STUDIES ON SOME PHYSICOMECHANICAL AND ANTIMICROBIAL PROPERTIES OF PREPARED CELLULOSE HYDROGEL FILMS

Thinzar Aye[1], Thinzar Nu[2], Cho Cho[3]

Abstract

In this research work, sugar cane bagasse waste (raw material) was used as a cellulose source which was chemically treated by sequential operation of sulphuric acid, sodium hydroxide and sodium hypochlorite. The yield percent of the purified cellulose fiber was 32.31 %. Fourier Transform Infrared (FT IR), Scanning Electron Microscope (SEM) and X-ray Diffraction (XRD) measurements were carried out to characterize the properties of raw material as well as cellulose fiber obtained from each sequential step. Cellulose fiber (1g) was dissolved in Dimethylacetamide /Lithium chloride (DMAc/LiCl) solution containing different lithium chloride concentration of 6, 8, 10 and 12 wt %. The different hydrogel films were obtained from above cellulose solutions of different solvent ratios leading to solid hydrogel in ethanol vapor by phase inverse method. The mechanical properties such as tensile strength, thickness, width, elongation, viscoelasticity and water content were measured to characterize the effect of LiCl concentrations on the resultant hydrogel films. Antimicrobial activities of cellulose solutions were tested for the purpose of using hydrogel films to biomedical applications.

Keywords: bagasse, cellulose hydrogel films, viscoelasticity, elongation, antimicrobial activities

Introduction

In recent years, there has been an increase in the level of research on the development of new biodegradable materials for use in packaging, agriculture, medicine and other areas. Generally, biodegradable polymer materials are increasingly important as environmental contamination and waste disposal problems associated with plastics and related products from synthetic polymers become more severe. Natural polymers have various advantages over synthetic polymers due to their low-cost, great availability and biodegradability (Zhou, *et al.,* 2008).

[1.] PhD Candidate, Assistant Lecturer, Department of Chemistry, Pyay University
[2.] Dr, Associate Professor, Department of Chemistry, University of Yangon
[3.] Dr, Professor and Head, Department of Chemistry, Yenangyaung Degree College

Cellulose hydrogel has become especially attractive to "tissuse engineering" as matrices for repairing and regenerating a wide variety of tissue and organs. Hydrogels consisted of hydrophilic polymer networks which can absorb from 10-20 % up to thousands of times of their dry weight in water. Various hydrogels from natural polymers were fabricated by using hyaluronate, chitosan and its derivatives, and cellulose in which there is a potential application in the biomaterials field. Among them, cellulose is the most abundant renewable resource on earth and may become a main chemical resource in the future. Therefore, this sustainable material in plants has numerous functional possibilities and can be expected with the demand for environmentally and biocompatible products (Svensson *et al.*, 2005).A wide range of lingocellulosic agricultural by-products has successfully been converted into cellulose hydrogel film including agave tequilana Weber bagasse (Karla *et al*., 2013), sugar cane bagasse (Kazuki and Kobayashi, 2016).

Sugarcane bagasse (SB), as the fibrous by-product remaining after sugar extraction from sugarcane, is one of the most important byproducts. About 54 million dry tons of SB are produced annually throughout the world (Ren*et al.*, 2006). Bagasses offer the advantages of being a cheap, plentiful and low polluting fuel (Mothe and Miranda, 2009). Commonly, all plant biomass consists of cellulose, hemicellulose, lignin, pectin and protein. Most of the plant biomass consists of about 33 % of cellulose as the major component of the rigid cell walls. Table 1 shows the percentage composition of sugarcane bagasse (Sun *et al.*, 2004)

Table 1: Percentage Composition of Sugar Cane Bagasse

Component	Composition (%)
Cellulose	44.6
Hemicellulose	33.5
Lignin	18.1
Ash	2.3
Wax	0.8
Other	0.7

Cellulose is a linear and high molecular weight polymer as well as natural, renewable and biodegradable material (Rachtanapun, 2009). Cellulose is aligned parallel to each other in fibrils, which are surrounded by a matrix of lignin and hemicellulose (Figure 1). In addition, cellulose has properties such as low density, good mechanical properties as well as biodegradability. Cellulose, the major chemical component of fiber wall and contributing 40-45 % of the dry weight. It is composed of linear chain of D-glucose linked by β -1,4-glycosidic bond with the degree of polymerization (DP) from 10,000 in native wood to 1,000 in bleached kraft pulps. Each D-anhydro glucopyranose unit possesses hydroxyl groups at C2, C3, and C6 positions, capable of undergoing the typical reactions known for primary and secondary alcohols. The molecular structure imparts cellulose with its characteristic properties: hydrophylicity, chirality, degradability, and broad chemical variability initiated by the high donor reactivity of hydroxyl groups.

Cellulose has a strong tendency to form intra - and inter-molecular hydrogen bonds by the hydroxyl groups on these linear cellulose chains, which stiffen the straight chain and promote aggregation into a crystalline structure and give cellulose a multitude of partially crystalline fiber structures and morphologies. Crystalline cellulose has very limited accessibility to water and chemicals (Edgar *et al.*, 2001).

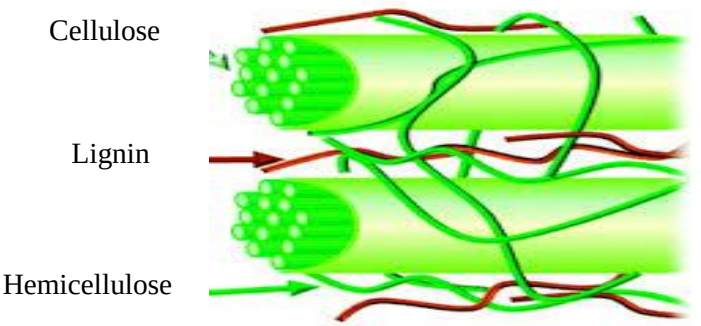


Figure 1: Components of primary cell wall

It is well known that native cellulose is very difficult to dissolve in common solvents. This is due to formation of strong hydrogen bond between abundant hydroxyl groups in the polymer chains. In addition, crystalline and

aggregated fibers of the cellulose also make it difficult in the solubilization. For this reason, studies on native cellulose hydrogel are fewer than cellulose derivatives. Therefore, solvents such as alkali-based aqueous system, N-methylmorpholine-N-oxide (NMMO), lithium chloride (LiCl)/ N, N-dimethylacetamide (DMAc) and ionic liquids (ILs) were developed for cellulose dissolutions. These solvent system provides great opportunities to prepare native cellulose hydrogel through physical cross-linking (Striegel, 1997).

For cellulose hydrogel films preparation has been reported by which a DMAc/LiCl system enables flexible cellulose hydrogel films using phase inversion processes. Hydrogels are networks of hydrophilic polymer chains in natural or synthetic polymers. They are highly water absorbent without being soluble in water. Here, the phase inversion process involves transformation from a liquid phase polymer solution to a solid state of the polymer (Kazuki and Kobayashi, 2016). In the present study, sugar cane bagasse was used to obtain cellulose hydrogel solution and the hydrogel film.

Materials and Methods

Collection of Sugar Cane Bagasse

Sugar cane bagasse was collected from Nawaday Sugar Mill, Pyay Township, Bago Region.

Bagasse Treatment

The bagasse was firstly washed with distilled water to remove remaining sugar components and then heated in oven at 50 ˚ C. For acid treatment, 10 g of bagasse was added into 300 mL of 4 vol % H_2SO_4 aqueous solution and stirred for 2 h at 90 ˚ C. Then, sample was washed with abundant distilled water five times to eliminate residues of the H_2SO_4 solution. And then 300 mL of 10 wt % NaOH solution was added and kept under stirring for 12 h at 90 ˚ C until a black liquor solution was obtained. The residues of fiber were washed with excess distilled water until neutral pH. After that the fiber was added into 300 mL of 10 vol % NaOCl solution and stirred for 3 h at 40 ˚ C. NaOCl was used as bleaching agent to obtain light coloured fiber for preparation of cellulose solution. Figure 2 shows sugarcane bagasse raw sample, acid treated sample, base treated sample and cellulose fiber. The

prepared cellulose fiber was used for the preparation of cellulose solution in DMAc/LiCl system.

(a) (b) (c) (d)

Figure 2: (a) Sugarcane bagasse raw sample (b) acid treated sample (c) base treated sample and (d) cellulose fiber

Preparation of Cellulose Hydrogel Solutions

The cellulose fiber (1 g) was stirred in 300 mL of distilled water at room temperature for 24 h to swell the fiber. After the swelling fiber was filtered by an adapter glass filter under vacuum, ethanol (300 mL) was added to the swelled fiber and the mixture was stirred for 24 h at room temperature. Ethanol was removed and the swelled fiber was added to 300 mL of DMAc. The mixture was then left overnight under stirring condition. Both dried LiCl and DMAc were added to the swelled bagasse fiber to dissolve the cellulose fiber and stirred at room temperature for 3 days. About 1 wt% cellulose in DMAc/LiCl containing different concentrations of LiCl (6, 8, 10 and 12 wt %) were obtained.

Preparation of Cellulose Hydrogel Films

Cellulose hydrogel films which containing different concentrations of LiCl (6, 8, 10 and 12 wt %) were prepared. 10 g of cellulose hydrogel solution was poured into a glass dish (9.1 cm diameter), and kept for 24 h in a plastic container filled with ethanol. In this step, cellulose was gradually progressed in the vapour at room temperature. Finally, the cellulose hydrogel films such as CHF-1, CHF-2, CHF-3 and CHF-4 were obtained by the phase inversion process from liquid to solid gel. The resultant transparent films as shown in Figure 3 were washed with excess distilled water and then placed in distilled water for 24 h to remove DMAc. The obtained hydrogel films were kept in plastic container filled with distilled water until further experiments.

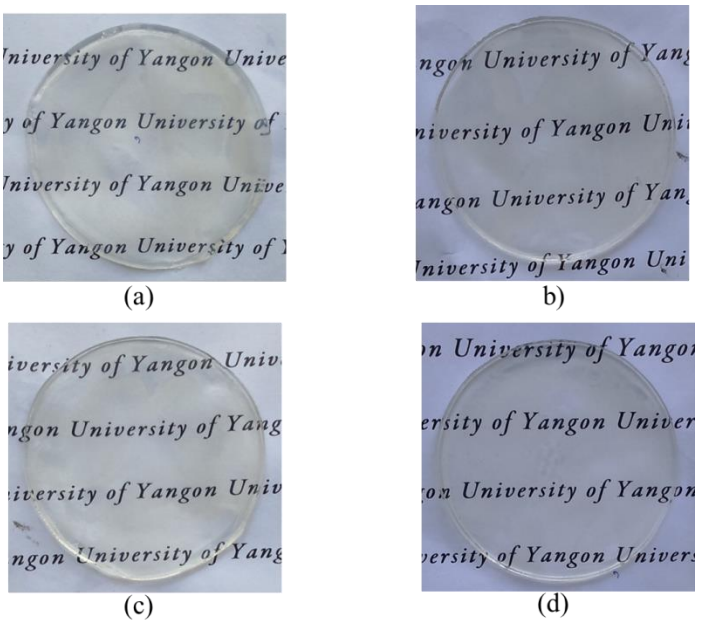


Figure 3: Prepared cellulose hydrogel films (a) CHF-1, (b) CHF-2, (c) CHF-3 and (d) CHF-4

Characterization of Raw Material and Treated Fibers

The formation of prepared cellulose fiber samples was monitored by FT IR, SEM and XRD. The structural changes of samples were analyzed by FT IR spectrometer (FT IR – 8400 SHIMADZU, Japan).FTIR analysis was in a range of wavenumber from 4000 to 400 cm^{-1}. Surface morphology of the samples was studied by SEM (JSM-5610 LV Scanning Microscope, JEOL, Japan). X-ray diffraction pattern of the sample was recorded on X-ray diffractometer (Rigaku, Tokyo, Japan), using CuK_{α} radiation (λ = 1.54 Å)at 40 kV and 40 mA. The diffraction angle ranged from 10˚ to 70˚ of 2θ .

Investigation of the Antimicrobial Activity of Cellulose Hydrogel Solutions

The cellulose hydrogel solutions such as 6 wt % cellulose hydrogel solution (CHS-1), 8 wt % cellulose hydrogel solution (CHS-2), 10 wt % cellulose hydrogel solution (CHS-3), 12 wt % cellulose hydrogel solution (CHS-4) and DMAc solvent were tested with *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and

Escherichia coli species to investigate the nature of antimicrobial activity. After preparing the bacteriological media, about 0.1 mL of sample was introduced into the agar –disc and incubated at 37 ºC for 24 h. The inhibition zone (clear zone) which appeared around the agar-disc indicated the presence of antimicrobial activity. The results are shown in Table 3.

Determination of the Properties of Cellulose Hydrogel Films

Tensile strength and elongation of the hydrogel films were measured on a LTS -500N - S20 (Minebea, Japan) with universal testing machine equipped with a 2.5 kN cell. Strips with a length of 50 mm and a width of 10 mm were cut from cast film with a razor blade. Strain was recorded by means of Zwick Makrosense clip-on displacement sensors. One set of samples (five strips each) was measured and each set was repeated 3 times. Only samples which ruptured near mid-specimen length were considered for the calculation of tensile strength. The values of the tensile strength and elongation were calculated. The results are shown in Table 2.

Water content of the resultant hydrogel films were determined by weighing dry and wet samples according to the following procedure. Disk samples with 5 mm of diameter were cut from cast films and dried in vacuum oven for 24 h and weighed. Then, samples were emerged in distilled water for 36 h. After 36 h the specimens were removed from water and wrapped with filtered paper on the surface in order to remove excess of water, and then weighed. Finally, the water content was calculated with the weights of the wet (Ws) and dried (Wd) hydrogel films.

$$\text{Water content (\%)} = (Ws - Wd)/ Wd \times 100$$

The water content of cellulose hydrogel films are shown in Table 2.

Viscoelasticity of the hydrogel films with 2 cm in diameter and having 5 mm of thickness was determined by Anton Paar- Reoplus equipment (Anton Paar Japan, Tokyo) in wet conditions at 37 °C. The results are shown in Figure 7.

Results and Discussion

In Figure 2, sugar cane bagasse raw sample, acid treated sample, base treated sample and cellulose fiber are shown. In acid treatment, hemicellulose is removed because hemicellulose is hydrolyzed easily by a dilute acid or base. Alkali treatment results in a higher amount of swelling. Cellulose fiber by treatment with sodium hypochlorite gets white colour from brown colour.

Figure 4 shows FT IR spectra of the sugarcane bagasse, treated fibers and cellulose fibers at different treatment conditions. The FT IR spectra of all sample show the strong broad band around 3400 cm^{-1}which is due to the O-H stretching vibration. The strong band at around 2900 cm^{-1} which is due to C-H stretching vibration referred to CH_2 group. The absorption band at around 1730 cm^{-1} indicates the C=O stretching in carbonyl group of pyrone. Appearance of the band around 1600 cm^{-1} is a relative pure ring stretching mode strongly associated with the aromatic ring C=C in benzene as well as in pyrone ring. From the comparison of the FT IR spectra, the appeared peaks due to aromatic skeletal vibration of lignin around 1510 cm^{-1} showed the presence of lignin and lignocellulose in the initial raw sample. Moreover, the C-O-C stretching vibration of ester group of hemicellulose around 1200 cm^{-1} disappeared in treated fiber which is assigned the removal of hemicelluloses in cellulose fiber by bleaching. This clearly indicated that the amount of lignin from the sugar cane bagasse raw sample was successfully reduced by the chemical and temperature treatments.

SEM microphotographs of the treated samples indicated clearly the appearance surface morphologies of the samples. Figure 5(a) is SEM micrograph of sugar cane bagasse sample which shows the major constituents of natural fibers such as cellulose, hemicelluloses and lignin. The SEM images of treated samples in Figures 5(b) and (c) have been changed in their morphologies due to the removal of lignin and hemicelluloses. Finally Figure 5(d) can be seen that the main structural unit of cellulose in the plant wall consists of cellulose microfibrils bonded together in a polymeric matrix.

XRD measurement was carried out to evaluate the effect of treatment condition on the crystalline structure of bagasse, treated fibers and cellulose fibers. Figure 6 shows the XRD patterns of the bagasse and the purified fibers. The patterns of (a) – (d) exhibited typical crystalline lattice of cellulose with

peaks at 22.3° and 16.4° . The crystalinity indices of sugar cane bagasse raw sample, acid treated sample, base treated sample and cellulose fiber were 44.1%, 58.8%, 59.1% and 60.2% respectively. The increment of crystalinity in cellulose fiber was due to the removal of hemicelluloses and lignin by NaOH and NaOCl treatment.

The crystalinity index (CI) percent was calculated by using the following equation:

$$CI\ (\%) = (\ I_{002} - I_{am}\ ) / I_{002} \times 100$$

I_{002} = the maximum intensity of the peak (002) lattice diffraction

I_{am} = the intensity of diffraction attributed to amorphous cellulose

As shown in Table 2, the tensile strength and elongation values of 8 wt % LiCl cellulose hydrogel film (CHF-2) showed the highest value other than the other hydrogel films such as 6, 10 and 12 wt % LiCl cellulose hydrogel films (CHF-1, CHF-3 and CHF-4). However, the values of CHF-3 and CHF-4 were higher than CHF-1. Therefore, the effect could improve the resistance to the applied force in the higher LiCl case increasing the elongation value of the hydrogel films.

Table 2 lists the properties of hydrogel films. The values of water content decreased from 320 % to 156 % with the increment of the LiCl content from 6 to 12 wt % respectively. Therefore, water contents in the film became higher when the LiCl content was lower. It was noted that the hydrogel films had very soft and flexible shape even though there was no chemical crosslinking treatment.

Figure 7 shows relationship between *G'* and *G''* for the viscoelasticity of the hydrogel films. The strain was varied from 10^{-1} to 10^{2} % at 25 °C and constant frequency of 1 Hz. The crossover point of *G'* and *G''* meant fracture of material or inability to follow deformation, since rigid polymer network may not flow. So, crossover point at lager strain indicated flexible structure of the hydrogel film. In the case of 8 wt % LiCl, the *G'* and *G''* values were overlapped at 35.71 % strain. However, crossover points of 6, 10 and 12 wt % LiCl were lower which indicated less flexible structure of the hydrogel films comparing to 8 wt % LiCl.

Antimicrobial activity of 6 wt % cellulose hydrogel solution (CHS-1), 8 wt % cellulose hydrogel solution (CHS-2), 10 wt % cellulose

hydrogel solution (CHS-3), 12 wt % cellulose hydrogel solution (CHS-4) and DMAc solvent are shown in Table 3. The tested organisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli.* As seen in Figure 8, antimicrobial tests of CHS-1, CHS-2, CHS-3, CHS-4 and DMAc solvent were used in the agar medium cultivation. DMAc solvent was seen as inactive against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*. CHS-1, CHS-2, CHS-3 and CHS-4 were observed to possess the antimicrobial activity against all six test organisms show the reaction. Among these, CHS-2 has the highest activity.

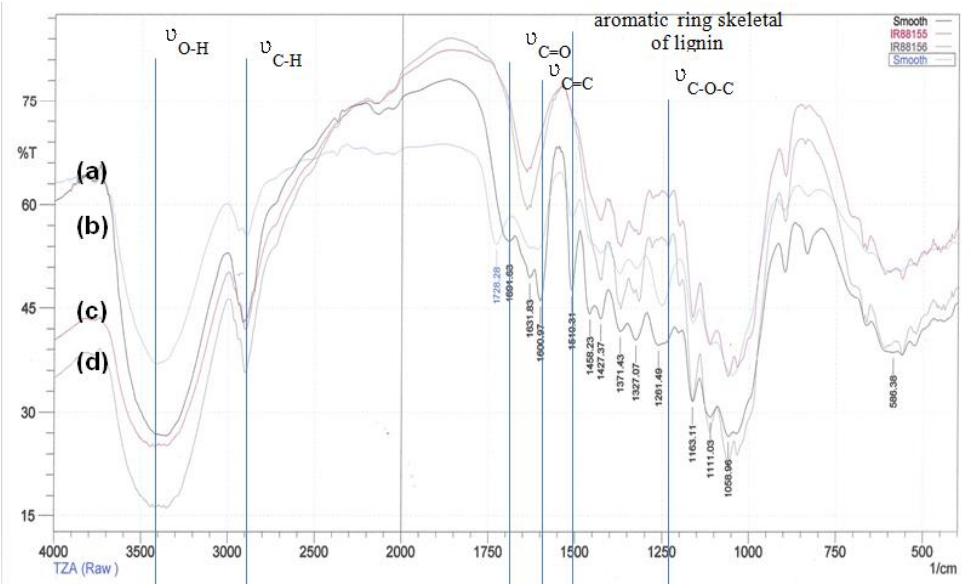


Figure 4: FT IR spectra of (a) sugar cane bagasse raw sample (b) acid treated sample (c) base treated sample and (d) cellulose fiber

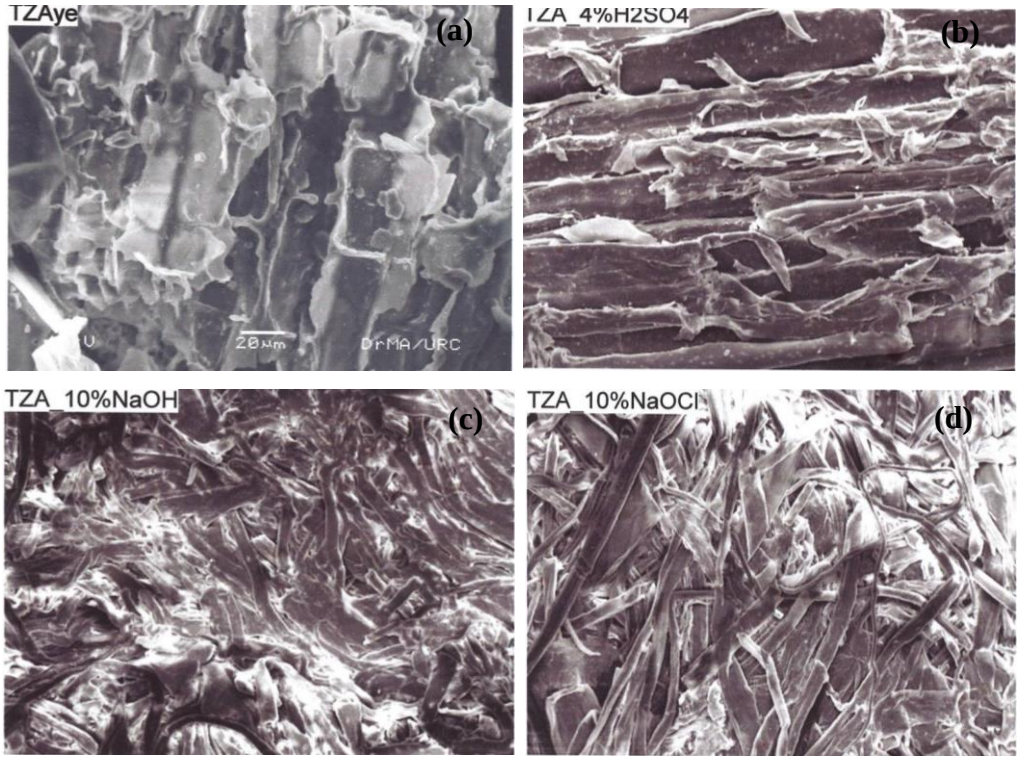


Figure 5: SEM photographs (20µ m magnification) of (a) sugar cane bagasse raw sample (b) acid treated sample (c) base treated sample and (d) cellulose fiber

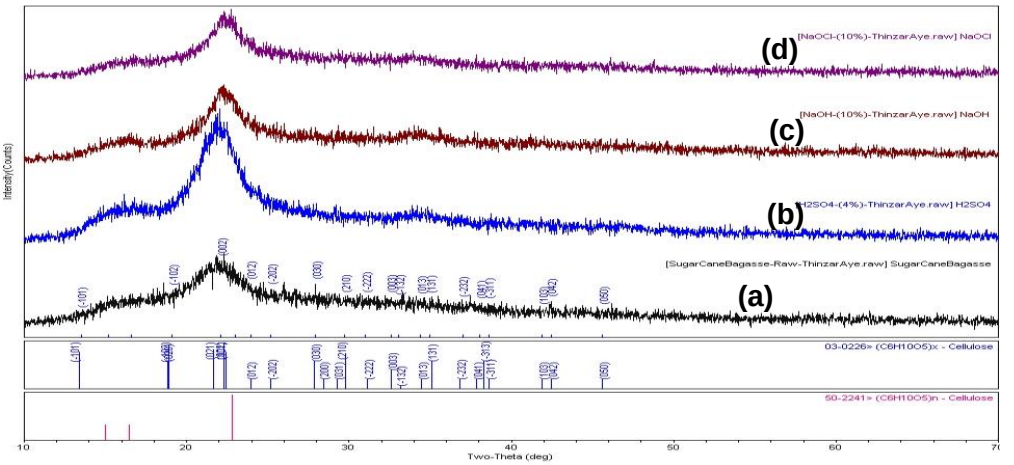


Figure 6: XRD patterns of (a) sugar cane bagasse raw sample (b) acid treated sample (c) base treated sample and (d) cellulose fiber

Table 2: Properties of Cellulose Hydrogel Films Prepared from Sugar Cane Bagasse

Type of Hydrogel Film	Width (mm)	Thickness (mm)	Tensile Strength (N/mm^2)	Elongation (%)	Water Content (%)
CHF-1	10.14	0.636	0.2499	22.58	320
CHF-2	9.96	0.592	0.3597	27.52	228
CHF-3	9.88	0.618	0.3113	24.82	186
CHF-4	10.02	0.612	0.3019	24.31	156

CHF-1 using 6 % LiCl in DMAc (w/w)
CHF-2 using 8 % LiCl in DMAc (w/w)
CHF-3 using 10 % LiCl in DMAc (w/w)
CHF-4 using 12 % LiCl in DMAc (w/w)

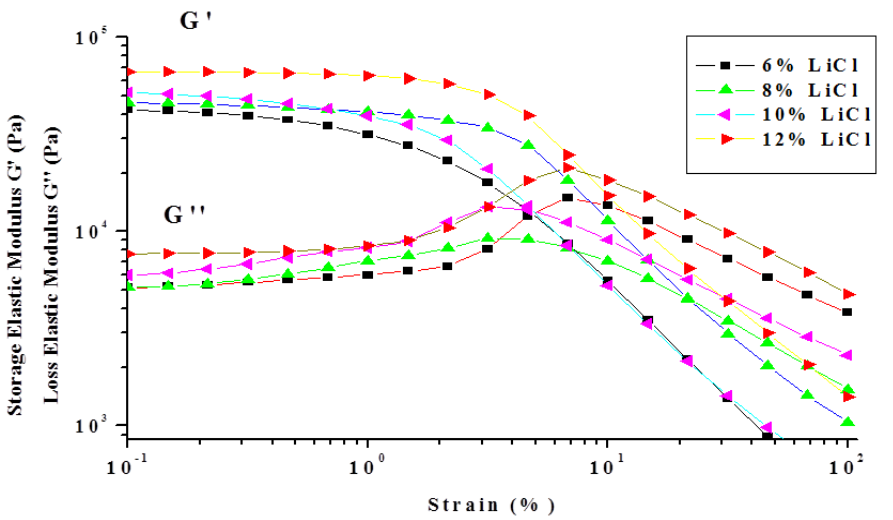
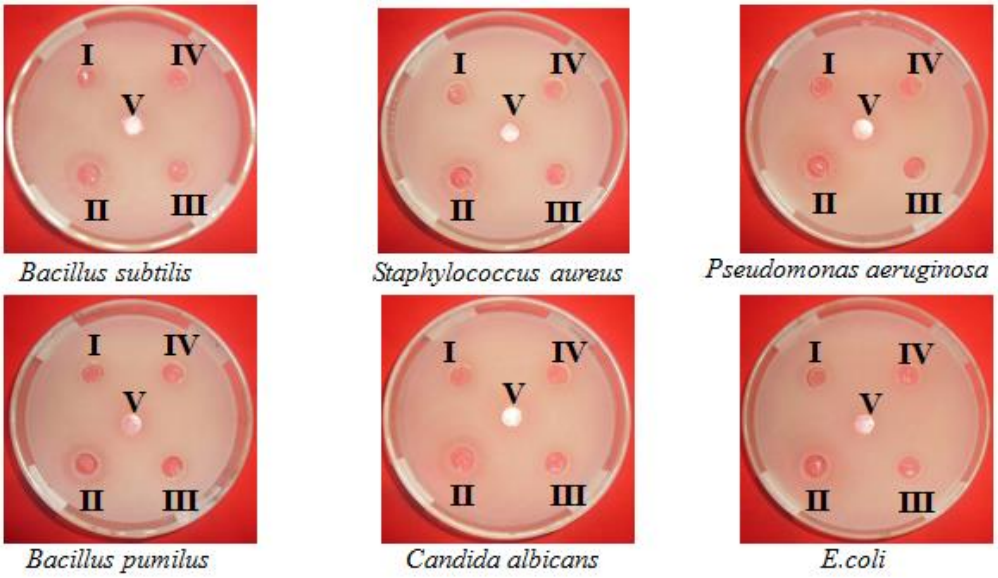


Figure 7: Viscoelasticity of cellulose hydrogels films of different LiCl contents for strain –G',-G" plots at 25 ˚ C



I = CHS-1, II = CHS-2, III = CHS-3, IV = CHS-4, V = DMAc

Figure 8: Antimicrobial activity of cellulose hydrogel solutions and DMAc solvent ondifferent bacterial strains

Table 3: Antimicrobial Activities of Cellulose Hydrogel Solutions

Type of Hydrogel Solutions	**Inhibition Zone Diameters of Different Cellulose Hydrogel Solutions Against Tested Organisms (mm)**				
	B. subtilis	***S.aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***
CHS-1	11 (+)	11 (+)	11 (+)	11 (+)	11 (+)
CHS-2	16 (++)	17 (++)	16 (++)	18 (++)	17 (++)
CHS-3	11 (+)	12 (+)	11 (+)	11 (+)	11 (+)
CHS-4	11 (+)	12 (+)	11 (+)	11 (+)	11 (+)
DMAc solvent	10 (+)	10 (+)	10 (+)	10 (+)	10 (+)

Agar Well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Conclusion

Cellulose hydrogel films were successfully prepared from sugar cane bagasse by phase inversion of the DMAc solvent with LiCl. The sugar cane bagasse was used as starting material which was treated using sulphuric acid, sodium hydroxide and then bleached with sodium hypochlorite. The treated samples were characterized by FT IR, SEM and XRD. From the XRD data, percent crystalinity indices of sugar cane bagasse raw sample, acid treated sample, base treated sample and cellulose fiber were 44.1 %, 58.8 %, 59.1 % and 60.2 %, respectively. SEM and FT IR analyses clearly showed that the amount of liginin and hemicellulose from sugar cane bagasse sample was successfully reduced by chemical treatment and also proved that the final product was cellulose fiber. From the observation, obtained bagasse fiber was pure cellulose fiber which was used for preparation of cellulose hydrogel films. The different LiCl concentrations such as 6, 8, 10 and 12 wt % were used to prepare different hydrogel films. These films had good mechanical properties, although there were no chemical crosslinking. Depending on the LiCl concentrations, these hydrogel films exhibited varying nature in the deformation, tensile strength, elongation and water retainable property. Among these, the best mechanical properties, antimicrobial activities and suitable water retainable property were observed in hydrogel film (CHF-2) prepared at 8 wt % of LiCl. Showing the antimicrobial activities of cellulose solutions against six test organisms, the cellulose hydrogel films are possible to be utilized for biomedical applications.

Acknowledgements

The authors wish to thank to Professor Dr Hnin Hnin Aye, Head of the Department of Chemistry, University of Yangon for her encouragement. Warmest thanks are also extended to Professor Dr Ye Chan, Head of Department, Universities' Research Centre, University of Yangon for providing necessary research facilities.

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