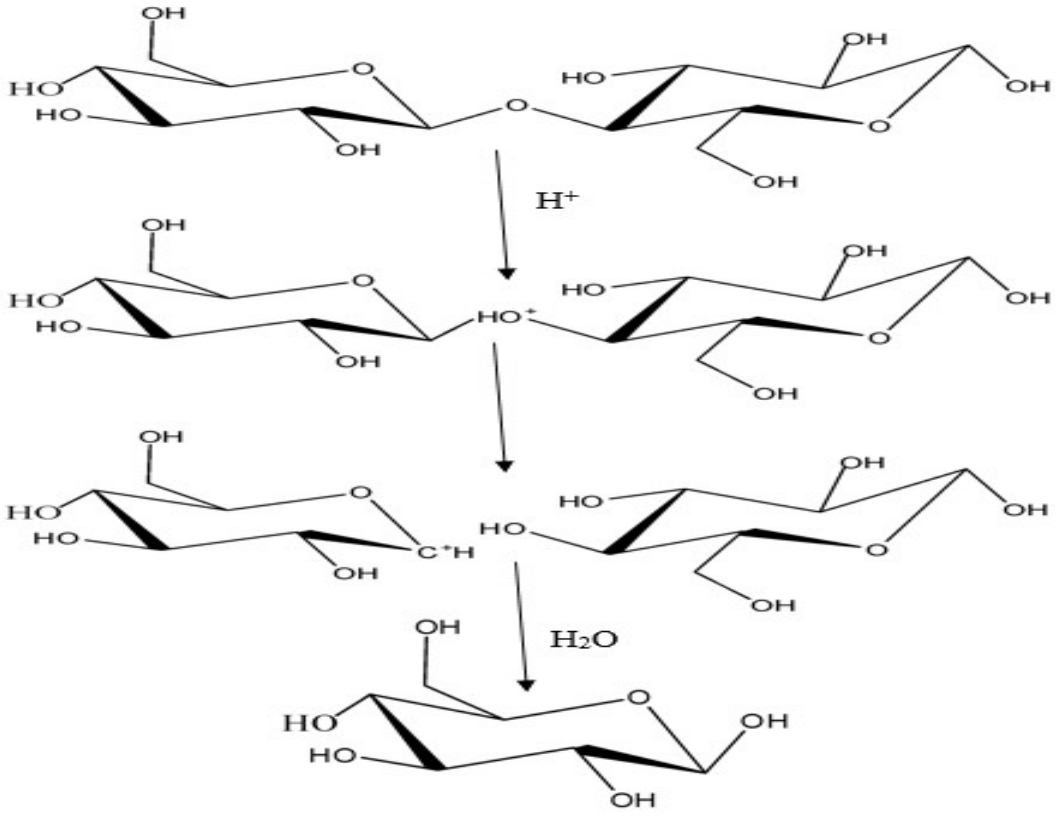


Fig. 4.1: Mechanism of acid-catalyzed hydrolysis of MCC by cleavage of β-1-4- glycosidic bond.

Similarly, other factors such as acid to MCC ratio, the temperature of the hydrolysis and time for hydrolysis were also investigated earlier in our research group [2]. These investigations clearly showed that the hydrolysis reaction should be carried out at 60^{o}C temperature for 90

minutes with 64% (w/w) H_2SO_4 at the acid to MCC ratio 20: 1 [3]. Although the application of ultrasonic energy after hydrolysis has been documented [1], the mechanism of the ultrasonic effect has not been clearly understood. Cellulose can form intra and inter molecular hydrogen bonds.

That is why they have a strong tendency to agglomerate to form larger particles. The aggregate formation manifested itself especially in the absence of surface charges when the repulsion forces between the individual nanocrystals were minimized. The agglomeration can be decreased by introducing surface charges on the surface of CNCs. Sulfuric acid is known to introduce negative charges on the surface of the CNCs via an esterification reaction with the sulfate anions [4-6].It has already proved that at high temperature (~100^{o}C) and under strong acid, hydrolysis of cellulose gives glucose or even carbonization [7, 8]. But under relatively mild conditions in the present study, the chemical degradation mechanism can be acid-catalyzed cleavage of β-1-4-glycosidic bond [9]. This degradation process of MCC has proceeded in three steps. Firstly,

a. The glycosidic oxygen linking two sugar units is rapidly protonated under acidic conditions, forming a conjugate acid.
b. Then the cleavage of the C–O bond and breakdown of the conjugate acid to the carbonium ion take place.
c. After a rapid addition of water, the resulted segments and a proton are liberated.

Assuming that the cleavage of the C–O bond takes place more rapidly at the end than in the middle of the polysaccharide chain, more monosaccharides and lower yields of NCC have resulted.

4.1.2. Characterization of prepared NCC through H_2SO_4 hydrolysis

To investigate the size and surface morphology of NCC, FESEM analysis was conducted.

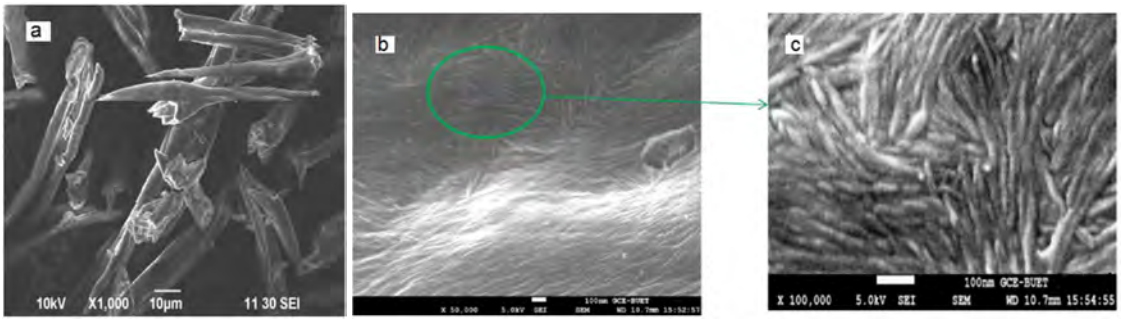


Fig 4.2: (a) FESEM image of MCC and (b) and (c) FESEM image of NCC

The diameter and length of fibrils of cellulose were reduced to nano-scale due to the removal of most the amorphous region of microcrystalline cellulose after hydrolysis. Fig. 4.2(a) shows the FESEM micrographs of a very dilute suspension of NCC, showing agglomerated 'cross-linked fiber' nanocrystals. From fig. 4.2 (b) it can be illustrated that the diameter of nanocrystals had wide range of distribution but the size of most of the 'cross-linked fiber' nanocrystals lies within the range 100-300 nm in length and 10-20 nm in diameter. Compact agglomeration of CNCs shows that cellulose chains have an intermolecular hydrogen bonding

and a strong hydrophilic interaction in between the cellulosic chains. On the other hand from Fig. 4.2(c), it can be explained the criteria for starting material MCC. It can say that MCC particles were irregular shapes and their average diameter was about 10 μm and the length was about 50 μm. The irregular shape of MCC compares to NCC comes for the more amorphous region. From the above discussion, it can be claimed that microcrystalline cellulose was successfully converted into nanocrystalline cellulose.

4.2. Preparation and characterization of functionalized nanocellulose

4.2.1. Preparation and characterization of dialdehyde nanocellulose (DANC)

Sodium meta-periodate is a selective reagent which converts the vicinal hydroxyl group to the aldehyde group. Periodate oxidation converts 1, 2-dihydroxyl groups (glycol) to paired aldehyde groups without significant side reactions [10, 11]. During the oxidation, 1 mol of NCC theoretically consumes 1mol of $NaIO_4$ [12].

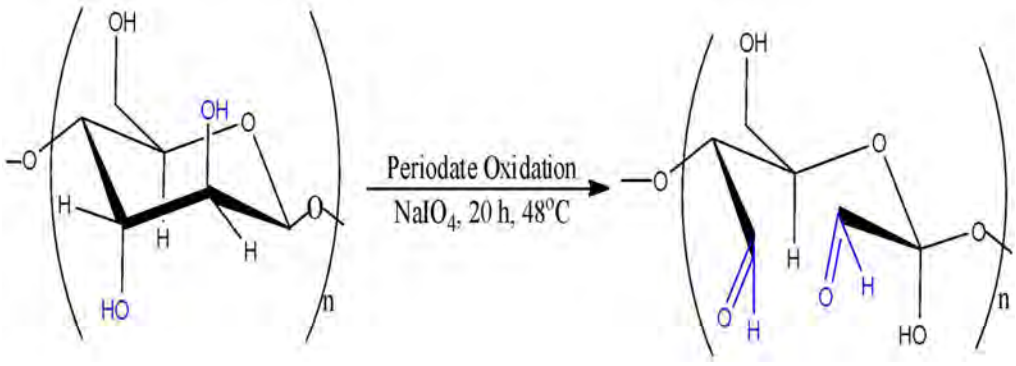


Fig.-4.3: Periodate oxidation of NCC to DANC

Oxime formation method was used to determine the degree of oxidation, which corresponds with the aldehyde content (AC). Since cellulose is polymer, only surface hydroxyl groups will react with the oxidant and 100 % conversion is not possible. In this study, it was found that about 40% conversion of vicinal hydroxyl groups to dialdehyde group through oxime formation method. Here different factors such as temperature, reaction time, concentration of the reactants strictly controlled. This oxidation is temperature sensitive and the increasing temperature is favorable for the periodate oxidization at the beginning due to the nature of the endothermic reaction. However, if the reaction temperature is higher than 55°C, decomposition of periodate will predominate thus the effect of the oxidant will lost more or less. That's why 48°C temperature was chosen for the periodate oxidation. Again if the reaction times exceed more than 20 h less amount of aldehyde content is found due to the decomposition of aldehyde containing molecules. But if time is less than 20h then less amount of hydroxyl group converts to aldehyde group. The presence of dialdehydic group was confirmed from the FTIR spectra (Fig. 4.4) as well as by the classical functional group test with hydrazine reagent gave orange precipitate (Fig.4.5).

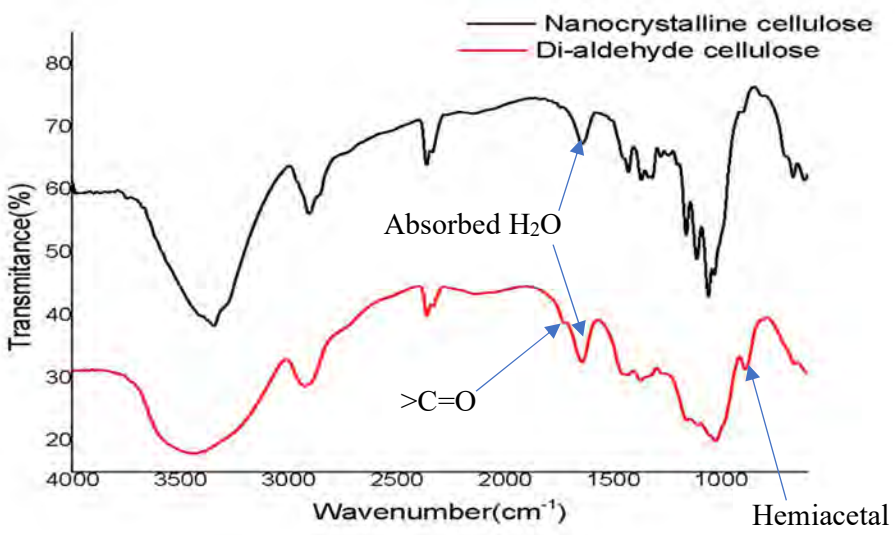


Fig.4.4: FTIR spectra of Nanocellulose and Dialdehyde Nanocellulose

In Fig. 4.4 the FTIR spectra of prepared nanocrystalline cellulose and periodate oxidized DANC are shown. The broad peak near 3400 cm^{-1} and the peak at 1300 cm^{-1} is due to the stretching and bending vibration of –OH groups respectively [13]. On the other hand, the peak at 2900 cm^{-1}, 1430cm^{-1} and 1020 cm^{-1} are assigned to -C-H stretching vibration, -CH_2 scissoring and -CH_2-O-CH_2- stretching respectively [14]. It is clear that two characteristic IR bands at ~1738 cm^{-1} (very weak) and 890 cm^{-1} regions appear in DANC. The carbonyl groups can hardly be detected by spectroscopic methods such as FTIR spectroscopy [15], likely due to hydration and acetalization effects although high degrees of oxidation can be obtained by periodate oxidation. Severe drying down to 7% relative humidity and high temperatures eventually increased the C = O vibrations [16, 17], which indicated a large extent of hydration of the carbonyl groups in these substrates. Other explanations are strong cross-linking by the formation of hemiacetals with neighboring hydroxyl groups. Cellulose has both reducing end and non-reducing end. The reducing end can form a hemiacetal bond from aldehydic group and an adjacent hydroxyl group. As a result, NCC always shows a weak peak at ~890 cm^{-1}. During oxidation, the intensity of that peak increased because there are more available aldehyde groups to form hemiacetal bonds with neighboring hydroxyl groups which is consistent with the reported FTIR spectra of periodate oxidized cellulose [15, 12, 18]. Generally, the peak near at 1740cm^{-1} is characteristic of aldehydic carbonyl groups but the band around 890 cm^{-1} is assigned to the formation of hemiacetal bonds between the aldehyde groups and neighbor hydroxyl groups. The results indicate that the carbonyl group has been introduced into the structure by selective periodate oxidation of NCC.

Again conversion of hydroxyl group to carbonyl group can be confirmed by 2, 4-dinitrophenyl hydrazine test. Carbonyl compound forms hydrazone with hydrazine which gives yellow precipitate. Here in figure 4.5, yellow precipitate confirm the conversion of hydroxyl group to aldehyde group.

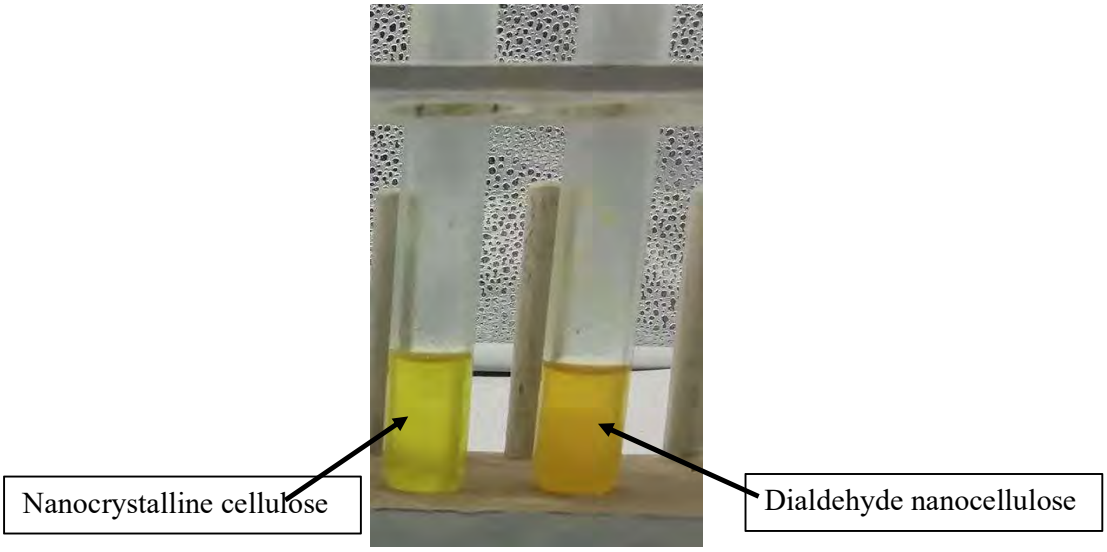


Fig. 4.5: Hydrazine test for carbonyl group detection of dialdehyde nanocellulose

4.2.2. Preparation and Characterization of dicarboxylated nanocellulose (DCNC)

DANC was used for the preparation of 2,3-dicarboxy cellulose. An aqueous suspension containing DANC was further oxidized with sodium chlorite and acetic acid in a reaction vessel kept at 48°C with vigorous stirring. For oxidation of aldehyde group to carboxyl group, chlorite (ClO,) and hydrogen peroxide were used according to the oxidation conditions.

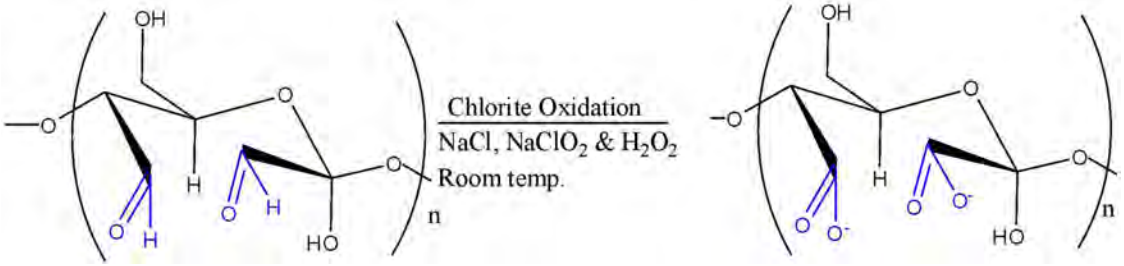


Fig.-4.6: Chlorite oxidation of DANC to DCNC

The functionality of the chlorite-oxidized dicarboxylated cellulose was confirmed by the disappearance of the aldehyde bands at 1738 cm^{-1} and weakening of the intensity of hemiacetal signal at 890 cm^{-1} and the appearance of new band at 1650 cm^{-1} in FTIR spectra in figure 4.7 [19]. This new band is associated with dissociated forms of carboxyl groups, which indicate the formation of the dicarboxylic group containing celluloses.

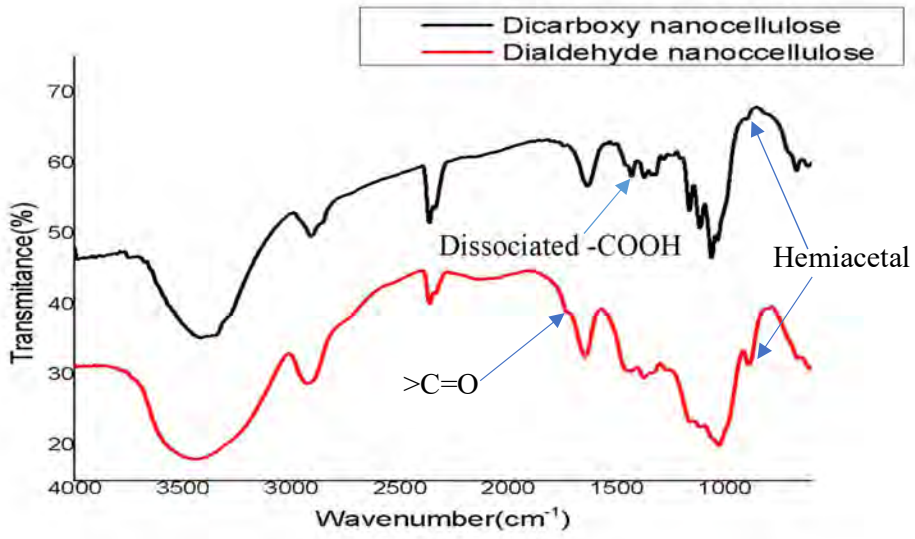


Fig.4.7: FTIR spectra of NCC and Dicarboxylated NCC

Again, the classical functional group test with hydrazine reagent gave orange precipitate for aldehyde group but clear transparent solution was formed due to carboxylate group.

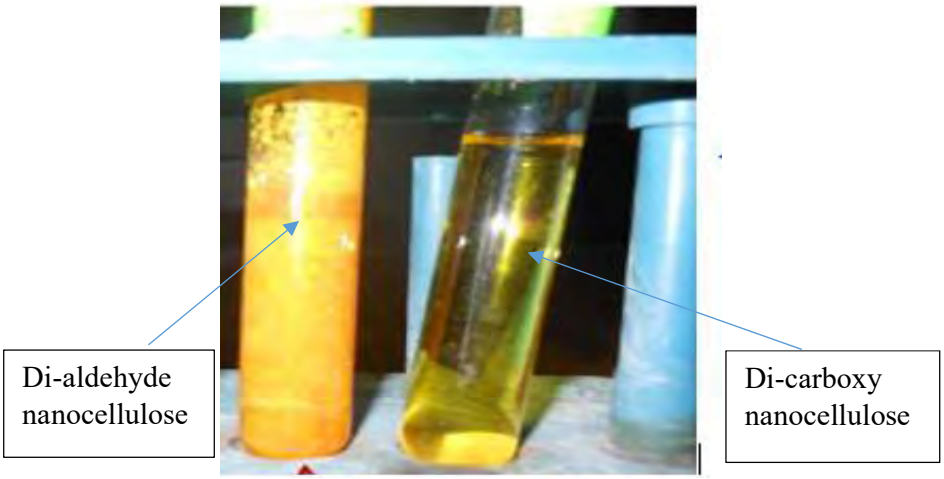


Fig.4.8: Hydrazine test for carbonyl group confirmation of DANC and Dicarboxylated NCC

4.3. Preparation and characterization of dicarboxylated nanocellulose-poly(acrylic acid) based hydrogel

4.3.1. Preparation of dicarboxylated nanocellulose-poly(acrylic acid) based hydrogel

Here, the hydrogel was prepared by a free radical mechanism. At first free radicals has formed by heating from ammonium persulfate as sulfate free radicals. These free radical can hit on hydroxyl group of dicarboxylated nanocellulose to form free radicals. Dicarboxylated nanocellulose free radical then bonded with acrylic acid to form another intermediate free radical which transforms to hydrogel when cross-linked with MBA. During the preparation

of hydrogel, different conditions were executed to synthesize hydrogels such as pH and monomer composition. It has been seen that in the presence of 0.029 mol PAAc monomer, the formation of hydrogel was better than other composition. Above this composition, DCNC nanoparticles were aggregated therefore hydrogel lost its homogeneity. Hence polymerization was not upright. On the other hand, below this composition, DCNC couldn't bring a remarkable change in the hydrogel matrix.

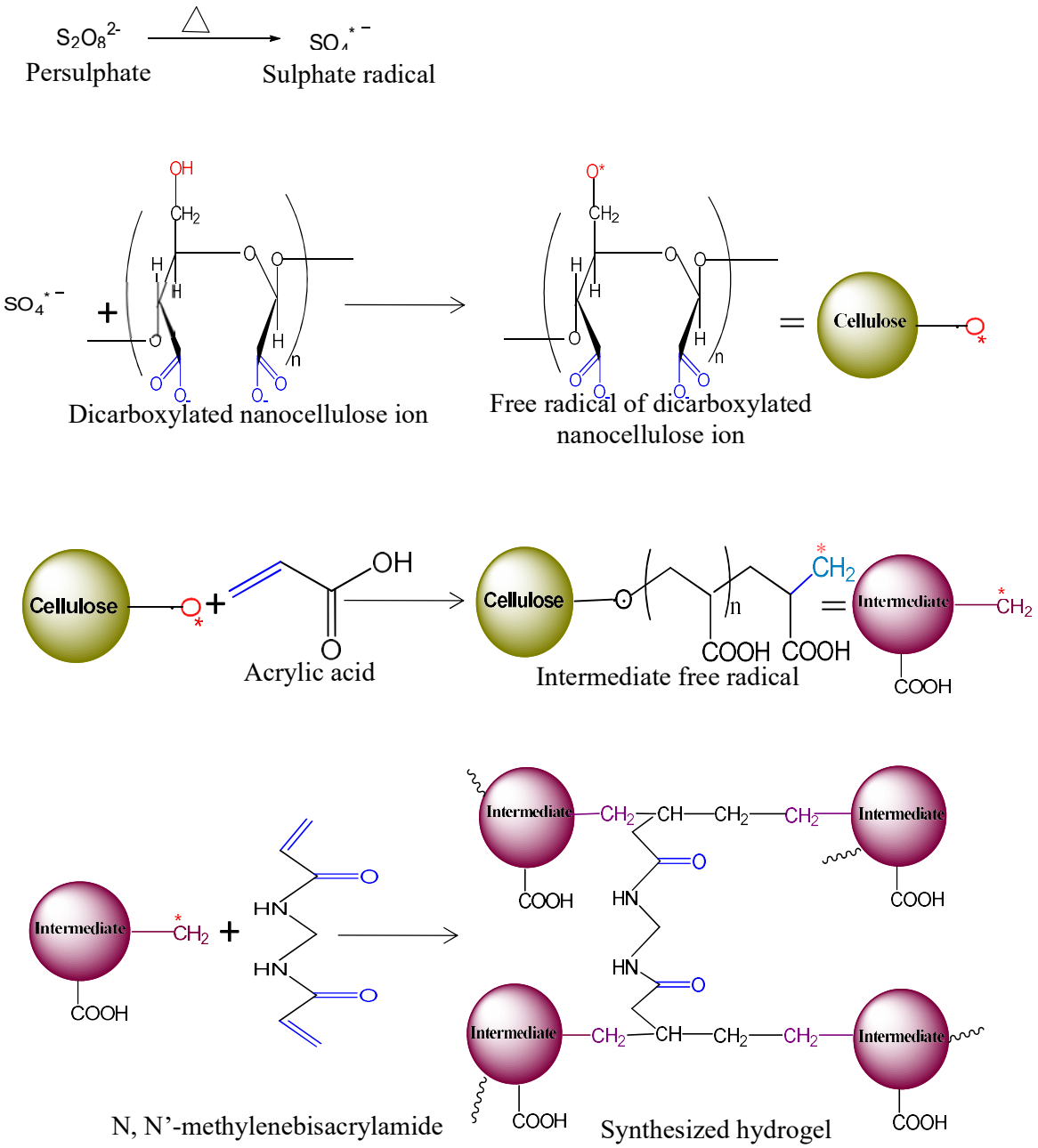


Figure-4.9**:** Proposed model mechanism for synthesis of hydrogel based on functionalized nanocrystalline cellulose.

Table-4.1: Optimization of monomer effect on polymerization

Serial no.	DCNC (wt. %)	AAc (mol)	APS (mol %)	MBA (mol %)	pH
1	0.058	0.021	0.12	0.089	3.0
2*	0.058	0.029	0.12	0.089	3.0
3	0.058	0.036	0.12	0.089	3.0
4	0.058	0.043	0.12	0.089	3.0
5	0.058	0.050	0.12	0.089	3.0
6	0.058	0.057	0.12	0.089	3.0

* Mark composition was respectable

Table-4.2: Optimization of pH for polymerization

Serial No.	DCNC (wt. %)	AAc (mol)	APS (mol %)	MBA (mol %)	pH
1	0.058	0.029	0.12	0.089	2.0
2*	0.058	0.029	0.12	0.089	3.0
3	0.058	0.029	0.12	0.089	4.0
4	0.058	0.029	0.12	0.089	5.0
5	0.058	0.029	0.12	0.089	6.0
6	0.058	0.029	0.12	0.089	7.0

* Mark composition was respectable

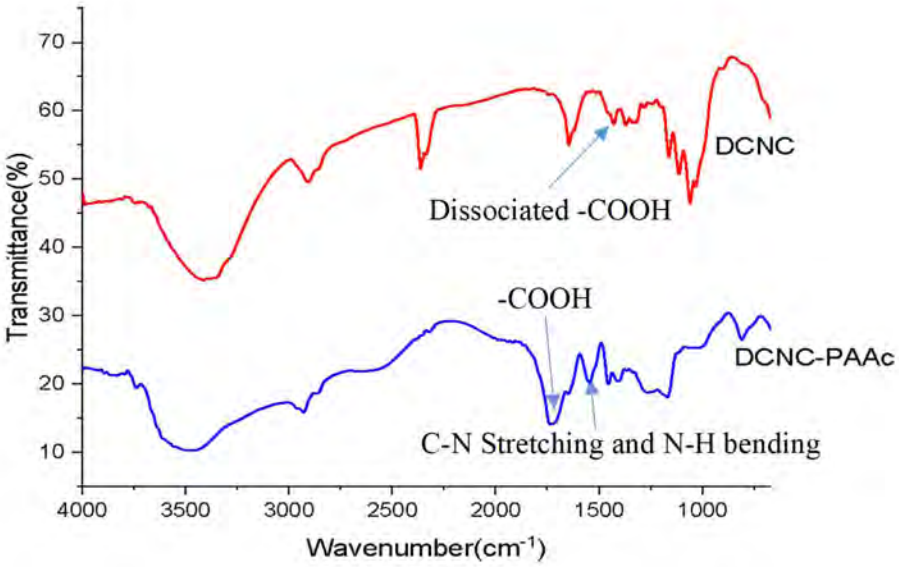
Again, pH effect was witnessed on the polymerization process. It has seen that pH-3 has a better impression on the polymerization process. Above pH-3, the charge screening effect was perceived due to protonation of carboxylate group. Therefore, molecules go too far from each other's. As the pH has increased their distance also increased. Hence, polymerization was not favorable at higher pH [20]. Again, at lower pH, carboxylate group containing monomer remains in compact condition. So, it was hard for them to go polymerization reaction [21].

Here other type of hydrogels were prepared are (a) poly(acrylic acid) hydrogel / PAAc hydrogel (b) poly(acrylic acid) - Nanocrystalline cellulose hydrogel / (PAAc-NCC) hydrogel (c) poly(acrylic acid) – Dialdehyde nanocellulose hydrogel / (PAAc-DANC) hydrogel following same Mechanism.

4.3.2. Characterization of dicarboxylated nanocellulose-poly(acrylic acid) based hydrogel

In spectra for DCNC-PAAc, a new band at 1550 cm^{-1} has induced which was undoubtedly associated with stretching vibrations of C–N groups and bending vibrations of N–H [22].

The sharp band at 1727 cm^{-1} has been assigned to stretching vibration of COO^-, which indicated the presence of carboxyl groups in the DCNC-PAAc hydrogel network. The new band at 1727 cm^{-1} and 1550 cm^{-1} has induced for PAAc and MBA respectively, which indicate that polymerization process has occurred between DCNC and PAAc through MBA cross linker.



4.10: FTIR spectra of DCNC and DCNC-PAAc

4.4. Grafting Percentage:

The grafting reactions were conducted by varying the DCNC concentration from 0.3 to 1.2 wt. % . It was observed that the grafting percentage increased as the concentration of DCNC was increased from 0.3 to 0.6. On further increasing the concentration grafting percentage decreased. Because initially with an increase in the DCNC concentration primary free radicals are formed in greater numbers thereby increasing the grafting percentage but beyond the cited concentration of DCNC, the formation of homopolymer increases as evident from the values. Due to the higher concentration of DCNC the viscosity of the reaction medium was higher. This restricts the movement of monomer molecules to the active sites on the backbone, thereby decreasing the grafting percentage [23-25].

Table -4.3: Grafting percentage (%) depending monomer on DCNC concentration

S.N.	DCNC (0.6mg/mL)	AAc (1.029g/mL)	Grafting percentage (%)
1	1.0	2.0	103.33
2	2.0	2.0	115.27
3	3.0	2.0	108.38
4	4.0	2.0	92.23

4.5. XRD Data Analysis:

The crystalline state of DCNC in the gel network was examined with the help of the XRD pattern. The diffractograms of both DCNC and DCNC-PAAc polymer were shown in Fig.-4.11DCNC exhibited a peak around 2θ=16.5° and 22.5° and 34.6° which are supposed to represent the typical cellulose structure. The cellulose crystals exhibit characteristic assignments of 110, 200, and 004 planes, respectively [26-29]. The DCNC has similar characteristic peaks in the same position. For the XRD pattern of DCNC-PAAc, the peak near 22.5 almost disappeared while a new peak arose at 32.5, which is indicating that the dicarboxylic nano-cellulose has been lost its crystallinity when incorporated in the polymer network. Meanwhile, the characteristic peak shifted and became broadened. These results exhibited a decrease in the crystallinity of the DANC due to polymerization**.**

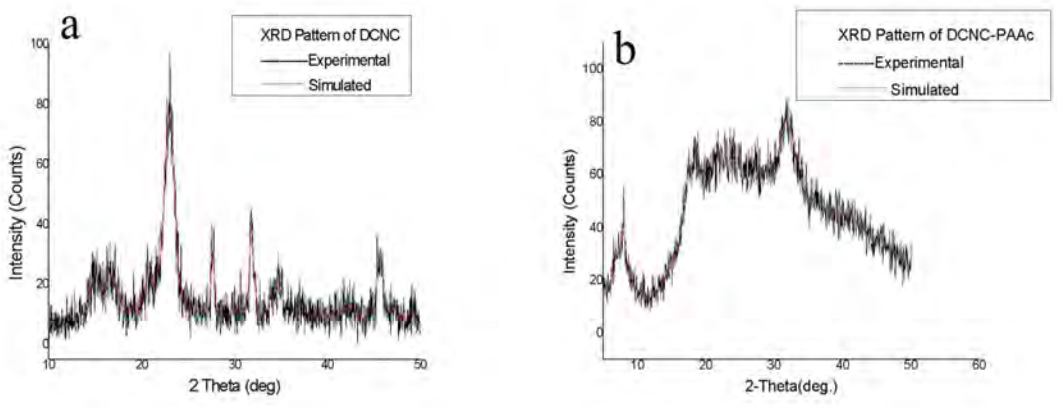


Fig.-4.11: XRD diffraction patterns of (a) the DCNC and (b) DCNC-PAAc hydrogel

4.6. Measurement of swelling property

Fig. 4.12 shows swelling characteristics of the prepared gel. The figure shows the swelling as a function of time for PAAc, NCC-PAAc, DANC-PAAc, and DCNC-PAAc. At the equilibrium time, the maximum swelling characteristics were observed for the DCNC-PAAc. There are two factors act here for showing maximum swelling ratio by DCNC-PAAc hydrogel.

First, there are large amounts of active groups, COOH group (water affinity COOH > CHO > OH) on the surface DCNC, which make the hydrogels more hydrophilic [30].

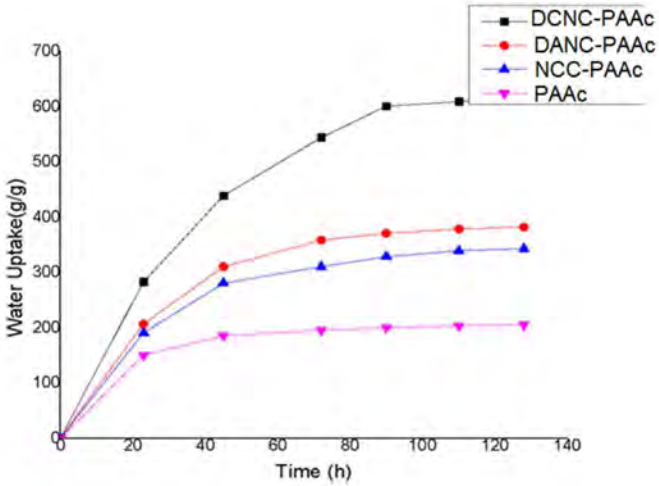


Fig.-4.12: Water uptake capacity of different hydrogels

Secondly, pH of the solution which vastly effects the water uptake capacity of the hydrogel especially for pH sensitive hydrogels.

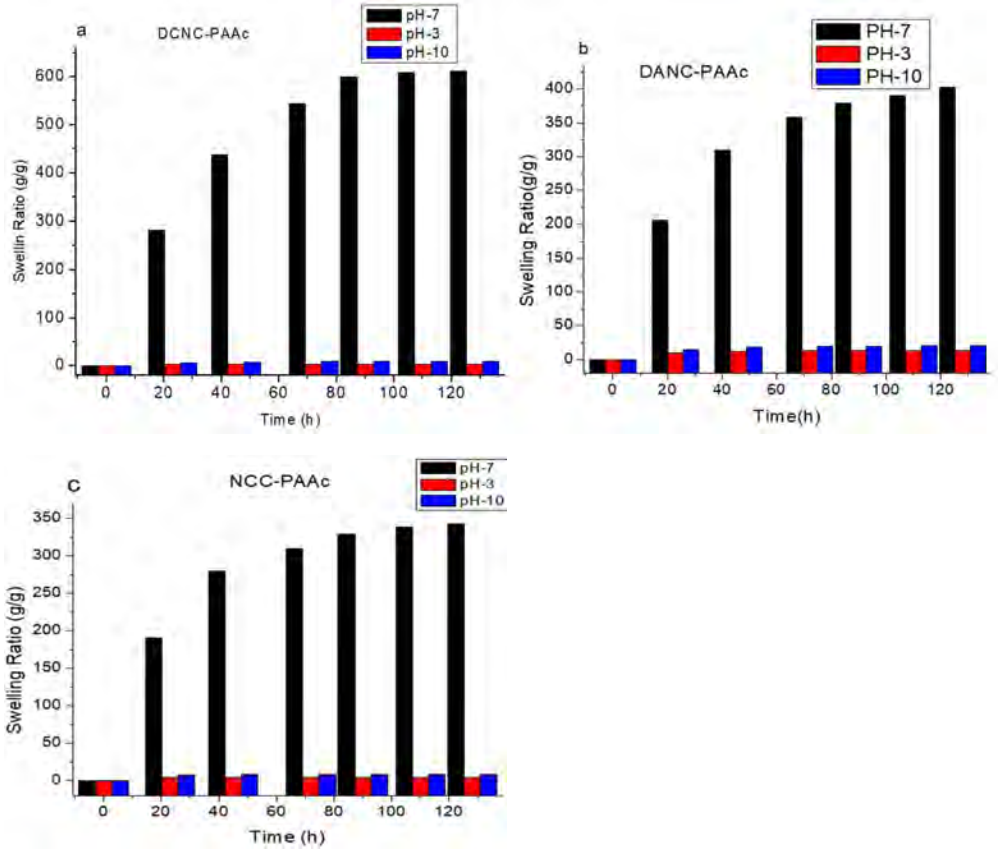


Figure-4.13: Effect of pH on water uptake capacity; (a) DCNC-PAAc, (b) DANC-PAAc, & (c) NCC-PAAc

The DCNC-PAAc nanocomposites contain substantial carboxylate groups (from DCNC and PAAc) and thus are mainly anionic-type absorbents. Under acidic conditions (pH < 5), most of the carboxylate groups are protonated (COO-⟶ COOH), which strengthen the hydrogen bonding interactions and generate an additional physical crosslinking in the network [31, 32]. Meanwhile, the electrostatic repulsions of the carboxylate are restricted and the network tends to shrink, and thus the swelling capacity is decreased. When pH is higher (6 < pH < 10), the hydrogen-bonding interactions are broken with decreasing effect of the H+, besides, the reinforcement of the repulsions among the carboxylate groups also make the hydrogels swell more. However, in highly basic solutions (pH > 10), the charge screening effect of the Na+ counter ions in the swelling medium prevents effective anion-anion repulsions and leads to decreased water absorption [33].

4.7. Mechanical properties of hydrogel

(a) Tensile test

Comparative mechanical properties of PAAc, NCC-PAAc, DANC-PAAc and DCNC-PAAcare shown in the figure-4.14. From the figure, it can be seen DCNC-PAAc hydrogel has the highest mechanical properties as compared toother hydrogels. In this case, two effects could be considered for higher mechanical properties of DCNC-PAAc hydrogel. One is the functional group effect and another one is the crystallinity effect.

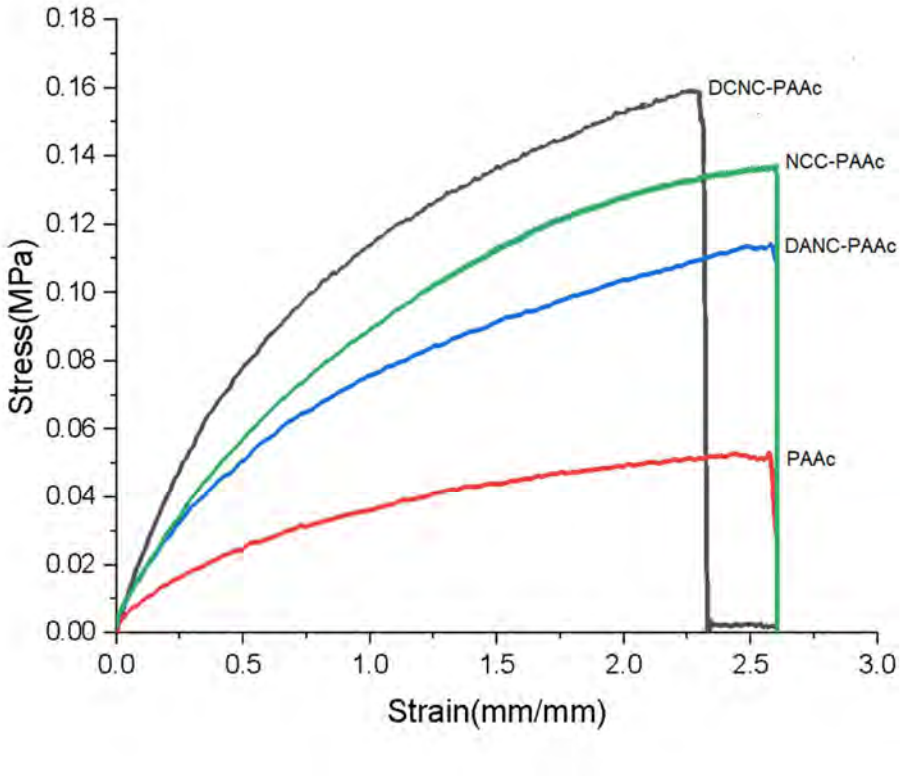


Fig. 4.14: Stress-strain curves for different hydrogels in case tensile test

Among DCNC, NCC, DANC & PAAc; DCNC contains additional carboxyl group. These extra carboxyl group in their matrix which strengthens the hydrogen-bonding interactions and

generate additional physical crosslinking in the network [34]. Again, the degree of crystallinity can have a significant influence on the mechanical properties because it affects the extent of the intermolecular secondary bonding. For crystalline regions in which molecular chains are closely packed in an ordered and parallel arrangement, extensive secondary bonding typically exists between adjacent chain segments. This secondary bonding is much less prevalent in amorphous regions, by virtue of the chain misalignment. As a consequence, for semi-crystalline polymers, tensile modulus increases significantly with the degree of crystallinity [35]. The order of crystallinity is NCC > DANC > DCNC [2]. But here mechanical properties of hydrogels maintain following order – DCNC-PAAc>NCC-PAAc>DANC-PAAc>PAAc. Because functional group effect has been dominated over the crystallinity effect.

In table-4.4 a comparison in mechanical properties such as Young modulus, toughness and tensile strength of different hydrogels are given.

Table-4.4: Mechanical properties of the different hydrogel in case of tensile test

Sample name	**Tensile strength (kPa)**	**Young's modulus (kPa)**
DCNC-PAAc	160.0	210.5
DANC-PAAc	124.0	170.0
NCC-PAAc	144.0	170.0
PAAc	53.0	90.2

(b) Compression test

Here, the result of the compression test of different hydrogels are mentioned graphically and the result is given in the table. From figure-4.15 it is seen that DCNC-PAAc hydrogel has higher compressive strength and toughness than PAAc based hydrogel but lower than NCC-PAAc and DANC-PAAc (NCC-PAAc > DA-PAAc). It is due to the effect of crystallinity in the polymer matrix and functional group. It is known crystallinity in a polymer modifies the modulus curve of amorphous polymeric materials by at least two mechanisms [36,37].

First, the crystallites act as cross-links by tying segments of many molecules together. Second, the crystallites have very high moduli compared to the rubbery amorphous parts, so they behave as rigid fillers in an amorphous matrix. Hard particles will stiffen into a soft matrix far more than that will a hard Matrix. We know from the literature review the order of crystallinity of cellulose and modified cellulose as NCC > DANC> DCNC >PAAc (amorphous).

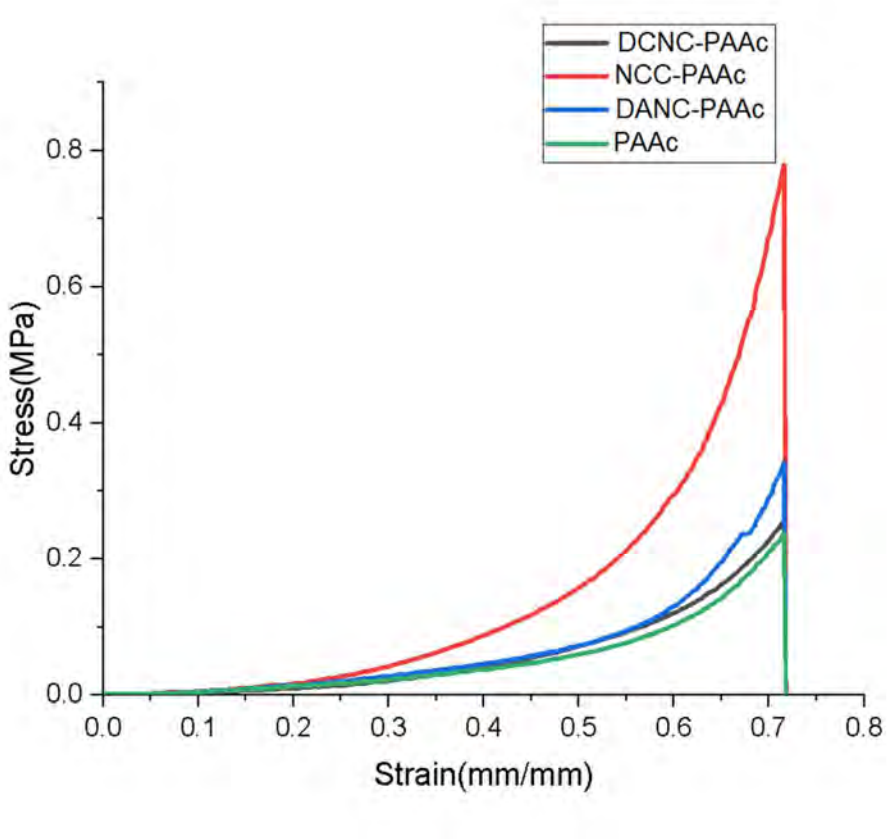


Fig. 4.15: Stress-strain curves for different hydrogels in the case compression test

As a result, it has been found following mechanical properties in compression test listed in the table-4.5.

Table-4.5: Mechanical properties of the different hydrogel in case of compression test

Sample name	Young's modulus (kPa)
DCNC-PAAc	40.35
DANC-PAAc	50.44
NCC-PAAc	60.52
PAAC	37.44

Here, the crystallinity effect has been dominated over the functional group which is vice versa in the tensile test.

4.8 Effect of different factors on mechanical properties of hydrogel

Once the mechanical properties of a material have been determined, it often needs to be optimized for the best results. Different factors viz. the amount of DCNC, carboxyl group density and the pH of the reaction medium under which the polymer is formed was changed and different outcomes were observed. It is expected that these will also affect other qualities of the material along with mechanical properties.

4.8.1. Effect DCNC content on hydrogel matrix

One of the first and simplest changes that were performed altering the composition of the comonomers used in preparing the hydrogel. In present work, hydrophilic DCNC contents are altered to see the effect of it on the polymeric network as well as mechanical properties.

Table-4.6: Composition of different hydrogel with varying amount of DCNC

Sample	DCNC (2mg/ml)	AAc (1.029g/mL)	MBA (4mg/mL)	APS (8 mg/ mL	H_2O (mL)	Total (mL)
PAAc	0.00	2.00	1.00	1.00	1.50	
DCNC-PAAc-1	0.25	2.00	1.00	1.00	1.25	5.50
DCNC-PAAc-2	0.75	2.00	1.00	1.00	0.75	
DCNC-PAAc-3	2.00	1.50	1.00	1.00	0.00	

From figure -4.16 we can see as the composition of DCNC is increased then it has been found elongated curves. Because with the increase of DCNC content hydrophilicity also increased in the polymer network. Therefore more water molecules entered into polymer network.

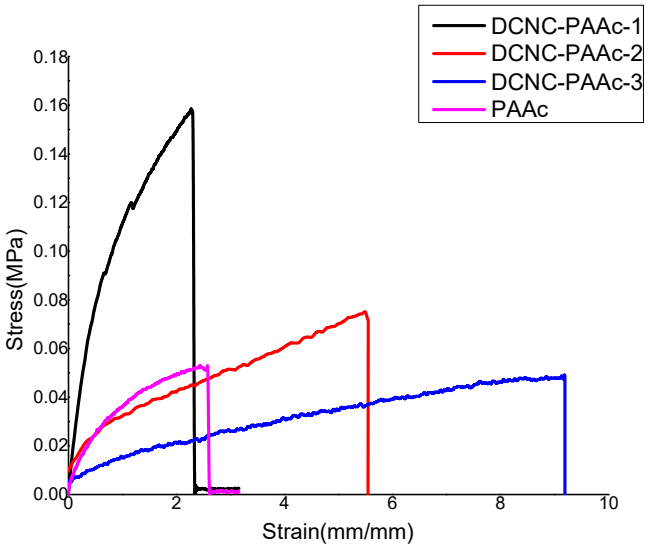


Fig.4.16: Stress-strain curves for hydrogel at different concentration of DCNC

As water molecules are surrounded to the network, polymers cannot form intra and inter molecular hydrogen bonds in their network. It forms hydrogen bond with water as –COOH group has great attraction to water.

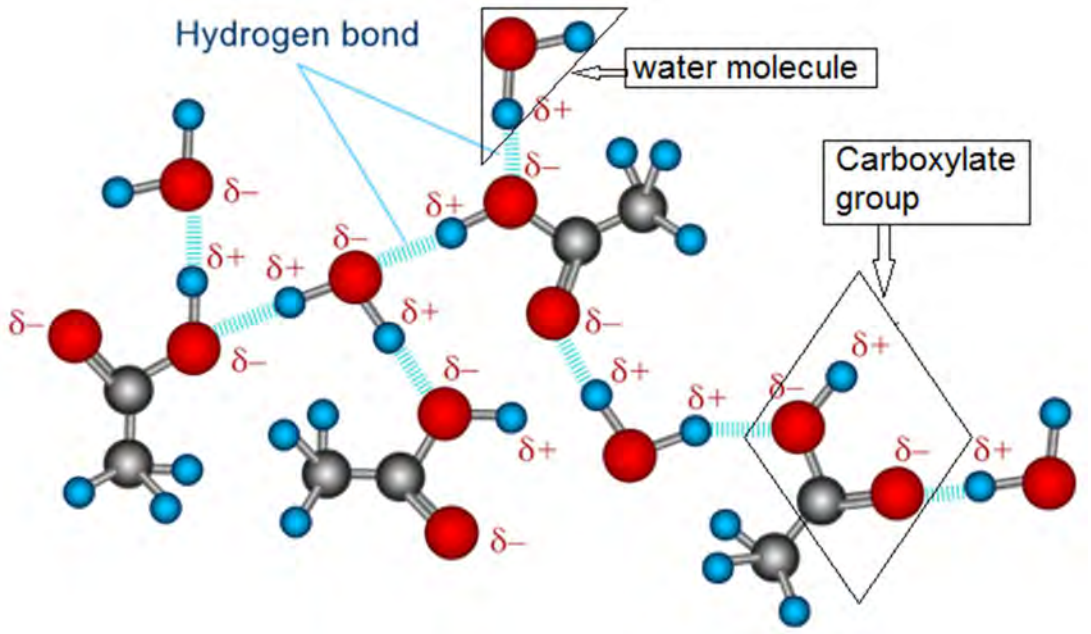


Figure- 4.17: Hydrogen bond formation of carboxyl group with water molecule

That's why we get an elongated curve with lower mechanical strength but high toughness as like as an elastomer.

4.8.2. Effect of pH of the reaction medium

The environment of the reacting medium has a great effect on polymerization process. Actually, it controls the polymerization reaction especially for pH-sensitive hydrogel [38].In present work, the influence of pH on polymerization process as well as mechanical properties is investigated. To investigate the effect of pH of reaction media on mechanical properties, DCNC-PAAc hydrogels were produced maintaining the pH value 3, 5 & 7. A pH of 3.0 was obtained simply by adding monomer with cross linker without any NaOH. Other pH values were obtained by the addition of NaOH (pH - 5.0 & 7.0).

From figure-4.18 it can be seen that at lower pH value of reaction medium the ultimate tensile strength and modulus is higher but ductility is lower. As the pH has increased, the material becomes less rigid and exhibits a larger deformation to rupture and lower ultimate strength. Because with the increasing pH value carboxyl group becomes deprotonated.

$$-COOH \xrightarrow{pH>3} -COO^- + H^+$$

Due to increasing negative charge repulsion force introduce on the polymer matrix. Therefore they get far from each other. As a result, inter and intra molecular hydrogen bond cannot form among the polymer matrix. Therefore polymer easily deforms when a tensile force is applied. Hence, ultimate tensile strength and modulus become lower. As they can easily deform their elongation is higher. That's why their ductility is higher [39].

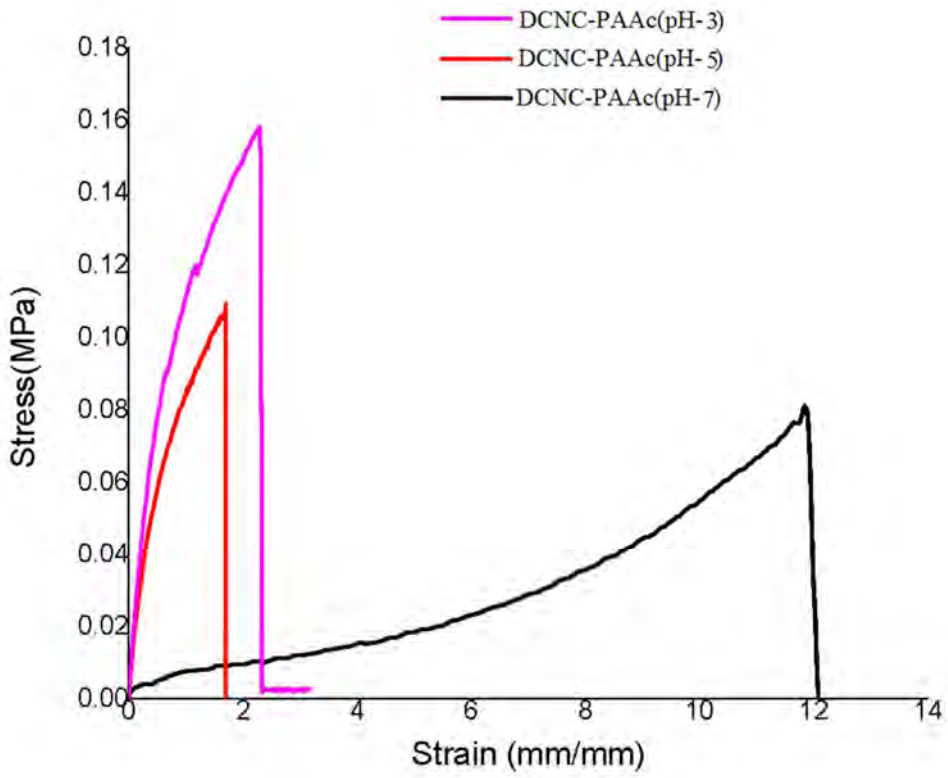


Fig.4.18: Stress-strain curves for hydrogel at different pH of reaction media

4.8.3. Effect of carboxyl group density

Another important factor of the mechanical properties of polymeric hydrogel is the density of carboxyl group in its network. From figure 4.20, we have noticed that as the percentage of carboxyl group increases in polymeric network mechanical properties such as tensile strength, modulus, toughness etc. increases. Because of carboxyl group forms intra and inter molecular hydrogen bond at acidic medium. The relative stiffness and rigidity of the DCNC molecule come from these hydrogen bonds. This property is reflected in its high tendency to crystallize, and its ability to form fibrillar strands. The chain stiffness property is further favored by the β-glucosidic linkage that bestows the linear form of the chain. The chair conformation of the pyranose ring also contributes to chain stiffness. Actually, carboxyl group has an -OH and an O^-. The sharing can occur between an -OH and another OH. Or an OH with the double bonded -O-. The presence of the extra O allows every carboxyl group to form three hydrogen bonds simultaneously.

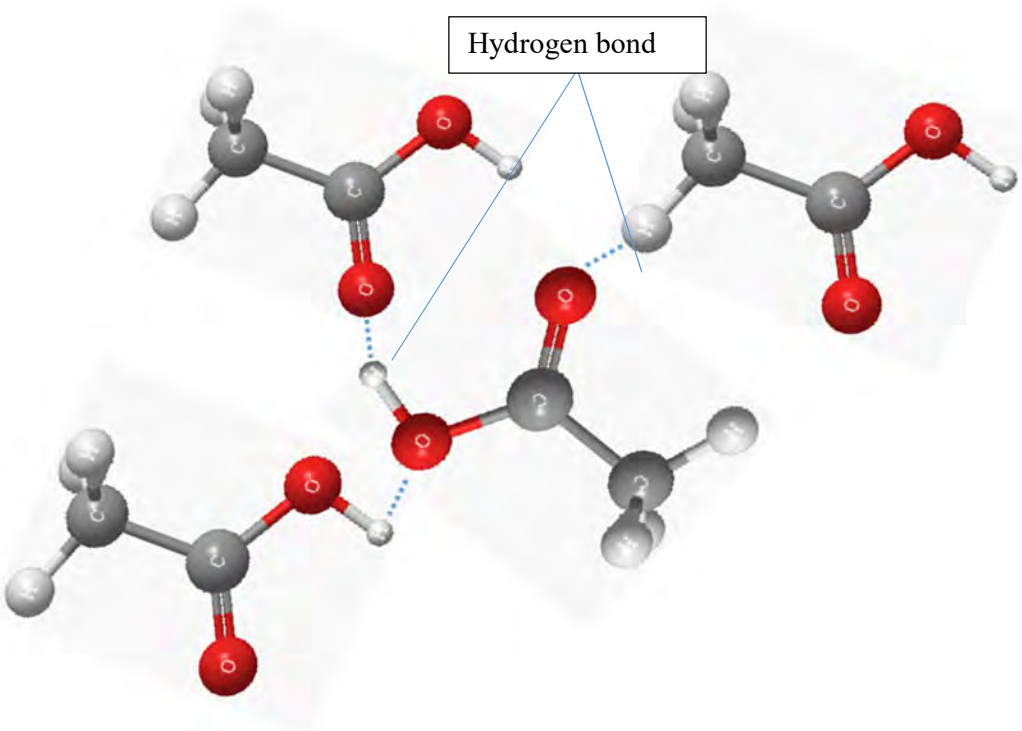


Figure-4.19: Formation of three hydrogen bond by carboxyl group

This allows carboxyl acids functionalized nanocellulose to have higher stiffness. As the percentage of carboxyl group increases additional intra and inter molecular hydrogen bonds are formed. Therefore, polymeric chain stiffness increases which contribute to mechanical properties of final products, hydrogels. The result are shown in the figure-4.20.

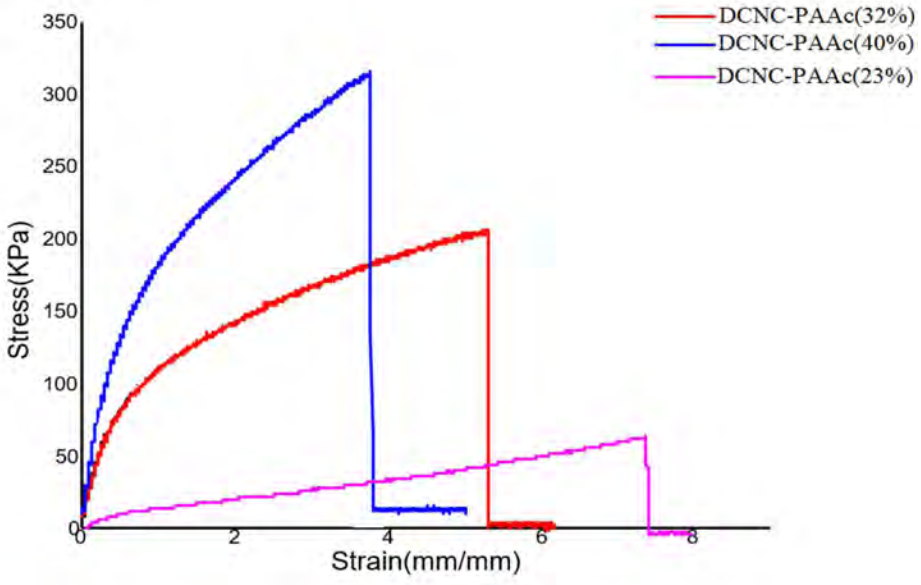


Fig.-4.20: Stress-strain curve of different hydrogel with varying carboxyl group content.

4.9. Conclusion :

a. In this research, MCC was successfully converted to DCNC with sodium chlorite via NCC and DANC. The conversion of microcrystalline cellulose to nanocrystalline cellulose was confirmed by FESEM micrographs. Again, the formation of carbonyl and carboxyl functional group on cellulose nanocrystal matrix was confirmed by different chemical methods and FTIR data analysis.

b. DCNC-PAAc based three-dimensional cross-linked hydrogels using N, N-methylenebisacrylamide (BIS) cross-linker were prepared successfully through free radical polymerization. The polymerization of DCNC and PAAc was confirmed by FTIR data analysis. The XRD results showed that DCNC and DCNC-PAAc are crystalline in nature where the crystallinity index of DCNC-PAAc was decreased compare to DCNC. In XRD the characteristic peak shifted and became broadened. These results exhibited the decrease in the crystallinity of the sample**.**

c. The swelling properties also investigated to determine the swelling ratio of the hydrogel matrix. It has been seen that the swelling ratio of synthesized hydrogel was tunable. It was seen when the hydrogel was tuned with sodium hydroxide it showed higher water uptake capacity up to 780g by per gram of its original weight.

d. Mechanical properties were determined by performing UTM machine. Remarkable strength, modulus and toughness of DCNC-PAAc hydrogel were found in the study of mechanical properties using UTM. It has been seen that the mechanical properties of synthesized hydrogel were tunable. It can be tuned by changing it different factors viz. DCNC content, pH of reaction content and carboxylate group density etc.

i. DCNC content has an impact on mechanical properties and with increasing DCNC hydrogel showed elastomer like behavior.
ii. The reaction medium has been controlled with pH from 3 to 7. At pH-3, monomer was highly polymerized. Therefore, tensile strength and young's modulus were high. With increasing pH of reaction medium it has turned to an elongated polymer with higher toughness.
iii. Carboxylate group has a great impact on mechanical properties. It has been seen that as the carboxyl group increases mechanical properties also increase. Because with increasing carboxylate group more hydrogen bonds have been made among the polymer network.

Therefore mechanical properties of hydrogel can be tuned considering different factors. It has been seen that DCNC-PAAc hydrogel has shown excellent tensile strength, Young's modulus and toughness of 160.0 kPa, 210.3 kPa and 251.34 kJ/m^{3}respectivly while PAAc showed 53.0KPa, 90.2 kPa and 97.4 kJ/m^3 respectively. On the other hand, in compression test Young's modulus and toughness of DCNC-PAAc are 41.03 kPa and 130.07 kJ/m^3 respectively but for PAAc are 37.03 kPa and 52.88 kJ/m^3.

So, it can be summarized as fabricated DCNC-PAAc hydrogel has higher mechanical properties including tensile and compression strength and modulus, toughness, ductility etc. than acrylic acid based hydrogel.

4.10 References

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