DEVELOPMENT OF BIO-DEGRADABLE PACKAGING FILM OF NANOCELLULOSE REINFORCED PVA FOR FOOD PACAKGING APPLICATION USING AGRO WASTE SUGARCANE BAGASSE

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF

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Submitted by

CHHAVI VERMA

2K16/PTE/02

Under the Supervision of

PROF. ARCHNA RANI

Department of Applied Chemistry

Delhi Technological University, Delhi-110042

Bawana Road, Delhi-110042

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DECLARATION

I **ChhaviVerma,** (2k16/PTE/02) of M.Tech. in Polymer Technology, hereby declare that the project dissertation titled "**Development of bio-degradable packaging film of Nanocellulose reinforced PVA for food packaging application using agro waste sugarcane bagasse"** which is submitted by me to the department of Applied Chemistry**,** Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology**,** is original and not copied from any other source without proper citation. This work has not been previously formed the basis for the award of any degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi

Chhavi Verma

Dated:

CERTIFICATE

I hereby certify that the project Dissertation titled "**Development of bio degradable packaging film of Nanocellulose reinforced PVA for food packaging application using agro waste sugarcane bagasse"** which is submitted by **Chhavi Verma (2K16/PTE/02)** in partial fulfillment for the award of degree of Master of Technology in Polymer Technology to Delhi Technological University, Delhi, is a record of the project work carried out by her under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or diploma of this university or elsewhere.

Signature of Supervisor

Place: Delhi

NAME OF SUPERVISOR**: Prof. Archna Rani**

Date:

Head of the department

Department of Applied Chemistry
Delhi Technological University
New Delhi -110042

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At the end I would like to express my sincere thanks to all my friends and others who helped me directly or indirectly during this project work.

Chhavi Verma

ABSTRACT

Sugarcane bagasse is the matter that remains after stalks of sugarcane are crushed to extract the juice. This is utilized as a reinforcing material in the matrix of Poly (vinyl alcohol),a synthetic polymer to develop a nanocomposite bio-degradable packaging film for few dairy products (milk, paneer and curd). The bagasse is acid hydrolyzed to obtain nano cellulose which is then used at different weight ratios to reinforce PVA films. Further turmeric is added to resist microbial attack onto the films and enhanced their durability. Moreover turmeric acts as natural pH indicator which helps to develop films as an intelligent and smart packaging material. Mechanical properties like tensile strength show increasing values with increasing weight ratios of nano cellulose without much affecting the elongation at break of films. Further swelling test, pH test, anti-microbial test, soil burial test, thermogravemetric analysis and optical test were carried out to characterize and analyze the films.

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List of symbols and abbreviations

PVA	Poly(vinyl alcohol)
CNC	Cellulose NanoCrystal
CNF	Cellulose Nanofiber
F1	5 wt. % PVA film
F11	5 wt. % PVA film with 1% (of PVA) nanocellulose reinforcement
F13	5 wt. % PVA film with 3% (of PVA) nanocellulose reinforcement
F15	5 wt. % PVA film with 5% (of PVA) nanocellulose reinforcement
F17	5 wt. % PVA film with 7% (of PVA) nanocellulose reinforcement
F2	10 wt. % PVA film
F21	10 wt. % PVA film with 1% (of PVA) nanocellulose reinforcement
F23	10 wt. % PVA film with 3% (of PVA) nanocellulose reinforcement
F25	10 wt. % PVA film with 5% (of PVA) nanocellulose reinforcement
F27	10 wt. % PVA film with 7% (of PVA) nanocellulose reinforcement
LB	Luria broth

CHAPTER 1

INTRODUCTION

The plastic waste management issues associated with petroleum derived synthetic polymers has attracted the researchers towards renewable resources i.e. the use of bio-based composite materials.

Sugarcane bagasse is the remnant produced in bulk amounts every year by the sugar and alcohol industries, and is utilized as a fuel to power the sugar mills [1].

At present bagasse has been used for the production of microcrystalline cellulose and nanocellulose. About 40–50% of bagasse is the glucose polymer cellulose, much of which is in crystalline structure. Also, 25–35% is hemicellulose, an amorphous polymer usually composed of xylose, arabinose, galactose, and mannose. The remainder is mostly lignin (18–24%); plus lesser amounts of mineral, wax (<1%), and ash (1–4%) [2].

Chemically, cellulose is a linear, stereoregular natural homopolymer composed of anhydroglucose units linked at one and four carbon atoms by glycosidic bonds. This is confirmed by the presence of three hydroxyl groups (secondary OH at the C-2, and at the C-3 and primary OH at the C-6 position) with different reactivities [3].Controlled acid hydrolysis of native cellulose fibers disrupts the fibers which can then be dispersed into their constituent rod shaped elementary crystalline microfibrils. Colloidal suspensions of these rods are known to exhibit birefringence and ordered liquid crystalline phases [4].

Poly (vinyl alcohol) is a water soluble polymer made by hydrolysis of poly vinyl acetate. The degree of solubility, and the biodegradability as well as other physical properties can be controlled by varying the molecular weight (MW) and the degree of hydrolysis (saponification) of the polymer[5].In recent times nanocomposites have attracted much attention due to their good electrical mechanical and also barrier properties. These are those composites in which one of the reinforcing materials in nanorange (1-100nm). PVA is a good choice to blend with cellulose as it is polar in nature and can be dispersed easily in water solutions.

1.1 Research Objective

Agro waste bagasse is utilized as a potential source of cellulose. Amid different available techniques for getting nano sized cellulose like homozenization, sonication, cryocrunching, oxidator agent (TEMPO), acid hydrolysis is used. PVA is a transparent polymer and utilizing nano range cellulose will help in maintaining its transparency to some extent.The main objective of this project work is:-

- Isolation of nano cellulose from Sugarcane bagasse by acid hydrolysis method using conc. sulfuric acid.
- To Study the thermal and morphological behavior of obtained cellulose by TGA, DSC and Scanning Electron Microscopy.
- This isolated nano cellulose is then utilized to reinforce the PVA matrix at different wt. % ratios and films are prepared by casting method.
- Swelling test, optical test, soil burial test, pH test, food spoilage test are done to study different properties of the films prepared.

1.2Organization of Thesis

Chapter 1: (Introduction) to provide a brief overview of the research objective and the problem to begin with.

Chapter 2: (Literature Review) to provide information about isolation methods of nano cellulose from Sugarcane bagasse and its properties, Poly(vinyl alcohol) nano composites films properties and their various applications.

Chapter 3: (Materials and methods) to report the materials and equipment used in the present research work followed by experimental methods which includes isolation of nano cellulose from bagasse and preparation of different PVA films reinforced with nanocellulose by casting method.

Chapter 4: (Results and discussion) in this chapter results of different characterization and testing have been discussed.

Chapter 5: (Conclusions) provides major findings in this research work and future scope.

CHAPTER 2

LITERATURE REVIEW

Cellulose build nano materials are derived from renewable and sustainable sources and have found applications in biomedical and other industrial applications. They have low density, less thermal expansion, good mechanical properties, toxicity is less, hydroxyl groups on surface that can be functionalized by chemical treatments[6-13].These nanomaterials have found multiple applications like barrier and filtration membranes ,protective coatings, solar cells, tissue engineering, drug delivery, transparent films packaging, lithium ion batteries, supercapacitors etc. [10,14].

Sugarcane bagasse is the fibrous matter that remains after sugarcane stalks are crushed to extract their juice. Bagasse is used as a fuel to power sugar mills and as in manufacturing of pulp and building materials [15].Utilization of agro waste sugarcane bagasse for polymer nano composites has found interest because their ecological and renewable features, and ease of availability of raw material.

Depending upon source like plant, animal, mineral cellulose nano materials may be classified as cellulose nanocrystal (CNCs) and cellulose nanofibers (CNFs). CNCs are highly crystalline material with one dimension equal to or less than 100nm. The main natural source of cellulose is plant fibers. The cell wall of fibers consists of primary cell wall and secondary cell wall. The primary wall consists of loosely bound microfibrils of length 100-200nms. The secondary wall contain three layers of 4-6 microns ,S1,S2,S3[10,16,17].These fibers along with cellulose consists of a amorphous matrix of lignin, hemicellulose, proteins, extractives organic substances and trace elements. There is a hydrogen bond between cellulose and hemicellulose whereas hemicellulose and lignin are linked by strong covalent forces.

2.1 <u>Cellulose: Introduction and structure</u>

Cellulose is a polysaccharide consisting of a linear chain of several thousand β(1→4) linked D-glucose units. Cellulose has no taste, is odorless, is hydrophilic, is insoluble in water and most organic solvent and is biodegradable. Each anhydroglucose unit (AGU) contains three hydroxyl groups. AGU forms internal hydrogen bond owing to equatorial positions of hydroxyls[18].The stiffness of cellulose chains is because of hindered free rotation of glucopyranosic rings around chemical glycoside bonds. Due to strong inter and intramolecular hydrogen bonding the crystallites is rigid, strong and insoluble in water. Also weak hydrogen bond of amorphous domains leads to hydrophilicity and approachability of cellulose.

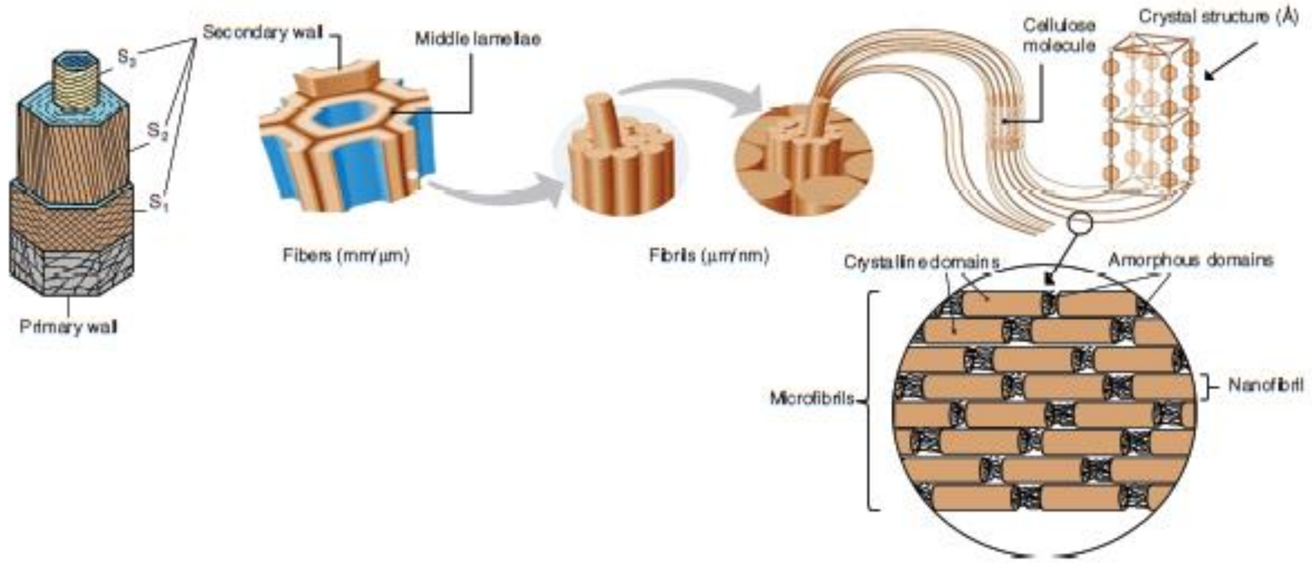


Figure 1: Cellulose in plant cell wall (S1, S2 and S3) and orientation of amorphous and crystalline domains [44 -46].

2.2. Classification of Nano cellulose

Based on their properties, function, dimension nanocellulose is classified depending on their origin source and processing methods. Below some nanocellulose types are explained in brief.

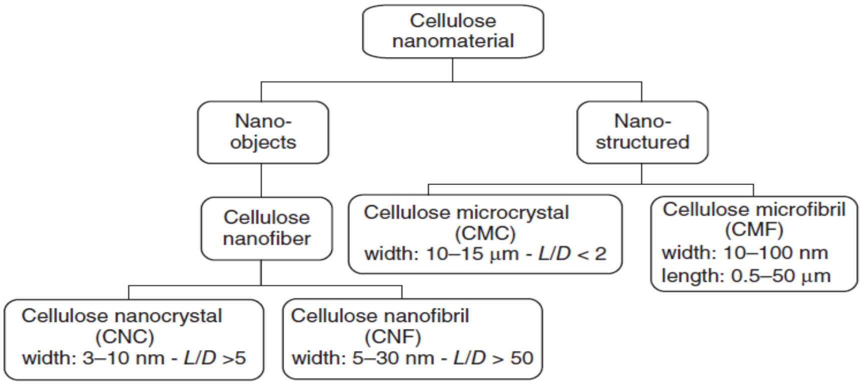


Figure 2: Cellulose nanomaterials classification [45]

- Micro crystalline Cellulose

  They have spherical rod like structure of diameter 10-100microns. This type of nanocellullose is prepared by hydrolysis of cellulose with weak mineral acid.
- Cellulose microfibrils

  These are prepared by action of strong mechanical forces on purified cellulose. This type of microfibrils have width of 20-100nm.
- Cellulose Nanocrystal

  These have rod like structure and are highly crystalline and less flexible. They are also called nanowhiskers, nano rods etc. Nano crystals are generated by separation of amorphous portions from nanofibrils which can be done by through strong acids with high mechanical and ultrasonic forces. These nanocrystals show high mechanical strength, Young's modulus 167.5 GPa which is comparable to Kevlar and higher than steel[19].
- Cellulose nanorayon

  This type of nanocellulose is produced by eletrospinning method by solutions of cellulose or its derivatives. They have diameter in range of 700-800 nm and are of lower thermal stability than initial grades.

2.3. Isolation techniques of cellulose

When cell wall of plant fiber is treated with high mechanical forces the original structure of cellulose breakdown giving fibrils in diameter range of 10-100 nm depending upon power of mechanical forces applied. Following are some processes to extract cellulose from their sources.

1. High Pressure Homogenization

In this process suspension is forced through a narrow channel under high pressure of 50-2000 MPa which is intended to homogenize the sample to reduce the particle size of any components within it.

The reduction in size of cellulose fibers can be possible with a large pressure drop, high shear forces turbulent flow and inter particle collision. The stretch of fibrillation depends on number of homogenization cycles and on applied pressure. Higher efficiency of disruption per pass through the machine is achieved at higher pressures [47].

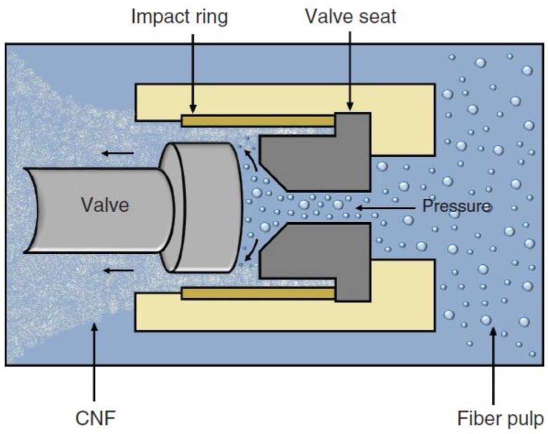


Figure 3:- Schematic of homogenizer

2. Micro fluidization

A micro fluidizer operates at a constant shear rate. The sample present in an inlet reservoir is taken by the Microfluidizer, and an intensifier pump creates pressures of up to 40,000 psi to push the sample via the interaction chamber. This interaction chamber exposes the sample to strong, continuous impact and high shear rates. Following this, the sample is cooled, and the nanoscale particles are either reclaimed for use or re circulated for numerous passes to obtain the required homogeneous particle size distribution.

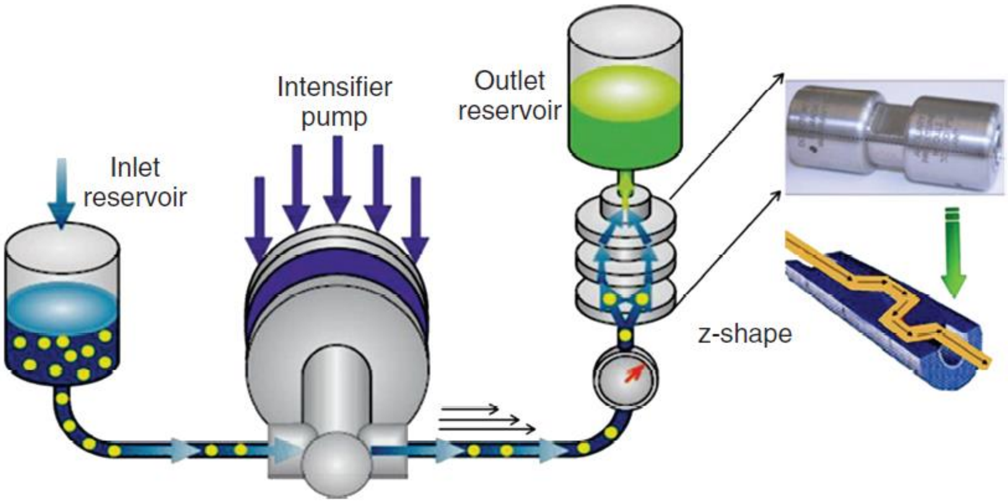


Figure 4:- A schematic of microfluidizer [48].

3. Grinding

A fiber fibrillation process in a grinder is carried between static and rotating grindstones revolving at 1500 rpm. Cellulose slurry is passed through grindstones which apply shear forces to degrade the cell wall structure and individualize the nanoscale fibrils [48].

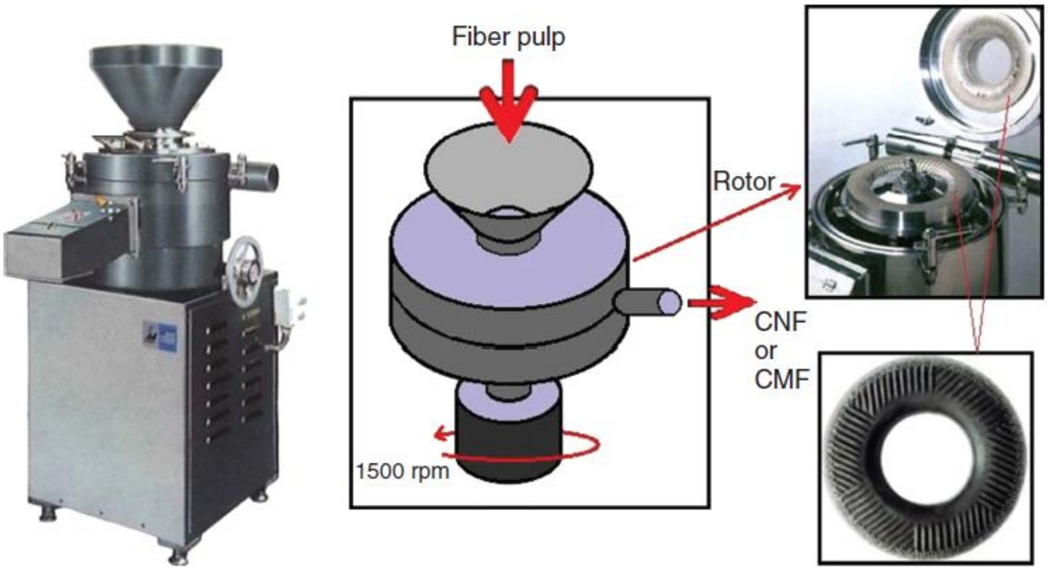


Figure 5:- A grinder system [50].

4. Cyrocrushing

Cyrocrushing is a frozen state mechanical fibrillation method for cellulose [50, 51]. Fibers with large diameters (0.1-1 microns) are produced with this method. Because of action of high impact forces the cell wall of frozen cellulose breaks which liberates nano fibers.

5. High Intensity Ultrasonification

In this process cell disruption is carried out in aqueous medium. Defibrillation of cellulose fibres is carried out by hydrodynamic forces of the ultrasound. Crystalline structure is altered sometimes due to ultrasonic treatment. Usually fibrils aggregate with broad width distribution are formed by this method.

2.4. Preparation steps for Nano Cellulose

It is a three step process as shown in figure below:-

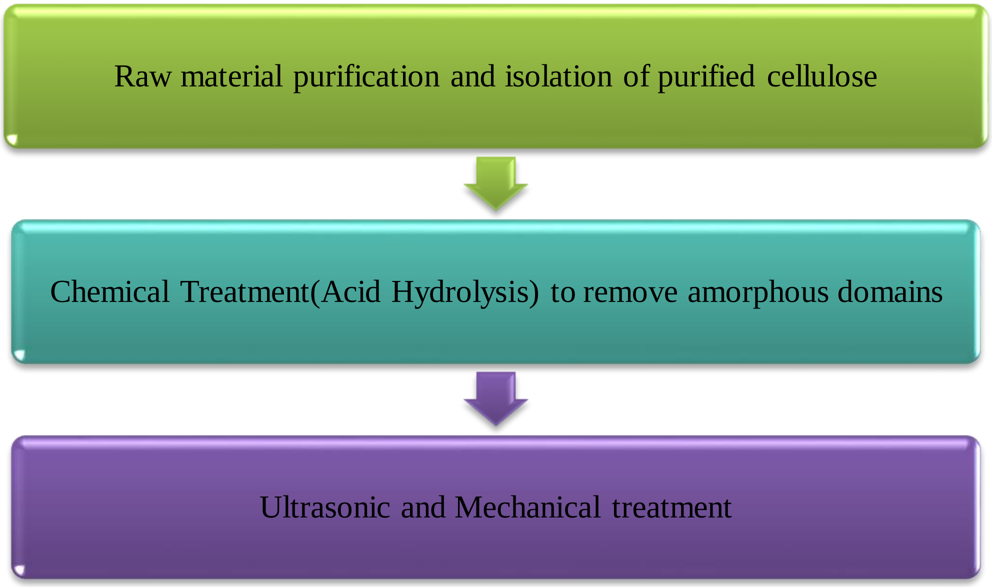


Figure: 6- Flowchart showing cellulose nanocrystal preparation

2.5 Acid Hydrolysis

To obtain nanocellulose, purified cellulose is hydrolyzed with different acids under controlled parameters like temperature of reaction, time of hydrolysis, agitation speed and acid to cellulose ratios. Various mineral acids are used for this purpose like sulfuric acid [20], hydrochloric acid [21, 22],nitric acid [23], formic acids[24], and amixture of hydrochloric acid and acetic or butyric acid is also reported [25].

For cellulose nanocrystal formation, Sulfuric acid is most commonly used acid. On hydrolysis, the amorphous domains are hydrolyzed leaving behind the crystal domains which are stiff and rod like structures [26]. The CNC dispersion after treatment with acid is washed with distilled water and is neutralized. Other steps like filtration[27], centrifugation [28], and mechanical disintegration have also been reported.

The disadvantage of using hydrochloric acid is that the uncharged nano crystalline particles tend to flocculate in dispersion [29]. Whereas when sulfuric acid is used it reacts with hydroxyl groupsof cellulose forming negatively charged sulfonic groups on the surface. This involves esterification of hydroxyl groups along with chain scission. Anionic charges stabilize the dispersion and the repulsion forces of electrical double layer prevent the flocculation of CNCs. The disadvantage of using sulfuric acid is that the thermal stability of nanocellulose is compromised [30] and to improve the thermal stability neutralization with sodium hydroxide is carried [31].

Usually product of lower crystallinity and lower thermal stability is obtained at higher acidic concentrations and reaction temperatures. A yield of 30% and less than 20% is obtained if cellulose is hydrolyzed with 63.5 wt. % and 65 wt. % of sulfuric acid respectively. If hydrolysis is carried at 60 wt% then the yield of cellulose nano crystal obtained is maximum, i.e. 65-70% [32,33].

It was observed from electron microscopes that at lower acidic concentrations aggregation of CNC take place with length dimension of 300-500 nm and at higher acidic values (about 65%) low yield is obtained with decrystallized structures. Therefore 60 wt.% of sulfuric acid is chosen for the hydrolysis process of starting cellulose to obtain maximum yield and highly crystalline nano cellulose [32].

2.6 Poly(vinyl alcohol)

PVA is a water soluble, hydrophilic, linear synthetic polymer which can be easily casted into films. PVA is made by dissolving Poly (vinyl acetate) into methanol and then treating with sodium hydroxide. This hydrolysis process removes acetate groups from side chain. The degree of hydrolysis indicates number of residual acetate groups present after hydrolysis.

Mostly the fillers used are hydrophilic in nature and the polymers are hydrophobic which leads to incompatibility of blend unless compatibilisers are not used. The combination of cellulose and PVA forms eco-sustainable composites. Cellulose and PVA material shows good compatibility as they form strong inter and intra molecular hydrogen bonding [34]. Both are polar materials and hence show excellent mechanical properties. The bond between matrix and reinforcement is one of the deciding factors for the final properties of the composite [35].

Direct addition of cellulose to PVA may not yield the composite with very good properties as hydroxyl groups of cellulose forms inter and intra molecular hydrogen bonding with one another and not with PVA matrix [36]. Thus to improve the compatibility between matrix and reinforcement they are usually cross linked by using suitable cross linker. Commonly used cross linker are glutaraldehye (toxic), glyoxal, hexamethylenetetramine (HMTA), Borax (non-toxic food additive) etc. Other than using cross linkers, modification of cellulose or the PVA matrix is one of the options. Chemical treatment of natural fibers with acids is one of the approaches to improve the mechanical properties of the composites [37,38].

Composites with aligned Nanocellulose

Agglomeration of nanocellulose in the matrix is one of the drawbacks for the final properties like strength and poor dispersion of the composite films, if used at higher concentrations. Hence, alignment of nanocellulose is a recent research area of interest. Nanocellulose particles align themselves under the action of electric, magnetic and shear forces [39, 40]. This make the whole process expensive but when compared to final performance it is believed that the production cost is attained.

Biodegradation of PVA films

PVA is a water soluble polymer. For a material to be biodegradable there must be some similar groups in the main polymer chain and natural substances [41].To increase the biodegradation of PVA composites they can be blended with other biodegradable polymers. The natural fibers undergo degradation on exposure to natural environment at certain temperature, moisture and other microbial conditions.

PVA resists certain organic solvents, grease and oils, but it absorb moisture. The barrier and mechanical properties required in packaging applications. The composites of potato starch, PVA and natural corn fibers are successful in replacing EPS (Expandable Polystyrene) foams owing to their biodegradability [42]. Clove and oregano oils are successfully incorporated in PVA cassava reinforced films to reduce their moisture sensitivity for food packaging applications [43].

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw materials and Chemicals used:-

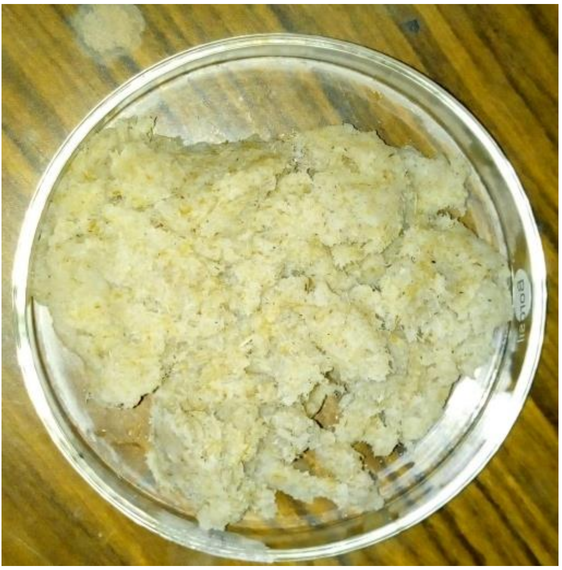
- Sugarcane Bagasse (from sugarcane mill in Uttar Pradesh)
- Sodium Chlorite(Loba Chemie, India)
- Acetic acid (Merck, India)
- Sodium Sulfite (Merck, India)
- Sodium Hydroxide (Merck, India)
- DimethylSulfoxide(Merck, India)
- Sulfuric acid(Merck, India)
- Distilled water
- Poly(vinyl alcohol)
- Turmeric (Commercially available)

3.2 Instruments Used:-

- Thermogravimetric Analyzer (Perkin Elmer)
- Differential Scanning Calorimeter
- Scanning Electron Microscopy(SEM)
- FTIR Spectroscopy (Thermo scientific 380 series)
- UTM (Instron Universal Testing Machine)
- UV Visible spectrophotometer(Agilent Technologies)

3.3: Procedure A:-Isolation of nanocellulose from sugarcane bagasse

- **Cellulose Delignification-**Firstly, the grounded cellulose was dried and then boiled for 5hrs to remove lignin, in 0.7% (w/v) of sodium chlorite solution in acidic medium maintaining pH around 4 by using 5% acetic acid. Then the product was washed with distilled water and dried. This neutral product was further boiled with 250 ml, 5% (w/v) of sodium sulfite solution for next 5 hours and the partial delignified product is washed with distilled water and dried.
- **Cellulose isolation process-**This neutral product was then boiled with 250 ml of 15% (w/v) for about 5hrs for complete delignification. This delignified insoluble product is filtered and washed with distilled water to make it neutral and was then air dried. This was then added to50 ml of dimethylsulfoxide solution for 3 hrs at 40˚C.Again the filtered product is washed and air-dried.
- **Nanocellulose preparation method-**The isolated cellulose was now acid hydrolyzed by refluxing it with a solution of 60% (w/v) of sulfuric acid with strong agitation for 3hr at 40˚C. By adding 5 fold excess water this process of hydrolysis is quenched and then the product is centrifuged and washed many times with distilled water to attain a pH around 5.This final obtained product was then sonicated for 15 mins and was stored at about 4˚C [59].


(a)

(b)

(c)

Figure 7: (a) Delignified cellulose, (b) Acid hydrolysis of delignified cellulose and (c) colloidal dispersion of acid treated cellulose

3.4. Procedure B: Preparation of nanocellulose reinforced PVA films

- 5 wt. % and 10 wt. % of PVA films are prepared by dissolving 5g and 10g of PVA in 100 ml of warm water. The dissolving process is carried out at about 90˚C with continuous stirring of solution.
- Now 1%, 3%, 5%, 7% isolated nano cellulose with respect to 5 wt. % and 10 wt. % of PVA are dispersed in PVA solution by strong agitation.
- These solutions were then sonicated for 15mins and poured into petridish and films are casted by solution casting method. These films were dried in oven at 40˚C for 5hrs.
- For antimicrobial activity of films to be used with food products, 0.01g of turmeric was added into the solutions of all films prepared.

Table1: Composition of films Prepared (PVA films are prepared in 100ml of distilled water)

Nanocellulose	0	1%	3%	5%	7%
FILM1-PVA (wt %)	5(F1)	5(F11)	5(F13)	5(F15)	5(F17)
FILM2-PVA (wt. %)	10(F2)	10(F21)	10(F23)	10(F25)	10(27)

(a) (b)

Figure 8: (a) 5 wt. % PVA (not reinforced) film (a) without sonication,

(b) With sonication.

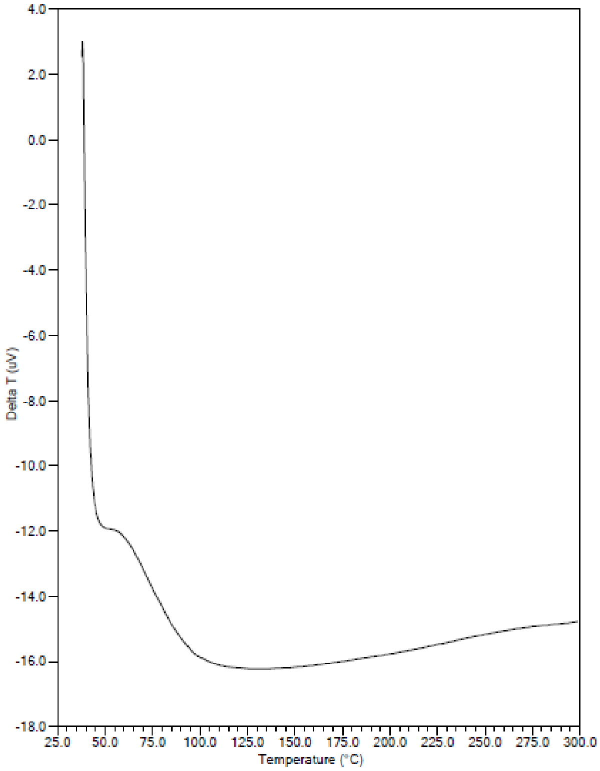
CHAPTER 4

RESULTS AND DISCUSSION

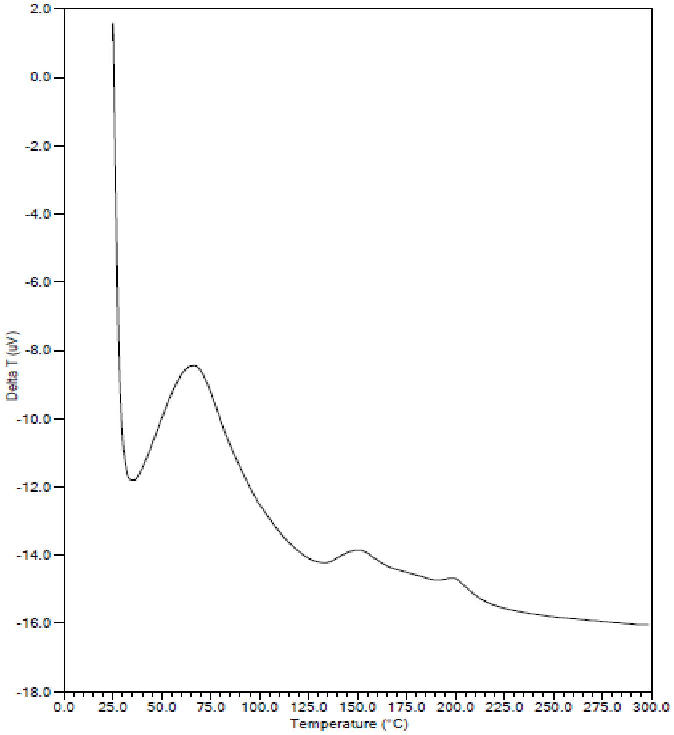
4.1 Characterization of isolated Nanocellulose

- ### 4.1.1 Differential Scanning Calorimeter

In DSC the sample and the reference are heated continuously to increase the temperature of both sample and reference but at the same time extra heat is supplied from a secondary power source either to the sample or the reference to maintain the temperature of sample and reference same.



(a)



(b)

Figure 9: DSC curve of (a) Raw sugarcane bagasse, (b) Acid hydrolyzed bagasse nanocellulose

The initial endotherm which occurs in both the cases at temperature much lower than 100 ◦C stands for the loss of moisture due to evaporation. Besides cellulose bagasse consists of hemicellulose, lignin and other non-cellulosic components. These hydrophilic groups help to retain moisture for longer time and hence process of moisture loss occurs at longer time period. The second endotherm gives idea about decomposition of crystallites which is higher than untreated bagasse and is narrower. Treated cellulose crystals get arranged and reoriented leading to a more compact crystal structure. This causes a higher onset of crystalline melting temperature [59].

- ### 4.1.2 Thermogravemetric Analysis

TG (Thermogravemetric and DTG (derivative thermogravemetric) are commonly used tools to measure thermal stability of polymers. This technique measures variation in mass of a sample undergoing temperature scanning in a controlled atmosphere.

This test was carried out on Perkin Elmer TGA at a heating rate of 10˚C per min from ambient temperature to 700˚C.

The initial mass loss observed in untreated bagasse around 25˚C and in acid hydrolyzed nanocellulose around 30˚C shows evaporation of loosely bound moisture on the surface.

The degradation of untreated sugarcane bagasse starts at around 270˚C and from DTG curve it was observed that rate of degradation becomes maximum at around 330˚C. The degradation of nanocellulose occurs at around 235˚C and maximum degradation is observed 320˚C from DTG curve.

The char formation of nanocellulose (27%) is more as compared to sugarcane bagasse (18%). Nanocellulose consists of more crystalline cellulose as most of the amorphous material is hydrolyzed in it whereas, sugarcane bagasse along with cellulose consists of hemi cellulose, lignin etc. which are amorphous in nature.

The high amount of char formation in nanocellulose can be a result of following factors:-

(a) Nanocellulose consists chains with more number of free ends with decompose at lower temperatures.
(b) Sulfuric acid act as dehydrating agent which again eases the decomposition of cellulose by mechanism esterification of OH groups present in cellulose [52].
(c) Nanocellulose is highly crystalline in nature which increases carbon content therefore with increase in carbon content char formation increases [53].

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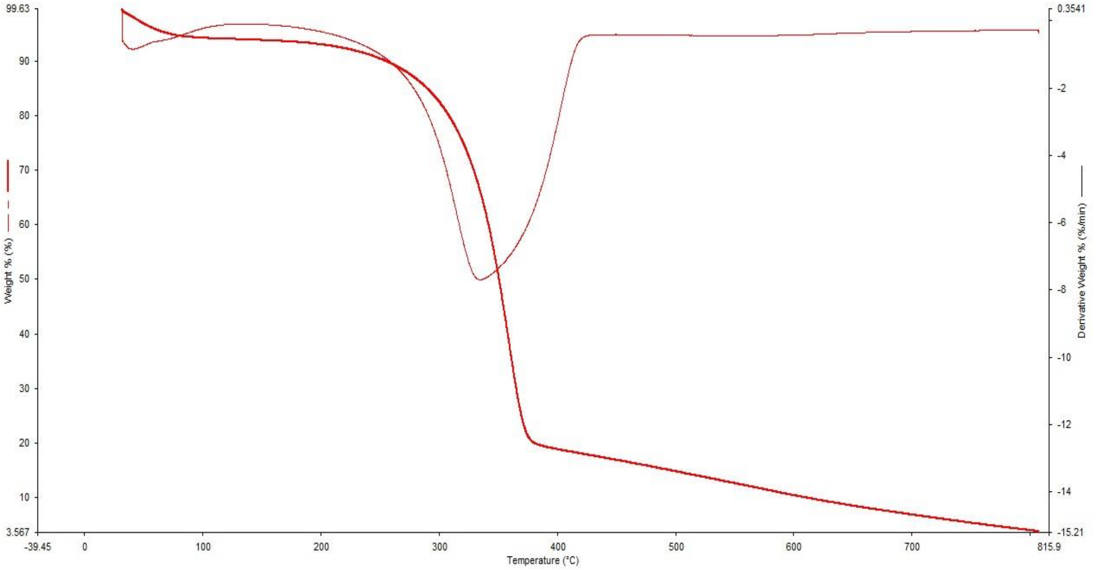
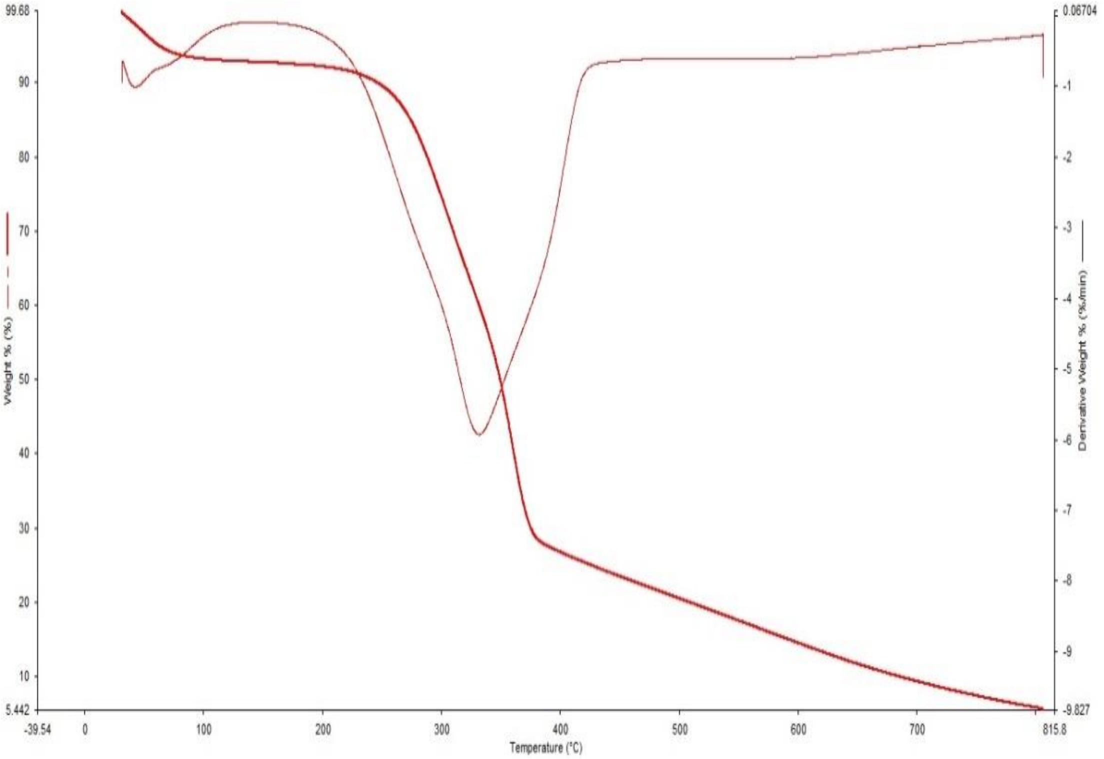


(a) Untreated bagasse



(b) Acid treated bagasse

Figure 10:- Study of thermal properties of (a) raw sugarcane bagasse and (b) acid treated bagasse i.e., nanocellulose by TGA

• 4.1.3Scanning Electron Microscope

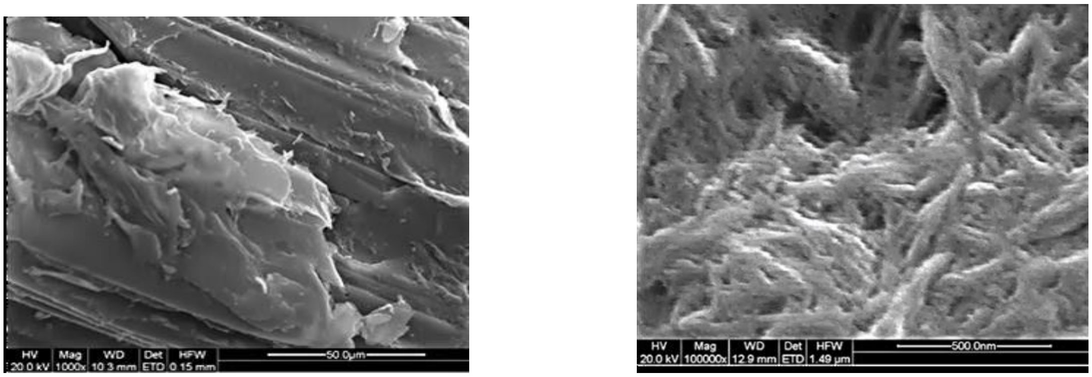


Figure 11:- (a) Sugarcane Bagasse (b) Acid treated Bagasse

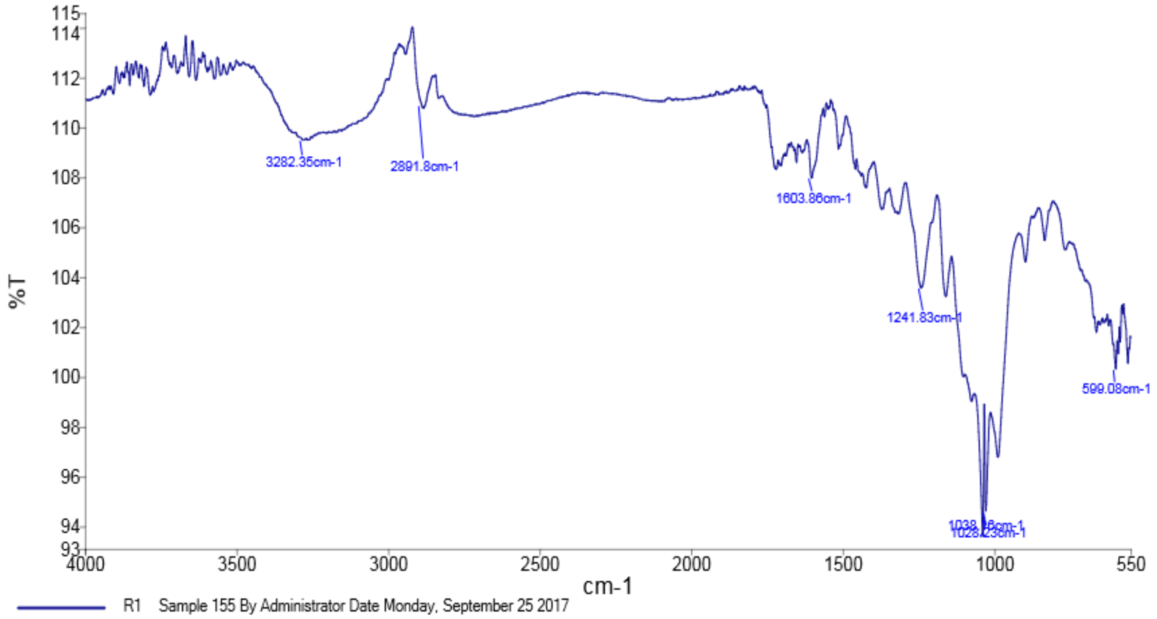
The diameter of the original sugarcane bagasse fiber was much bigger and each fiber appears to be composed of several microfibrils. On treatment with alkali the hemicellulose is hydrolyzed and becomes water soluble. These help in defibrillation of the fibrils and result in micrograph whereby the diameter of the fibrils is reduced to a great extent.

4.1.4 FTIR Spectroscopy

FTIR spectroscopy has been extensively used in cellulose research, since it presents arelatively easy method of obtaining direct information on chemical changes that occur during various chemical treatments.

The FTIR spectrum of sugarcane bagasse before chemical treatment shows peak at 3282cm^{-1} corresponding to O-H stretching of intermolecular Hydrogen bond, peak around 2851cm^{-1} for C-H stretching, and 1730cm^{-1} C-O stretching for ester linkages in lignin and hemicellulose.1603cm^{-1} corresponds to aromatic ring present in lignin.1241cm^{-1}indicates C-O out of plane stretching due to aryl group of lignin.

After acid hydrolysis the spectrum shows bands at, is observed at 1644cm^{-1} is due to O-H bending of absorbed water [54]. 1366cm^{-1} peak for O-H in plane bending.1313 cm^{-1} band is CH_2 wagging. 1026cm^{-1}band may be for glucose residues of disaccharides, 906cm^{-1} associated with cellulosic β glycosidic ether. On comparing we can relate that less amorphous content is present in the nanocellulose as compared to the sugarcane bagasse.



(a) Raw Sugarcane Bagasse

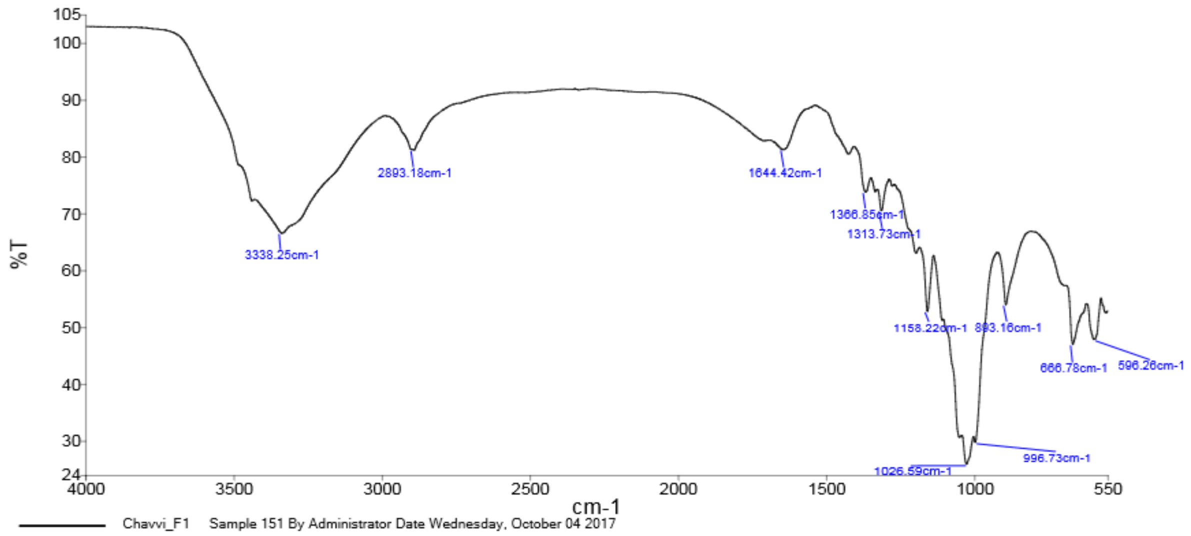


Figure 12 (b):-Acid Hydrolyzed Sugarcane bagasse i.e., isolated nanocelllose

4.2 Testing and characterization of PVA nano cellulose composites

4.2.1 Mechanical properties

Universal Testing Machine is used for measuring the tensile strength, elongation at break of the composite films prepared. The stain rate is 100 mm/min. Following graphs are obtained for the films tested:

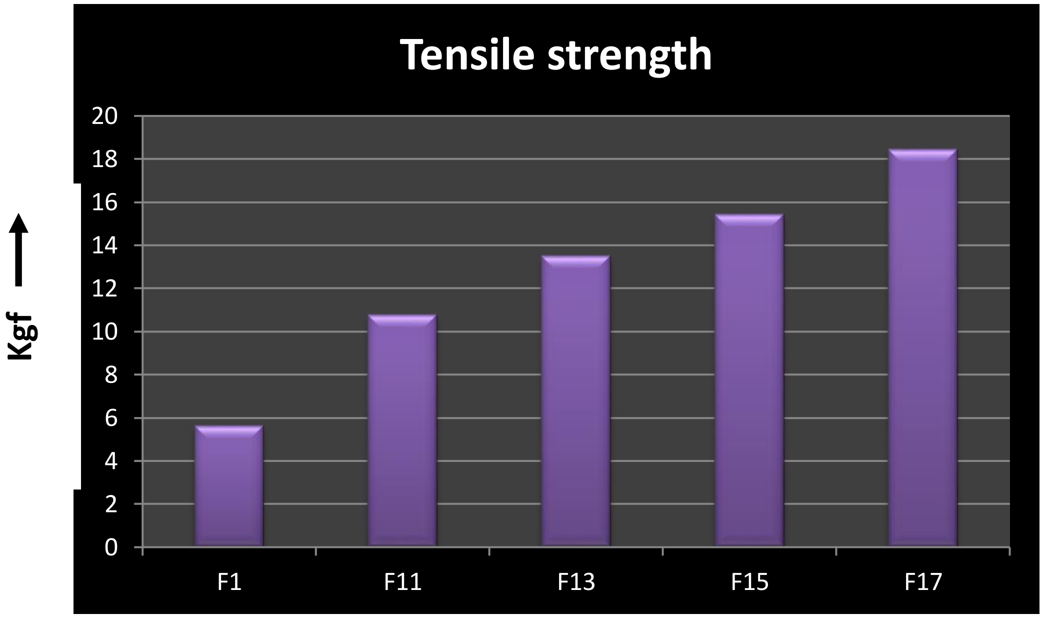


Figure 13:- Tensile strength of PVA (5 wt. %) film and its reinforced grades

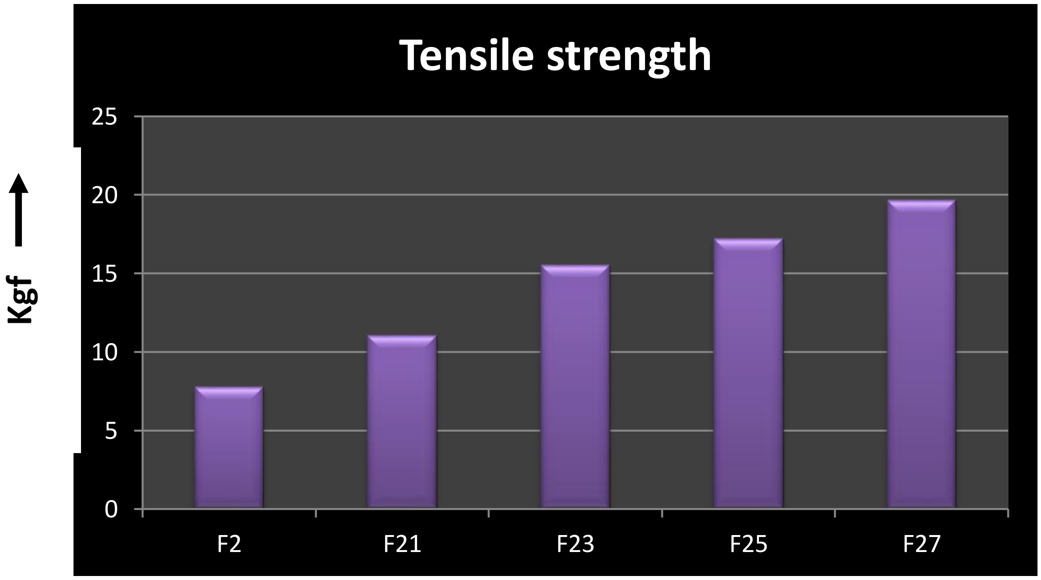


Figure 14:- Tensile strength of PVA (10 wt. %) film and its reinforced grades

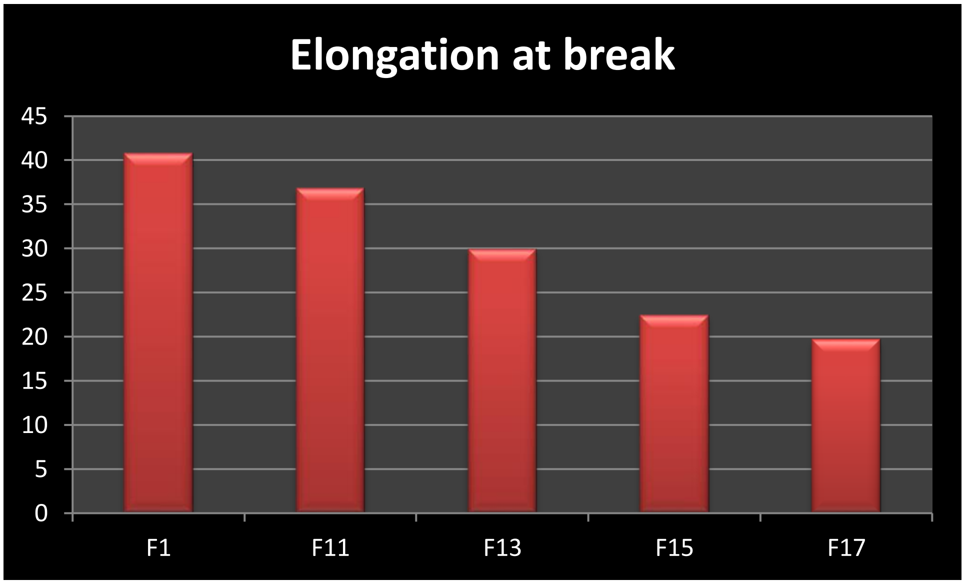


Figure 15:- Elongation at break of PVA (5wt. %) film and its reinforced grades

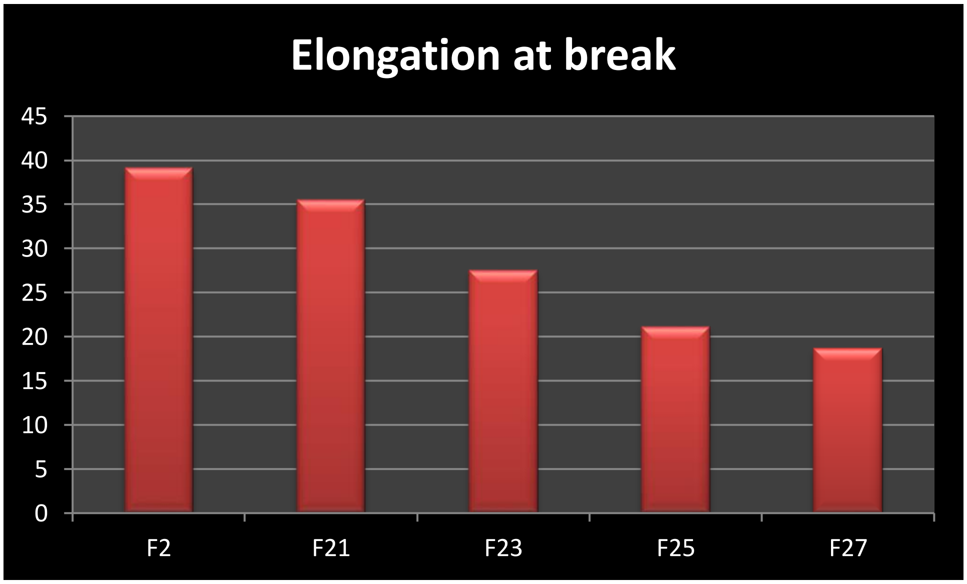


Figure 16:- Elongation at break of PVA (10 wt. %) film and its reinforced grades

Figure 13 and 14shows tensile strength of neat PVA and PVA/Nanocellulose reinforced grades with 1, 3, 5 and 7 wt% of nanocellulose. The tensile strength of neat PVA (5 wt. %) is 5.67 kgf. As the loading of nanocellulose is increased from 1, 3, 5 and 7 wt% (based on PVA weight), tensile strength of composite films also increases.

This might be possible due to inter molecular interaction between PVA and nano cellulose. PVA shows strong affinity towards the hydroxyl groups of cellulose leading to strong interactions [55]. Further compared to neat PVA the positive impact on mechanical properties may be attributable to high mechanical properties of nano cellulose in comparison to matrix used [56].

Figure 15 and 16 shows effects of nano cellulose on neat PVA film and the reinforced grades. A decreasing trend is observed in elongation at break with increasing nano cellulose loading. This might be possible due to increase in rigidity of films with increasing reinforcement levels [57]. Also the strong interaction between PVA and nano cellulose can restrict the chain movement and reduce the ductility of the films [57, 58].

As F2 (F27) grades shows better mechanical properties then F1 grades therefore further many test are performed with F27 grade only.

Table 2:- Values of Tensile strength and elongation at break for PVA and its reinforced grades

S. no.	Sample ID	Tensile strength(Kgf)	Elongation at break (%)
1	F1	5.63	40.77
2	F11	10.77	36.83
3	F13	13.51	29.93
4	F15	15.44	22.45
5	F17	18.45	19.69
6	F2	7.78	39.12
7	F21	11.05	35.52
8	F23	15.51	27.54
9	F25	17.24	21.14
10	F27	19.68	18.64

- ### 4.2.2 Swelling Behavior

The swelling behavior of the film PVA (5 wt. %), PVA (10 wt. %), and the reinforced grades are observed for 24 hours of time period. The dried films of 2cm^{x}2cm were immersed in distilled water at room temperature 30˚C. Moisture present on the film surface was allowed to dry and then their weight is recorded after 24 hr. It is seen that with increasing reinforcement (i.e. nanocellulose) degree of swelling decreases. This might be possible because the void spaces in PVA polymer matrix which absorbs water molecules decreases with increasing weight ratios of nanocellulose, thus decreasing the water holding capacity of the film. Degree of swelling (DS) is calculated by following formula:-

$$DS= (w_x-w_o)/w_o,$$

Where w_x is the final weight of the sample dipped in distilled water for 24hr and w_o is the initial weight of the sample.

Table 3: Swelling behavior of PVA films and its reinforced grades

Composite films	Initial wt.	Final wt.(after 24hr)	D.S of films
F1	0.0131	0.0151	0.1526
F2	0.0136	0.0159	0.1691
F11	0.0135	0.0147	0.0888
F21	0.0140	0.0159	0.1397
F13	0.0170	0.0184	0.0823
F23	0.0176	0.0191	0.085
F15	0.0215	0.0229	0.0651
F25	0.0225	0.0238	0.0577
F17	0.0245	0.0256	0.0448
F27	0.0249	0.0258	0.0361

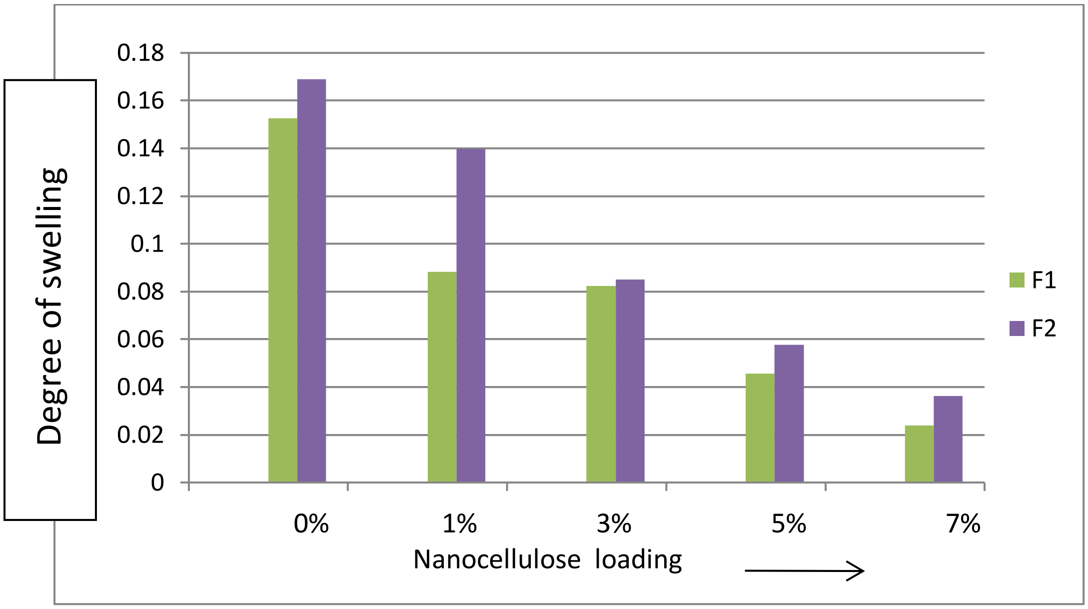


Figure 17:- Graph showing degree of swelling of film

• 4.2.3 pH Test

As turmeric was added to the films for anti-bacterial effect, it also serves the purpose as a natural pH indicator which shows different color in acidic and basic medium.

To study the effect of pH on the color of the prepared samples 1cm^{x}1cm square of the films were emerged in solution of hydrochloric acid (pH 2 and 4) and sodium hydroxide (pH 8 and 9) for different pH ranges. The investigation revealed that film changes its color after 10 min from light yellow to complete brown under strong acidic conditions (pH=2). At highly basic condition (pH=9) color changes to light orange.

These changes in color at different pH ranges help to develop an intelligent packaging material. If due to food spoilage the pH of food changes, the change in color of these packaging films can help to distinguish between fresh foods and spoiled one.

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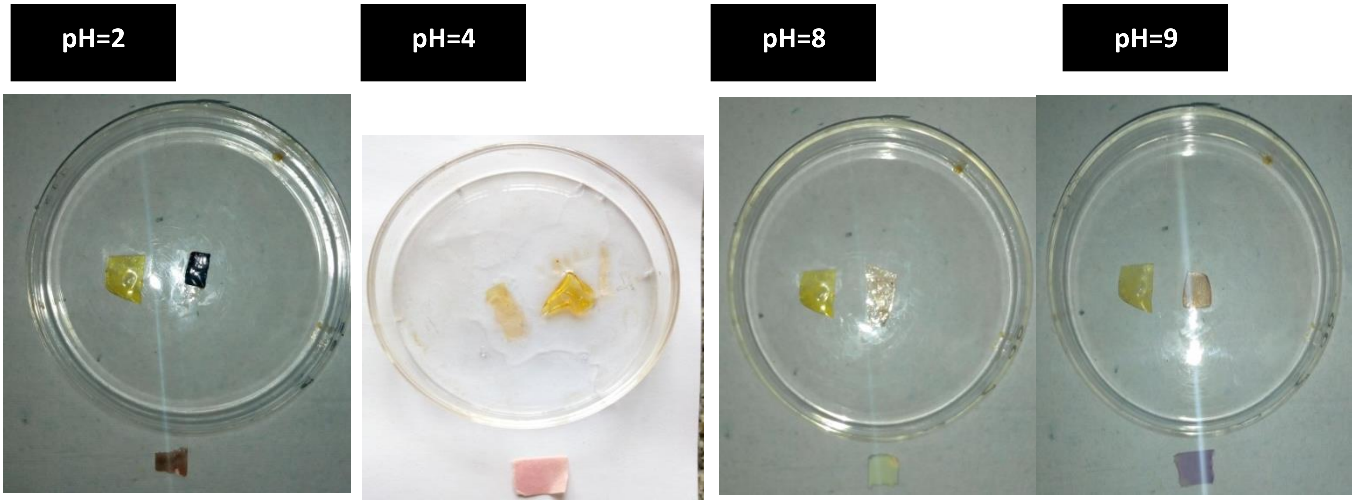


Figure 18: Color response of the films at pH 2, 4, 6 and 9 by immersing films in acidic and basic conditions (F27).

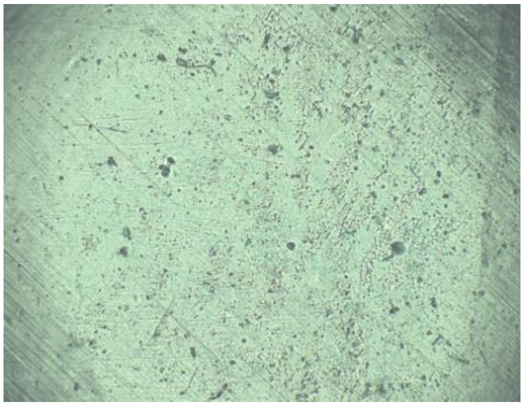
- **4.2.4 Soil Burial Test**

The soil burial test was carried out in small plastic cups where films samples were cut into 2cm^{x}2cm square are buried in soil for 60 days and their weight loss is recorded in comparison to initial weight.

The soil (pH about 6) was kept uncovered and moist by sprinkling water from time to time. Weight loss of film samples was used to indicate the degradation rate of samples in the soil burial test. The film samples become soft after 60 days of time period. Then nanocellulose reinforced grades show less weight loss than PVA film. Also with increasing reinforcement percent weight loss is less, indicating that it will require more time to degrade.

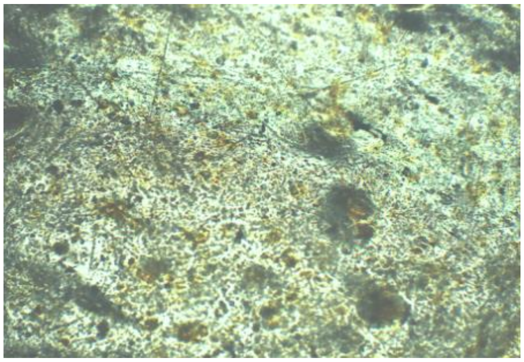
Figure 19:- Soil burial test in plastic cups containing film samples buried in soil

From images obtained by optical microscope it can be observed that F17 sample degrades more ascompared to F27 sample (estimated by more are covered underblack spots in the sample).One reason for this can be less thickness of sample F17 which allows more rubing action of soilonto the surface of the film.



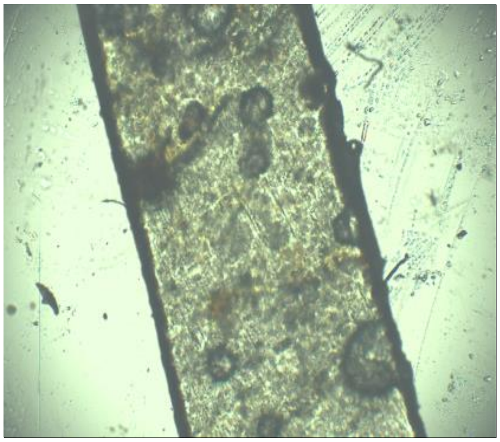
Optical microscope view of F17 film sample at beginning of soil burial test

(4X resolution)



Optical microscope view of F27 film sample after 60 days of soil burial test

(4X resolution)



Optical microscope view of F17 film sample after 60 days of soil burial test

(4X resolution)

Figure 20:- These images are obtained by optical microscope after 60 daysof soil burial test

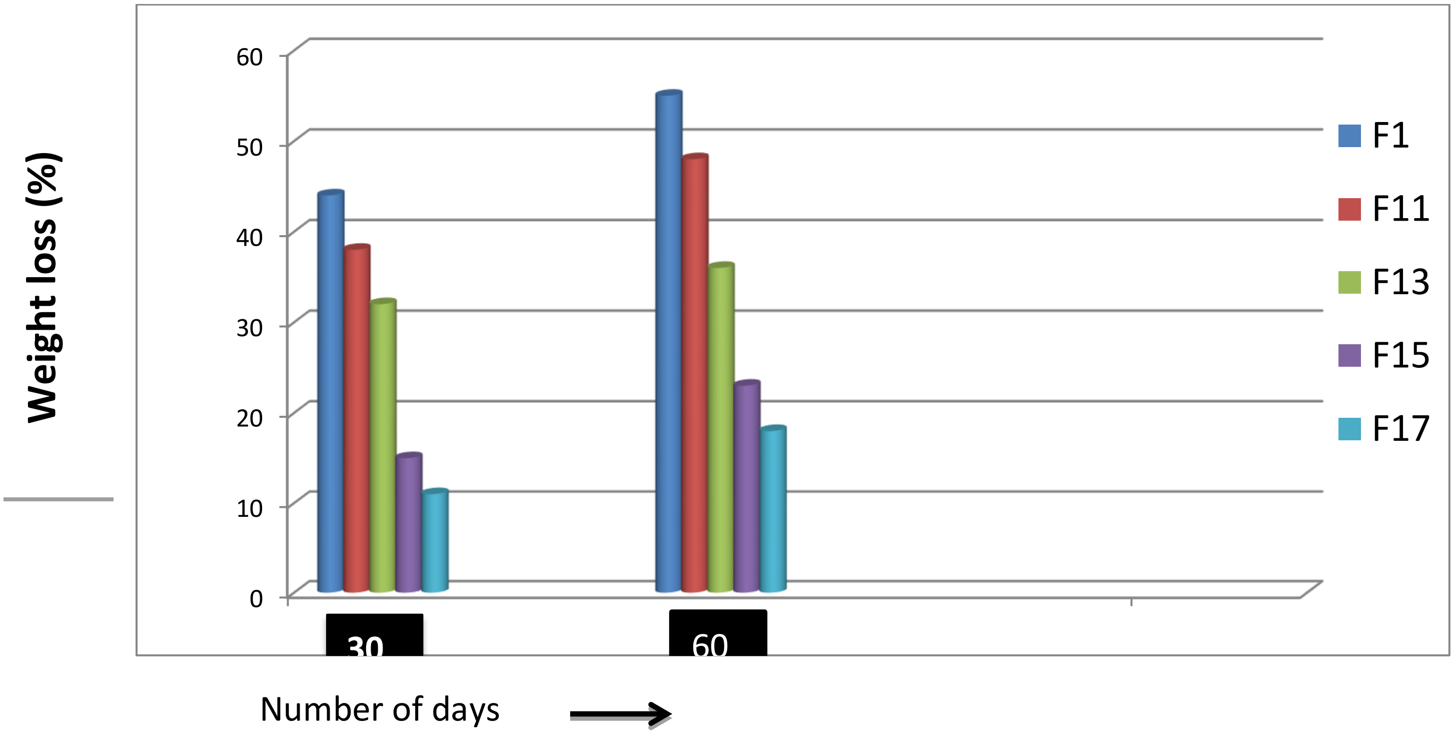


Figure 21: (a) Graph showing weight loss in film samples (F1 and its reinforced grades)

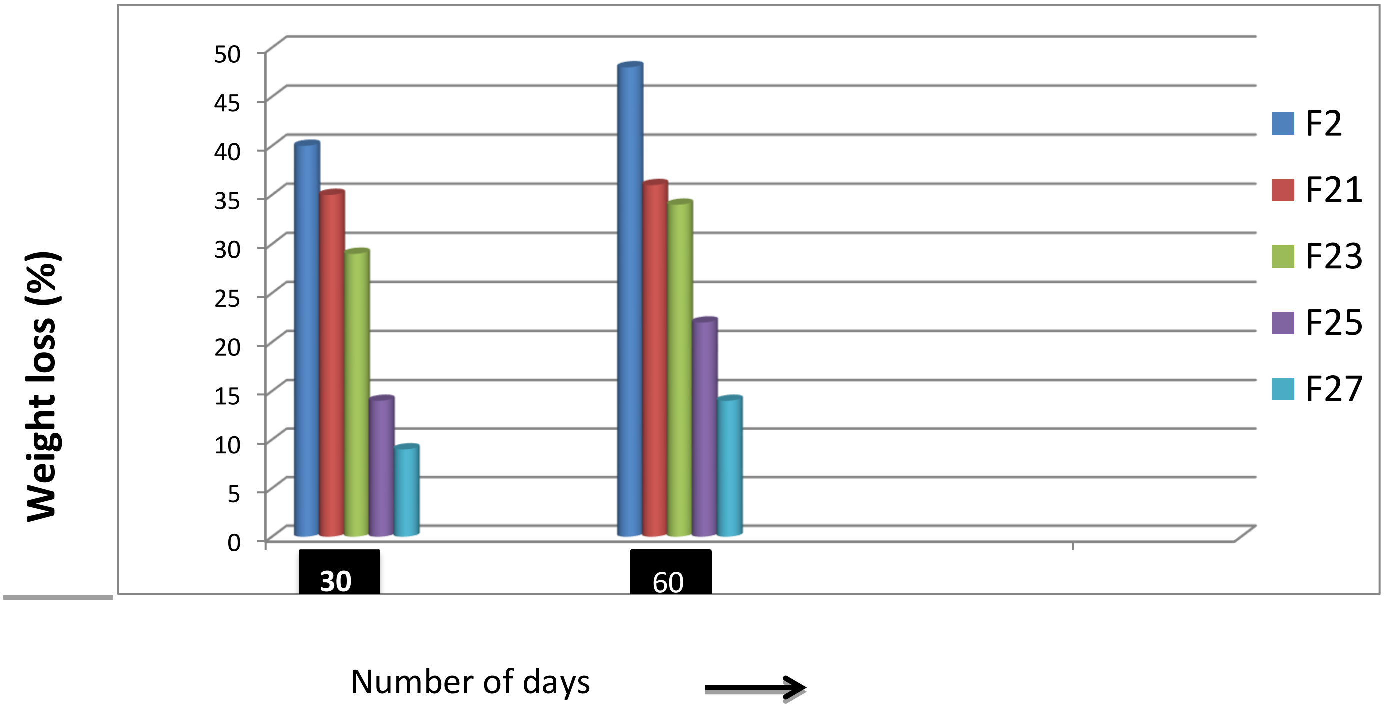


Figure 21: (b) Graph showing weightlossin film samples (F2 and its reinforced grades)

<u>TGA Analysis</u>

Thermogravimetric study of film sample F27 is carried out before and after soil burial process with Perkin Elmer TGA instrument at 10˚C heating rate from ambient to 700˚C.

The rate of degradation for sample before soil test starts at around 300˚C with maximum weight loss at around 330˚C (as observed by DTG peak) is 50%. This sample is buried in soil for 60 days and its TGA is carried out after 60 days of time with maximum period. Now the degradation of sample starts at a much lower temperature i.e. around 140˚C where the weight loss is maximum in a broad range of 155-165˚C and is 60%. Hence it can be assumed that after soil test the thermal stability of film decreases whereas the percentage of weight loss increases

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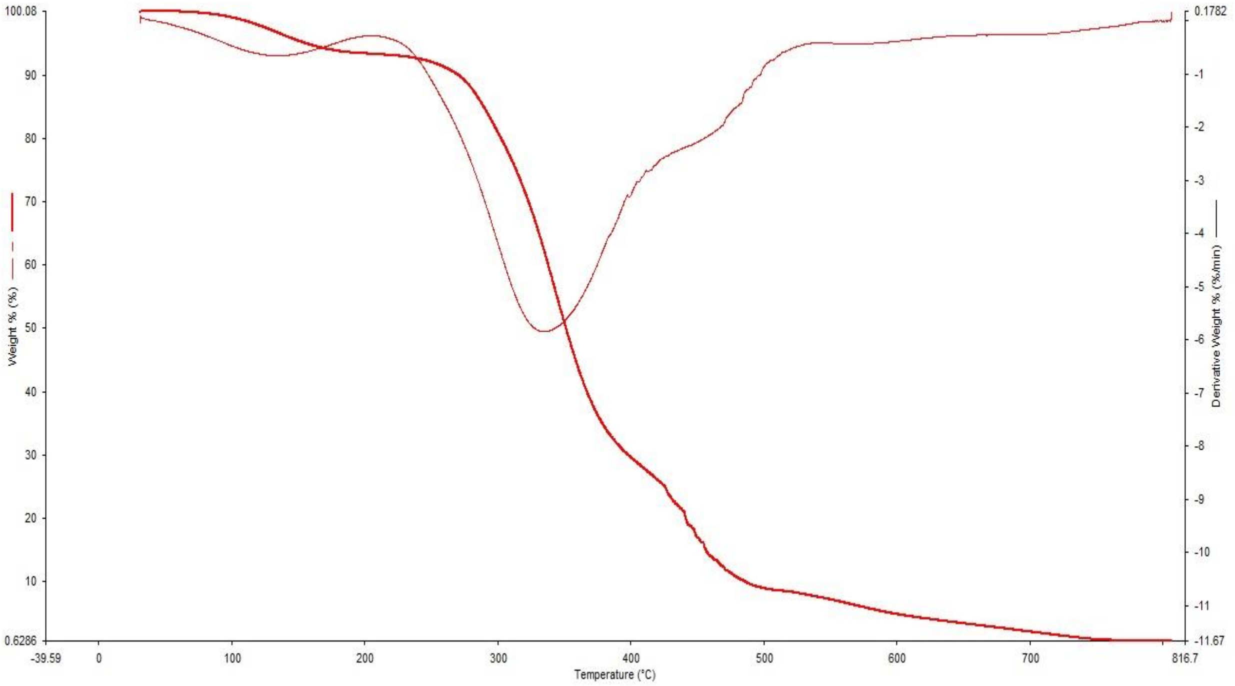
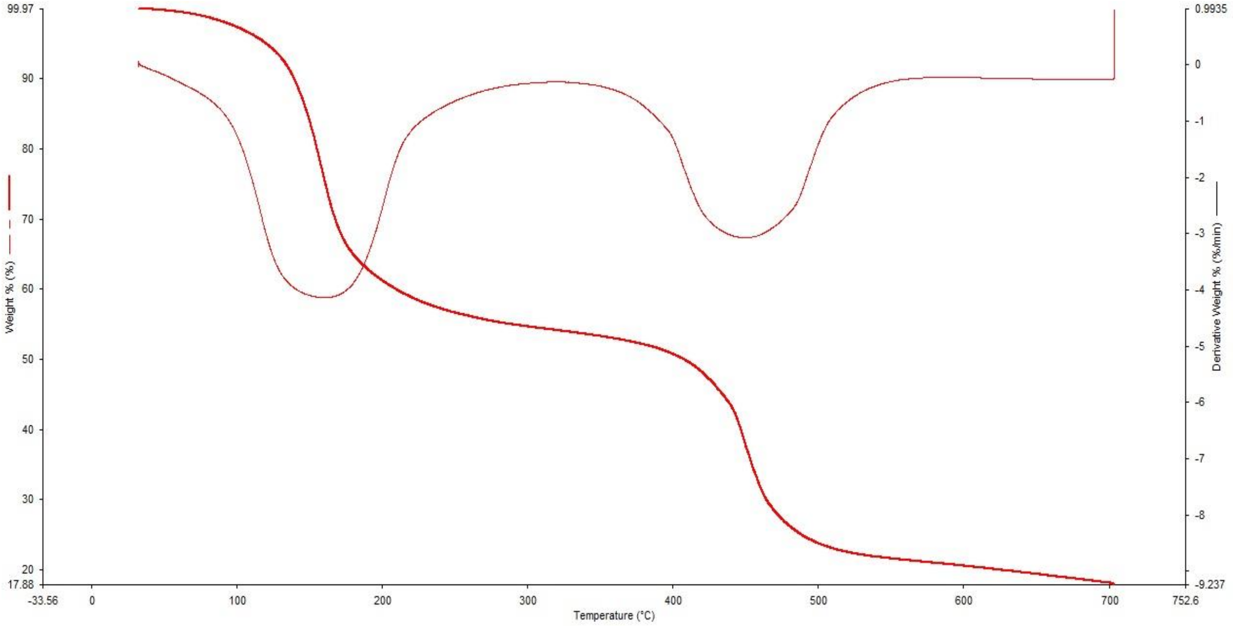


(a)



(b)

Figure 22:-TGA analysis of F27 sample (a) before soil burial and (b) after 60 days of soil burial

Table 4:-Weight loss (%) of film samples in soil burial test for 60 days

Film samples	Wt. loss (in %) after 30 days	Wt. loss (in %) after 60 days
F1	**44**	**55**
F11	**38**	**48**
F13	**32**	**36**
F15	**15**	**23**
F17	**11**	**18**
F2	**40**	**48**
F21	**35**	**36**
F23	**29**	**34**
F25	**22**	**14**
F27	**14**	**9**

- **4.2.5 Food spoilage Test**

For food spoilage test the films were tested with milk, paneer and curd.

Milk: Small covered bottles filled with milk (about 10 ml) having neutral pH (taken as blank) were used. The films samples of 3cm^{x}3cm square of PVA and nano cellulose reinforced PVA films were placed on cap of bottles. With time period of 24 hours the images of films were taken and were monitored. The pure PVA samples (F1and F2) were solubilized within 1-2 hours and the reinforced grades (F11,F13,F15,F17 and F21,F23,F25,F27) were examined and reported as under:-

Table 5:- Changes observed in samples subjected to milk

Composite films	**Changes observed**
F1	Solubilizes within 2 hrs
F11	Solubilizes within 2 hrs
F13	Solubilizes within 3-4 hrs
F15	Swell and then solubilizes within 3.5 hrs
F17	Swell and is stable for 4 hrs
F2	Solubilizes within 2 hrs
F21	Solubilizes within 2 hrs
F23	Swell and Solubilizes within 3.5 hrs
F25	Swell and stable for 3.5-4 hrs.
F27	Swell and stable for 4hrs

Low stability of films in milk than in water may be due to the presence of similar groups in milk and PVA reinforced grades.Milk contain carbohydrates like lactose,glucose and other oligosaccharides.

The film samples contain nanocellulose and some glucose groups formed by breakdown of cellulose during mechanical and chemical reactions. Thus presence of similar groups increases the commatibility between milk and film samples thereby reducing their resistance towards milk.

Figure 23: Covered milk bottle with PVA (10 wt. %) film at beginning of test.

Paneer: Some paneer pieces were kept in open and taken as a reference at pH around 5.5. Small pieces of paneer were packed in cones of films samples (F21 and F27) and kept for 12 hours of time period at room temperature. These film samples are also compared with reinforced film (nanocellulose content =7%) but without use of turmeric.

Up to 6 hours no odor and color change is observed in the packed food as shown in figure 24, but for 12 hours the paneer pieces in F21 film sample develop some odor and the films got more flexible and dimensionally unstable as observed by physical changes like color and odor, whereas in sample F27 no change in stability of films was observed and the paneer pieces packed in F27 remained durable for more time than other film samples. The uncovered paneer pieces loose their freshness in about 6 hours of time as observed by change in color from white to yellow patches on it. Also without use of turmeric, film can maintain the freshness of paneer for about 10 hours, after which it smell sour and rancid.

So among these samples, F27 film is more suitable for paneer packaging. This may be possible because of the antimicrobial agent turmeric present in reinforced grades which resist microbial attack on paneer with high nanocellulose content (F27) film stability can be maintained for longer time period.

Figure 24: Cones of film samples prepared for packing of paneer.

<u>Curd</u>: Two samples were tested for curd packaging, PVA nanacellulose reinforced, (F21) and (F27). Film specimens of 1cm*1cm square were placed in petri dish having 5g of curd for 12 hours of time period. At the end of test PVA film F23 swell to some extent whereas the higher reinforced grade F27 shows no effect at all. Higher concentration of nanocellulose provides more dimensional stability to the film.

Figure 25 (a): Film samples F27 immersed in Curd at beginning of test.

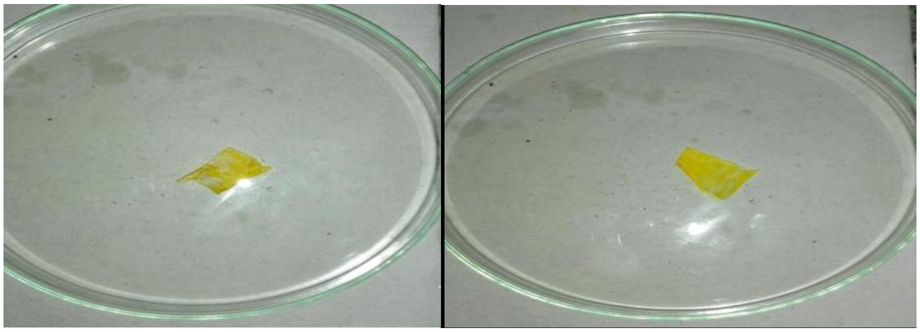


Figure 25 (b): Films samples after 12 hrs of food spoilage (curd).

- **4.2.6 Antibacterial Test**

The pure PVA film and the nanocellulose reinforced PVA films were tested against Gram negative (E. Coli) and Gram positive bacteria. Firstly the bacteria were cultured and identified as gram positive and gram negative by gram staining after 24 hrs time period.

(a) MIC method

Different concentrations of PVA film sample (F27) are prepared to find MIC (lowest concentration of antimicrobial agent that inhibits the visible micro-organisms growth).Following concentrations were prepared 0%,1%,1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5%. All these solutions generate some turbidity after 24hrs time period at room temperature. Hence further 10%, 15%, and 20% solutions are prepared and left overnight. Then 15% concentrated solution shows no bacterial growth. Hence for further accuracy 11%- 15% solution have to be studied to know the exact MIC value for film.

(a) Different concentration of solution at beginning of tesr

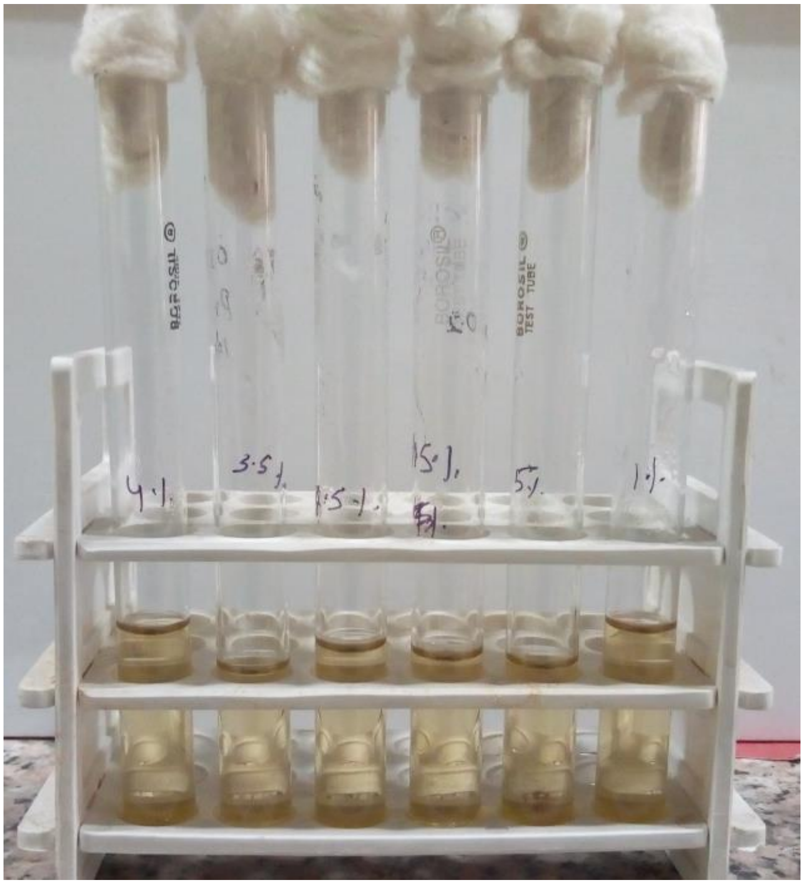
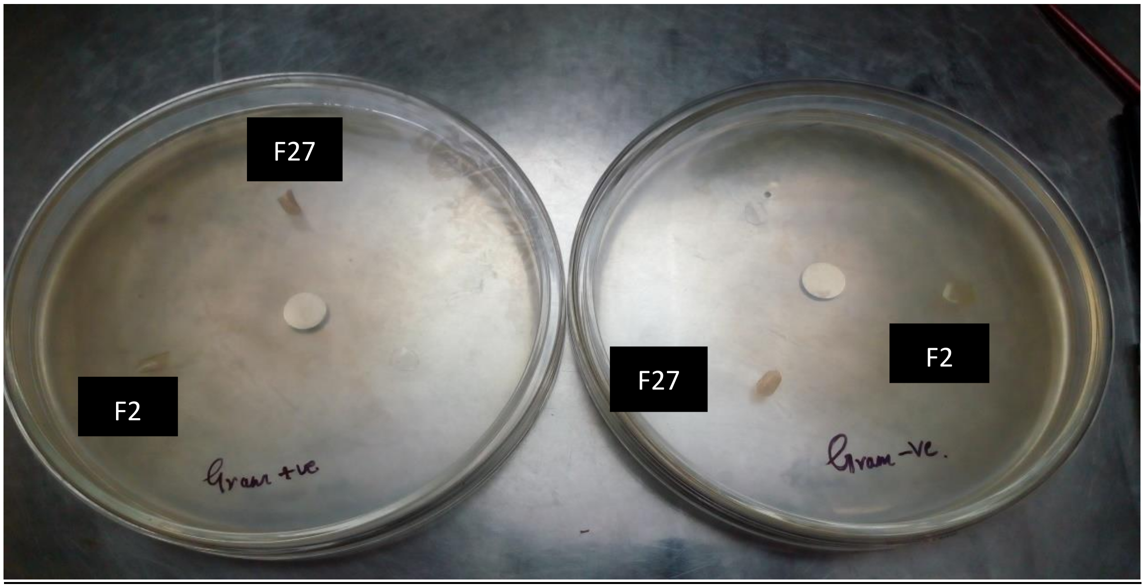


Figure 26: (b) Different concentrations prepared to find MIC (after 24 hr time period).

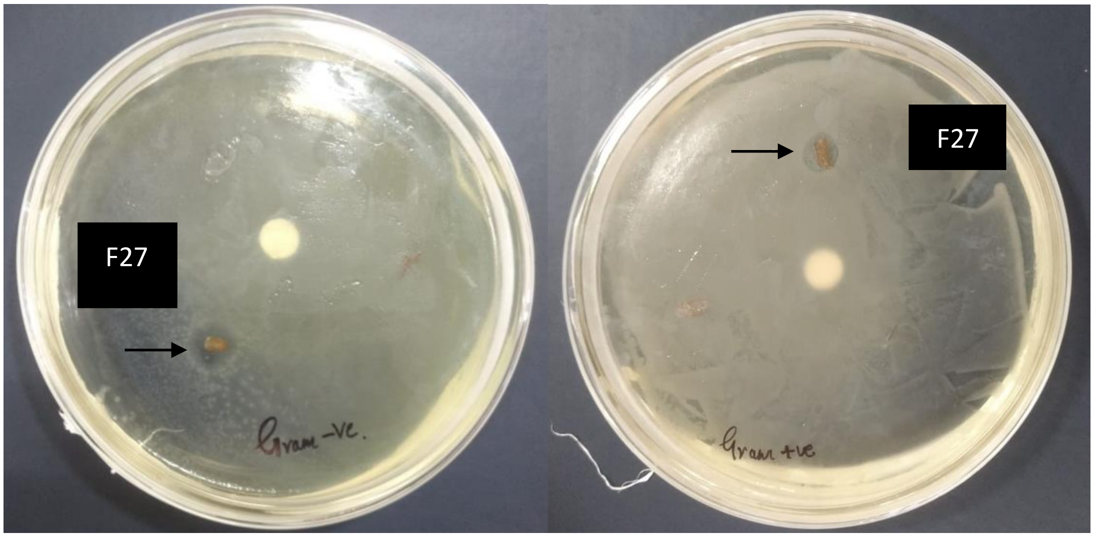
(b) Zone of inhibition method

Zone of inhibition is studied using this method. Agar and LB coated petridish are inoculated with inoculums (gram positive and gram negative). Test samples (F2, and F27) are cut in squares of 2mm*2mm and are placed in agar plates which were further incubated overnight. A 10 wt% PVA film sample of 7% nanocellulose without turmeric is also tested. The antimicrobial agent used diffuses into the agar and inhibits growth of micro-organisms and the diameters of zone of inhibition are recorded.

Only film sample with 7 wt. % (F27) nanocellulose inhibits the growth of both gram positive and gram negative bacteria, the diameter of inhibition for F27 is more in gram negative than gram positive .



(a)

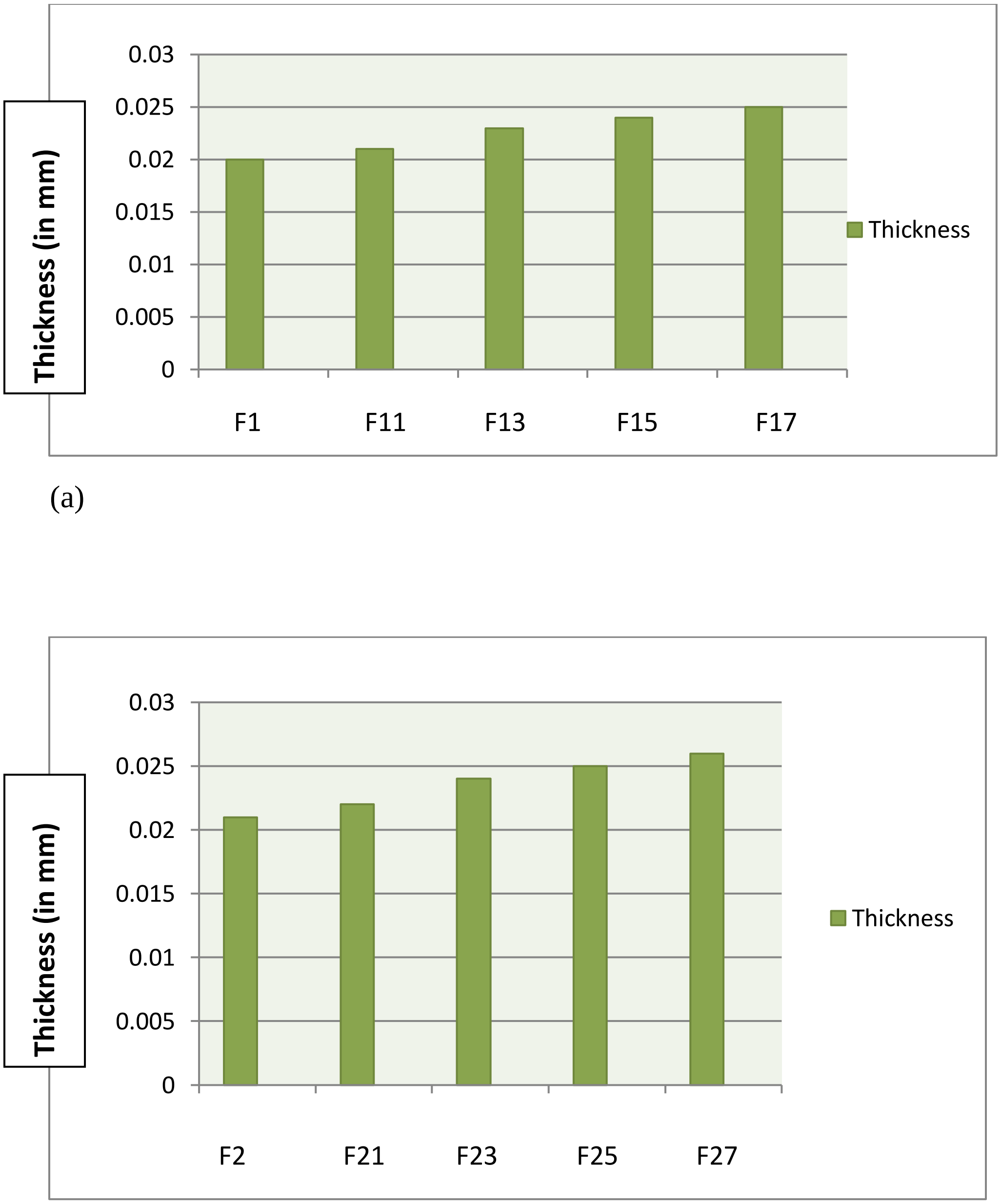


(b)

Figure 27: Zone of inhibition: (a) Action of gram positive and gram negative bacteria on film samples (b) overnight action of bacteria on film samples

- ### 4.2.7 Thickness of the films

With increasing reinforcement ratios in the films, a slight increase in thickness of PVA composite films is observed. This might be because of increase in solid content in the films.



(a)

(b)

Figure 28: Graph showing variation in thickness for(a) 5 wt% PVA and (b) 10 wt% PVA films and their respective composite grades.

- ### 4.2.8 Optical properties (Transmittance analysis)

UV visible spectrophotometer was used to see the percent transmission of the reinforced films samples. PVA films were transparent in nature. By adding nano cellulose to PVA the transparency of the films decreases. This decrease in transparency is higher with increasing levels of nano cellulose used. Transparency of films can provide idea about the particle size of dispersed phase. As nano range particle is smaller than that of wavelength of light therefore it should not make the film translucent or opaque. The pure PVA (5 wt. %) film has shown highest transparency as there are no particle to hinder light transmission. With increasing conc. of nano cellulose the transparency of film decreases. This might be due to the poor dispersion of nano cellulose into the matrix.

Hence the agglomeration of nano cellulose has led to decrease in film transparency.

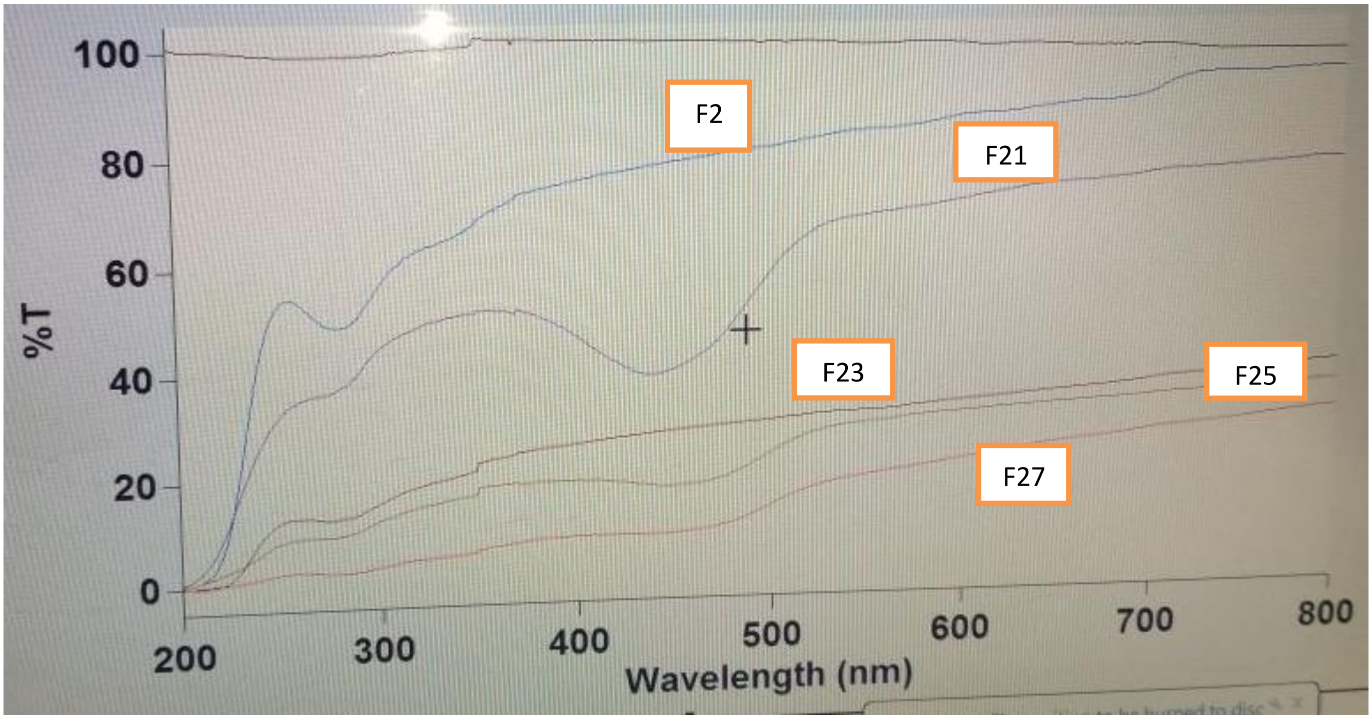


Figure 29: Transparency of PVA (F2) and other reinforced composite films.

Conclusion and future scope

Agro waste sugarcane bagasse is utilized to obtain nanocellulose using acid hydrolysis method. This report provides brief information about use of acid hydrolyzed nanocellulose in PVA matrix for food packaging applications. Owing to their versatile and advantageous characteristics these films are suitable for food packaging. In this project packaging of some dairy products like milk, curd and paneer was studied using PVA/nanocellulose composite films. To resist microbial attack and indicate pH change in packed food material turmeric was also added in the matrix as a natural pH indicator which shows different color at different pH ranges. These films can be successfully used for packaging of paneer and curd and are stable for about 12 hours of time period. These films were not found as a good packaging candidate for packaging of milk.

For improving mechanical properties of these films some steps can be taken like alignment of nanocellulose in the films, using a cross linker for better interaction of films and nanocellulose etc. These films are water soluble and hence are disposed easily.

In place of turmeric some other natural pH indicator can be utilized having more specific color range as compared to turmeric.

Hydrophilicity of films can be decreased by incorporating nanosilica particles with nanocellulose in the films.

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