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Synthesis and characterisation of cellulose & its derivatives to be used a template for bio-glass

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Abstract
Cellulose is an organic compound, a polysaccharide consisting of a linear chain of linked D- glucose units. In this paper, we study how the templates of cellulose & its derivatives were prepared. The characterization of cellulose and its derivatives was done through FTIR, SEM, NMR and XRD machines. Interaction between urea and cellulose in NaOH/ urea medium was also investigated and evidence was provided to support it. The objective of this paper was to determine the effectiveness of cellulose to be used as a template for bio-glass.

Keywords: FTIR, SEM, NMR and XRD machines, cellulose

1. Introduction

1.1 Bio-glass

A glimpse through the history of development of materials used in dentistry, specifically replacement materials, shows that the aim has been to create materials that were as chemically inert as possible. The materials used in 1960s were mostly metallic. In the late 1960s and early 1970s, the search for better biocompatibility of implant materials resulted in the new concept of bio-ceramic materials that would mimic natural bone tissue. Hydroxyapatite, a naturally occurring ceramic mineral, was also the mineral component of bone. Thus, only synthetic hydroxyapatite was believed to be entirely compatible with the body. During this period, Professor Hench came up with a new biocompatible material using silica as a base material that could be mixed with other ingredients such as calcium to unite fractured bones. By using this material, the trend of implant materials was shifted to stimulate body's own regenerative capabilities. This new glass material on dissolving, in normal physiological environment, activates genes controlling osteogenesis and growth factor production with bone produced of equivalent quality to natural bone. The trabecular bone growth and quantity were much more than produced by synthetic hydroxyapatite. After implantation of this material in bone tissue, these glass materials resisted removal from the implant site -- which was coined as “bonded to bone” by Hench. Hench used the term “bioactive glass”. A bioactive material is defined as a material that elicits a specific biological response at the interface of the material, which results in the formation of a bond between the tissue and that material. The gene activation, bone regenerative capability with better quality and quantity of bone equivalent to normal bone, and high level of bioactivity are unique only to bio-glass when compared with synthetic hydroxyapatite and any other allograft, which more than justifies the use of bio-glass.

The advantages of bio-glass over synthetic hydroxyapatite are the biological fixation, and the capability of bonding to both hard and soft tissues, whereas hydroxyapatite binds only to hard tissues and also needs an exogenous covering to hold the implants in place. Bio-inspired synthesis is taken for preparation of bio-glass.

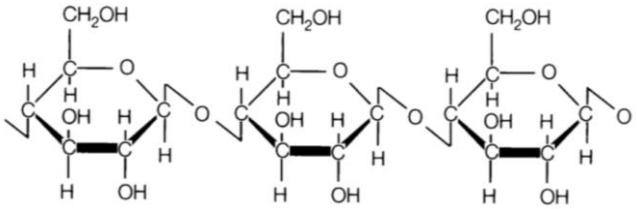
1.2 Bio-Inspired Synthesis

A number of fascinating advances, technologies and possibilities have emerged in recent years in the burgeoning field of nanotechnology. Metal nano-materials (MNMs) including common nanoparticles (NPs), nano-clusters (NCs), nano-wires (NWs) and related nano-structures have received tremendous attention owing to their unique catalytic, electrical, magnetic and thermal properties. ‘‘Top-down’’ and ‘‘bottom-up’’ approaches in nanotechnology are also generally applicable to the fabrication of MNMs. In contrast to ‘‘top-down’’ approaches, ‘‘bottom-up’’ protocols enable a comparatively flexible and inexpensive preparation.

MNMs have long existed in natural environments. Some metal ions might be adsorbed and further reduced to elemental metals by microorganisms, plants, biomass, etc. Bio-inspired syntheses have emerged as innovative and alternatively attractive synthetic protocols for MNMs. Seminal reports on bio- inspired synthesis date back to the late 90s when Ag and Au NPs were prepared from Pseudomonas stutzeri AG259 and alfalfa plant biomass, respectively. Along with microorganisms and plants, viruses, proteins and DNA have also become potentially useful candidates for bio-inspired synthesis of MNMs. Herein, based on these biological candidates, bio-inspired synthesis encompasses a combined application of biological concepts, mechanisms and functions for the design and development of innovative bio derived (nano) materials with a number of applications. Such combined application could be applicable to the synthesis or assembly of a wide range of inorganic NMs, bio-glass being one of them. We require a template for making bio-glass through bio-inspired route, thus we use Cellulose and its derivatives, namely Methyl Cellulose and Grafted Cellulose.

1.3 Cellulose

Cellulose probably is the most abundant organic compound in the world which mostly produced by plants. It is the most structural component in herbal cells and tissues. Cellulose is a long chain of linked sugar molecules that gives wood its remarkable strength.



Cellulose Structure

It is the main component of plant cell walls, and the basic building block for many textiles and for paper. Cotton is the purest natural form of cellulose. In the laboratory, ash less filter paper is a source of nearly pure cellulose. Cellulose is a natural polymer, a long chain made by the linking of smaller molecules. The links in the cellulose chain are a type of sugar: ß-D-glucose. Two unlinked molecules of ß-D-glucose are pictured at right. The sugar units are linked when water is eliminated by combining the -OH group and H. Linking just two of these sugars produces a disaccharide called cellobiose. Cellulose is a polysaccharide produced by linking additional sugars in exactly the same way.

The cellulose chain bristles with polar -OH groups. These groups form many hydrogen bonds with OH groups on adjacent chains, bundling the chains together. The chains pack regularly in places to form hard, stable crystalline regions that give the bundled chains even more stability and strength. Cellulose is a major component of wood. Cellulose fibres in wood are bound in lignin, a complex polymer. Cellulose is found in large amounts in nearly all plants, and is potentially a major food source. Crystallite cellulose is added to some foods to reduce the caloric value.

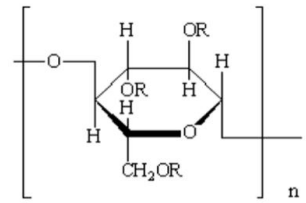
Cellulose is a natural long chain polymer that plays an important role in human food cycle indirectly. This polymer has versatile uses in many industries such as veterinary foods, wood and paper, fibres and clothes, cosmetic and pharmaceutical industries as excipient. Cellulose has very semi-synthetic derivatives which is extensively used in pharmaceutical and cosmetic industries. Cellulose ethers and cellulose esters are two main groups of cellulose derivatives with different physicochemical and mechanical properties. These polymers are broadly used in the formulation of dosage forms and healthcare products. These compounds are playing important roles in different types of pharmaceuticals such as extended and delayed release coated dosage forms, extended and controlled release matrices, osmotic drug delivery systems, bio-adhesives and muco-adhesives, compression tablets as compressibility enhancers, liquid dosage forms as thickening agents and stabilizers, granules and tablets as binders, semisolid preparations as gelling agents and many other applications. These polymeric materials have also been used as filler, taste masker, free-flowing agents and pressure sensitive adhesives in transdermal patches. Cellulose and cellulose based polymers have gained a great popularity in pharmaceutical industries owing to production of the new derivatives and finding new applications for existed compounds.

1.3.1 Methyl Cellulose

Methyl cellulose is a chemical compound derived from cellulose. It is a hydrophilic white powder in pure form and dissolves in cold water, forming a clear viscous solution or gel. It is sold under a variety of trade names and is used as a thickener and emulsifier in various food and cosmetic products, and also as a treatment of constipation. Like cellulose, it is not digestible, not toxic, and not an allergen.

Methyl cellulose has an extremely wide range of uses, of which several are described below.

Methyl cellulose is very occasionally added to hair shampoos, tooth pastes and liquid soaps, to generate their characteristic thick consistency. This is also done for foods. It is also an important emulsifier, preventing the separation of two mixed liquids because it is an emulsion stabiliser. It is used as a variable viscosity personal lubricant; it is the main ingredient in K-Y Jelly. The lubricating property of methyl cellulose is of particular benefit in the treatment of dry eyes. Dry eyes are common in the elderly and is often associated with rheumatoid arthritis. The lacrimal gland and the accessory conjunctival glands produce fewer tears.



where R = H or CH_3

Methyl cellulose structure

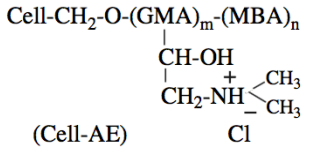
Methyl cellulose may be used as a tear substitute. Methyl cellulose is used in the manufacture of capsules in nutritional supplements; its edible and nontoxic properties provide a vegetarian alternative to the use of gelatin. When eaten, methyl cellulose is not absorbed by the intestines but passes through the digestive tract undisturbed. It attracts large amounts of water into the colon, producing a softer and bulkier stool.

It is used to treat constipation, diverticulosis, haemorrhoids and irritable bowel syndrome. It should be taken with sufficient amounts of fluid to prevent dehydration. Methylcellulose finds a major application as a performance additive in construction materials. It is added to mortar dry mixes to improve the mortar's properties such as workability, open and adjustment time, water retention, viscosity, adhesion to surfaces etc. Methylcellulose can be employed as a mild glue which can be washed away with water. This may be used in the fixation of delicate pieces of art as well as in book conservation to loosen and clean off old glue from spines. Methyl cellulose is the main ingredient in many wallpaper pastes. It is also used as a binder in pastel crayons and also as a binder in medications. Methyl cellulose is used as sizing in the production of papers and textiles as it protects the fibres from absorbing water or oil. Aqueous methyl cellulose solutions have been used to slow bacterial cell motility for closer inspection. Changing the amount of methyl cellulose in solution allows one to adjust the solution's viscosity.

1.3.2 Grafted Cellulose

Cellulose is one of the most abundant and low cost natural polymers, used as column packing materials for liquid chromatography. The adsorption properties of native

cellulose are not constant and vary according to its origin. Their adsorption capacity for metal ions remains very low in comparison to activated carbon. For modifying natural and synthetic polymers, grafting is preferred to physical blending because the grafted polymer chains are covalently linked and interposition don the backbone polymer. Grafting of synthetic polymers onto solids followed by functionalisation is a well-known method for the modification of the physical & chemical properties of the adsorbent. Cellulose derivatives have been used as ion exchangers or chelate resins because of their high chemical & mechanical stability & hydrophilic character. The substitution of tertiary and quaternary ammonium functional groups on cellulose backbone increases the number and changes the nature of reaction sites capable of adsorbing certain metal ions in solution.



Glycidyl methacrylate (GMA) has a reactive epoxy group, which on opening generates new functional groups that find uses in ion exchange, chelate formation and as pseudo-affinity ligands.

Since GMA is a hydrophobic monomer, its grafting into cellulose results in increased water repellence of the backbone, but cross-linker like N, N' -methylene-bi-acrylamide (MBA) being hydrophilic, increases water uptake by the copolymers. This work concerns the graft polymerization reaction of cellulose with GMA using MBA as a cross linker and benzoyl peroxide as an initiator, followed by amination and acid treatment, and its application in adsorption of As (V) from aqueous media.

Solubility Cellulose is very difficult to dissolve in common solvents because there is large amount of –OH in cellulose chains, forming strong intra- and intermolecular hydrogen bond interactions. The most widespread and dominant industrial process for the production of regenerated cellulose materials such as fibres and films is the viscose process, which is harmful to the environment and human health, so much effort has been made to develop a non-polluting solvent. One of the most interesting alternatives is the NaOH/urea aqueous solution developed by Zhang's research group. They reported that the dissolution of cellulose can be improved by adding moderate urea to aqueous alkali solutions. Zhang *et al*. suggest that the NaOH hydrates are directly associated with cellulose, and then the urea hydrates are possibly self-assembled at the surface of the NaOH hydrogen-bonded cellulose to form an inclusion complex.

However, the dissolution mechanism of cellulose in this solvent is still elusive, such as the role of urea and the interaction between cellulose and the solvent components. Much scientific research has been conducted to investigate whether there is a direct interaction between cellulose and urea. Based on the differential scanning calorimetry (DSC) results, Egal *et al.* propose that urea is not interacting with cellulose, but plays a role of binding water to make cellulose–NaOH links more stable. Xiong *et al*. suggest that urea has no strong direct interaction with cellulose as well as NaOH, and it may accumulate on the hydrophobic region to prevent the re-association of dissolved cellulose. On the contrary, results from solid-state 13C CP/MAS NMR showed that urea molecules interact directly with the cotton cellulose surface under dry conditions. Bergenstrahle-Wohlert *et al.* also find that there is a direct interaction between urea and cellulose through both hydrogen bonds and favourable dispersion interactions under wet conditions as well. Cai *et al.* suggested that a dominant hydrogen bonding pattern exists between cellulose and urea.

Alkali solutions were used to treat cellulose since Mercer discovered the process of mercerization of cellulose in 1844, and NaOH and LiOH with certain concentration range were found to dissolve cellulose. When additives such as urea or thiourea were added, the solubility and stability of the cellulose solution increased. Zhang's group did very intensive study on the dissolution of cellulose using alkali/additive solvent system. They found urea or thiourea with proper concentration can improve the dissolution of cellulose in aqueous alkali solutions. The aqueous solution of 7 wt% NaOH/12 wt% urea, pre-cooled to low temperatures, was found to be very adequate to the cellulose dissolution both in solubility and solution stability. In this solvent system, NaOH is found to interact with hydroxyl groups of cellulose, while the role of urea in cellulose dissolution is studied with different opinions.

2. Instrumentation

2.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. FTIR provides the following information:

- It can identify unknown materials
- It can determine the quality or consistency of a sample
- It can determine the amount of components in a mixture

Principle

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000 – 600cm^{-1}. The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The resultant absorption

spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range.

Sample Preparation

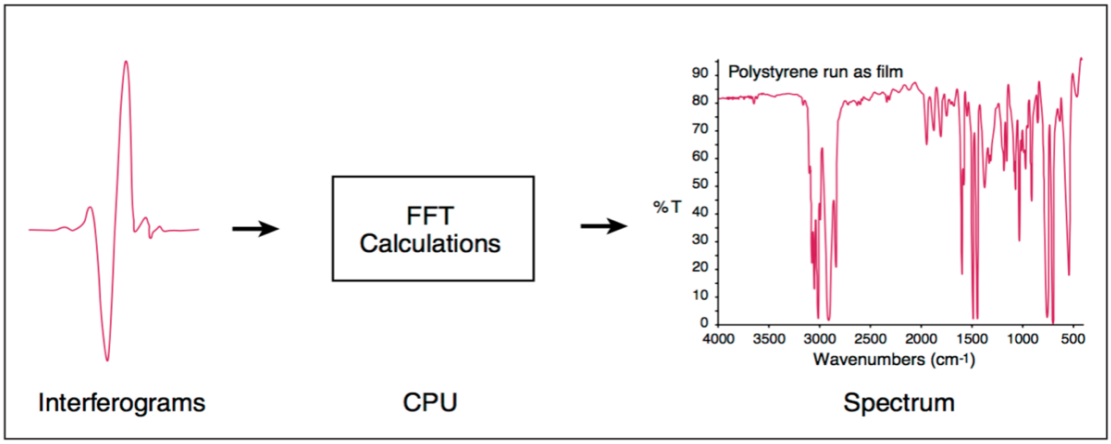
- Horizontal ATR – attenuated total reflectance. Allows measurement of aqueous solutions, elastic and viscous samples which are difficult to grind
- Specular reflectance – allows measurement of thin films on metals
- KBr Discs – allow suspension of powders or contaminants in IR transparent KBr so they may be analysed
- Gas cells for head space analysis.
- Solvent extractions of low level bulk organic compounds and surface contaminants.
- Solution cells – for measuring liquid sample in transmission mode.

Working

Infrared spectroscopy has been a workhorse technique for materials analysis in the laboratory for over seventy years. An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the same infrared spectrum. Infrared spectroscopy can result in a qualitative analysis of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present. With modern software algorithms, infrared is an excellent tool for quantitative analysis.

FTIR spectrometry was developed in order to overcome the limitations encountered with dispersive instruments. The main difficulty was the slow scanning process. A method for measuring all of the infrared frequencies simultaneously was needed. A solution was developed which employed a very simple optical device called an interferometer. The interferometer produces a unique type of signal which has all of the infrared frequencies "encoded" into it. The signal can be measured very quickly, usually on the order of one second. Time element per sample is reduced to few seconds rather than several minutes.

Most interferometers employ a beam splitter which takes the incoming infrared beam and divides it into two optical beams. One beam reflects off of a flat mirror which is fixed in place. The other beam reflects off of a flat mirror which is on a mechanism which allows this mirror to move a very short distance away from the beam splitter. The two beams reflect off of their respective mirrors and are recombined when they meet back at the beam splitter. Because the path that one beam travels is a fixed length and the other is constantly changing as its mirror moves, the signal which exits the interferometer is the result of these two beams "interfering" with each other. The resulting signal is called an interferogram having a unique property that every data point which makes up the signal has information about every infrared frequency which comes from the source.



Spectrum Generation

As the interferogram is measured, all frequencies are being measured simultaneously. Thus, the use of the interferometer results in extremely fast measurements.

Because the analyst requires a frequency spectrum, in order to make identification, the measured interferogram signal cannot be interpreted directly. A means of "decoding" the individual frequencies is required. This can be accomplished via a well-known mathematical technique called the Fourier transformation. This transformation is performed by the computer which then presents the user with the desired spectral information for analysis.

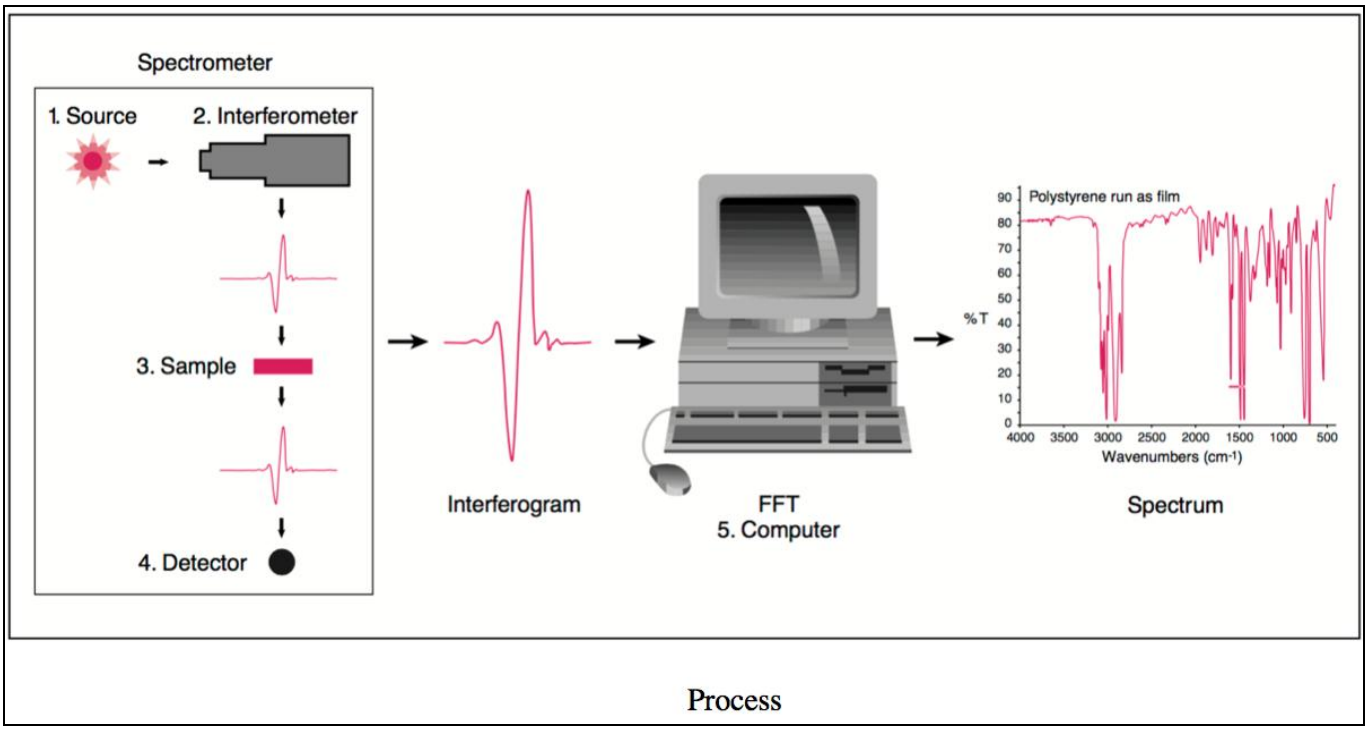
Process

The normal instrumental process is as follows:

1. The Source: Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample.
2. The Interferometer: The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer.
3. The Sample: The beam enters the sample compartment

where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. The Detector: The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.
5. The Computer: The measured signal is digitized & sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented for interpretation.



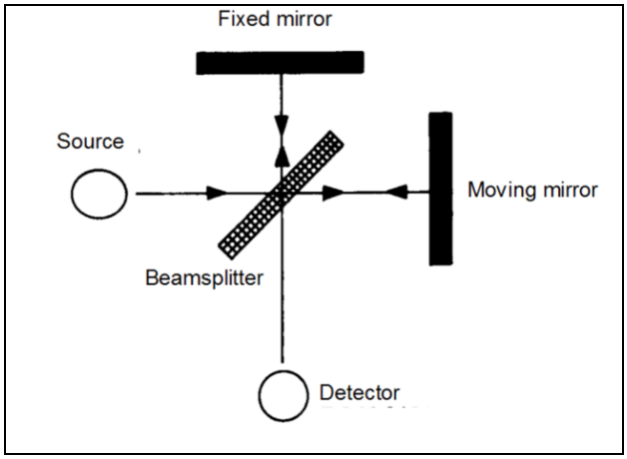
Process

Because there needs to be a relative scale for the absorption intensity, a background spectrum must also be measured. This is normally a measurement with no sample in the beam. This can be compared to the measurement with the sample in the beam to determine the "percent transmittance." This technique results in a spectrum which has all of the instrumental characteristics removed. Thus, all spectral features which are present are strictly due to the sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of the instrument itself.

The Michelson Interferometer

The Basic Michelson Interferometer Consists Of:

- a broad-band light source which emits light covering the mid-IR range,
- a beam splitter made of KBr or CsI,
- Two front surface coated mirrors – one moving and one fixed, and a detector.



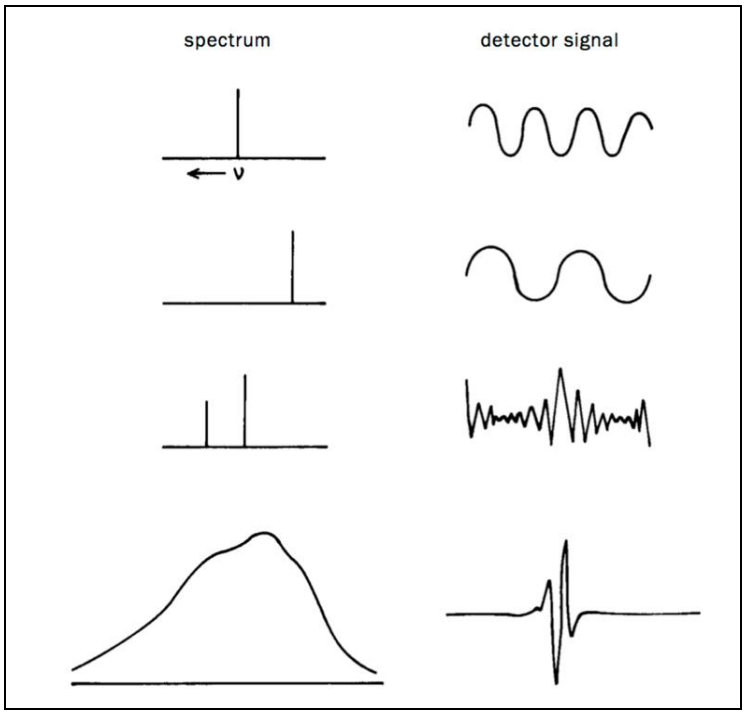
Schematic of Michelson Interferometer

The working principle of Michelson Interferometer

a) Light from the light source is directed to the beam splitter. Half of the light is reflected and half is transmitted.
b) The reflected light goes to the fixed mirror where it is reflected back to the beam splitter. The transmitted light is sent to the moving mirror and is reflected back towards the mirror.
c) At the beam splitter, each of the two beams (from the fixed and moving mirrors) is split into two: one goes back to the source and the other goes towards the detector. Hence the detector sees two beams: one from the moving mirror and the other from the fixed mirror.
d) The two beams reaching the detector come from the same source and have an optical path difference determined by the positions of the two mirrors, i.e. they have a fixed phase difference. Therefore the two beams interfere.
e) The two beams may interfere constructively or destructively for a particular frequency by positioning the moving mirror. If the moving mirror is scanned over a range, a sinusoidal signal will be detected for that frequency, with its maximum corresponding to constructive interference and the minimum corresponding to destructive interference. This sinusoidal signal is called interferogram – detector signal (intensity) against optical path difference.
f) Assume a light source which emits only two frequencies, each frequency will produce its own sinusoidal interferogram. Both interferograms will have maximum at optical path difference δ equals to zero. But the other maxima will not coincide, since their positions are determined by the equation,

$$\delta = 2\Delta x = 2n\lambda i,\ \lambda i = \lambda 1, \lambda 2$$

Where Δx is the difference in the distances between the beam splitter and the two mirrors.
g. Because the source emits a range of frequencies, the detector output is the sum of all the interferograms. The resulting inteferogram will have a maximum at Δx = 0 (centre burst) and tails off rapidly away from the centre burst.



Relation between spectrum and interferogram

Generating the Spectrum

- Interferogram is determined experimentally in FTIR spectroscopy, and the corresponding spectrum – frequency against intensity plot, is computed using Fourier transform. This transformation is carried out automatically and the spectrum is displayed.
- The detector sees all the frequencies simultaneously, whereas in a dispersive spectrometer, only one frequency can reach the detector at one time.
- FTIR spectrometer is inherently a single beam instrument. It is imperative to record a relevant background spectrum for each sample examined.
- Background spectrum: The empty beam background (no sample in the light path) is recorded first. This spectrum shows the instrument energy profile, which is affected by the characteristics of the source, the beam splitter, the absorption by the air (mainly due to CO_2 and water vapour) in the beam path, and the sensitivity of the detector at different wavelengths.
- Sample spectrum: The sample is placed in the combined beam. The sample spectrum is the ratio of the spectrum containing sample against that of the background.
- In recording the background spectrum, the light path should be made as close to that of the sample spectrum as possible. For example, in transmission experiment, the background may be recorded with nothing in the light path whereas it should be recorded with KBr powder in the DRIFT experiment.

Advantages

Some of the major advantages of FT-IR over the dispersive technique include:

- Speed: Because all of the frequencies are measured simultaneously, most measurements by FT-IR are made in a matter of seconds rather than several minutes.
- Sensitivity: Sensitivity is dramatically improved with FT-IR for many reasons. The detectors employed are much more sensitive, the optical throughput is much higher which results in much lower noise levels, and the fast scans enable the co-addition of several scans in order to reduce the random measurement noise to any desired level.
- Mechanical Simplicity: The moving mirror in the interferometer is the only continuously moving part in the instrument. Thus, there is very little possibility of mechanical breakdown.
- Internally Calibrated: These instruments employ a HeNelaser as an internal wavelength calibration standard.

These advantages make measurements made by FT-IR extremely accurate and reproducible. Thus, it a very reliable technique for positive identification of virtually any sample. The sensitivity benefits enable identification of even the smallest of contaminants. This makes FT-IR an invaluable tool for quality control or quality assurance applications whether it be batch-to-batch comparisons to quality standards or analysis of an unknown contaminant. In addition, the sensitivity and accuracy of FT-IR detectors, along with a wide variety of software algorithms, have dramatically increased the practical use of infrared for quantitative analysis. Quantitative methods can be easily developed and calibrated and can be incorporated into simple procedures for routine analysis.

Applications of FTIR

- Identification of simple mixtures of organic & inorganic compounds as solids or liquids.
- Identification of polymers and polymer blends.
- Indirect verification of trace organic contaminants on surfaces.
- Routine qualitative & quantitative FTIR Analysis.
- Thin film analysis.
- Analysis of adhesives, coatings and adhesion promoters or coupling agents.
- Small visible particle chemical analysis.
- Analysis of stains and surface blemishes remnant from cleaning and degreasing processes combined with optical microscopy, SEM/EDX, XPS and SIMS techniques.
- Analysis of resins, composite materials and release films.
- Solvent extractions of contaminants, plasticisers, mould release agents and weak boundary layers coupled with XPS surface chemical analysis techniques.
- Identification of rubbers and filled rubbers.
- Determination of degrees of crystallinity in polymers (eg. LDPE and HDPE).
- Extent of thermal, UV or other degradation or depolymerisation of polymers and paint coatings.

FTIR technique has practical advantages to infrared spectroscopy. It has made possible the development of many new sampling techniques which were designed to tackle challenging problems. It has made the use of infrared analysis virtually limitless.

2.2 X-Ray Powder Diffraction (XRD)

It is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenised, & average bulk composition is determined.

Principle Max von Laue, in 1912, discovered that crystalline substances act as three dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). This law relates the wavelength of electromagnetic radiation tote diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of diffraction peaks to d-spacing's allows identification of the mineral because each mineral has a set of unique d-spacing's.

All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation.

Sample Preparation in x-ray diffraction work we normally distinguish between single crystal and poly-crystalline or powder applications. The single crystal sample is a perfect (all unit cells aligned in a perfect extended pattern) crystal with a cross section of about 0.3 mm. The single crystal diffract meter and associated computer package is used mainly to elucidate the molecular structure of novel compounds, either natural products or man-made molecules.

In powder or polycrystalline diffraction it is important to have a sample with a smooth plane surface. If possible, we normally grind the sample down to particles of about 0.002 mm to 0.005mm cross section. The ideal sample is homogeneous and the crystallites are randomly distributed. The sample is pressed into a sample holder so that we have a smooth flat surface.

We have a random distribution of all possible h, k, l planes. Only crystallites having reflecting planes (h, k, l) parallel to the specimen surface will contribute to the reflected intensities. If we have a truly random sample, each possible reflection from a given set of h, k, l planes will have an equal number of crystallites contributing to it.

Working X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the

electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor. The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from ~5° to 70°, angles that are pre-set in the X-ray scan.

Advantages X-ray powder diffraction is most widely used for the identification of unknown crystalline materials. Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include

- Characterization of crystalline materials
- Identification of fine-grained minerals that are difficult to determine optically
- Determination of unit cell dimensions
- Measurement of sample purity

With specialized techniques, XRD can be used to:

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
  i) Determining lattice mismatch between film & substrate & to inferring stress &strain
  ii) Determining dislocation density & quality of the film by rocking curve measurements
  iii) Measuring superlattices in multilayered epitaxial structures
  iv) Determining the thickness, roughness and density of the film using glancing incidence

X-ray reflectivity measurements

- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

2.3 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance is a branch of spectroscopy that deals with the phenomenon found in assemblies of large number of nuclei of atoms that possess both "magnetic moments" and "angular momentum" is subjected to external magnetic field.

Principle the NMR phenomenon is based on the fact that nuclei of atoms have magnetic properties that can be utilised to yield chemical information. Quantum mechanically subatomic particles have spin. In some atoms (e.g. 12C, 16O, 32S) these spins are paired and cancel each other out so that the nucleus of the atom has no overall spin. However, in many atoms the nucleus does possess an overall spin. To determine the spin of a given nucleus one can use the following rules:

If the number of neutrons and the number of protons are both even, the nucleus has no spin. If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin (i.e.1/2, 3/2, 5/2). If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3).

The principle of NMR usually involves two sequential steps:

- The polarization of the magnetic nuclear spins in an applied, constant magnetic field.
- The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field and the nuclei of observation.

Sample Preparation

Samples for NMR analysis should be prepared in 5 mm NMR tubes. Select the right type of NMR tube. Tubes should be clearly labelled with an appropriate concentric label. Solvent selection is dictated by a number of factors. It is general practice to use deuterated solvents for two reasons.

a) The deuterium can be used for field frequency lock, making spectral resolution easier to assure and thereby saving you valuable time.
b) Solvents containing regular hydrogen yield large signals that may swamp those of your sample: the situation is made worse by saturation effects that broaden large signals. We recommend the use of deuterated solvents wherever possible.

The sample must be sufficiently soluble to yield an NMR spectrum. For 1H observed NMR, it is recommended to dissolve between 2 and 10 mg in between 0.6 and 1 mL of solvent so that the sample depth is at least 4.5 cm in the tube. For high molecular weight samples, more concentrated solutions are sometimes recommended. However, too concentrated a solution leads to lower resolution due to saturation and/or increased viscosity.

To achieve maximum resolution, choose a solvent with low viscosity. While dissolving, utmost care should be taken to prevent any dust particle from entering the NMR tube.

Working NMR spectrometers have now become very complex instruments capable of performing an almost limitless number of sophisticated experiments. However, the really important parts of the spectrometer are not that complex to understand in outline, and it is certainly helpful when using the spectrometer to have some understanding of how it works.

Broken down to its simplest form, the spectrometer consists of the following components:

- An intense, homogeneous and stable magnetic field
- A "probe" which enables the coils used to excite and detect the signal to be placed
- A high-power RF transmitter capable of delivering short pulses

- A sensitive receiver to amplify the NMR signals
- A digitizer to convert the NMR signals into a form which is stored in computer memory
- A "pulse programmer" to produce precisely times pulses and delays
- A computer to control everything and to process the data

Applications

- Solution structure: The only method for atomic-resolution structure determination of bio-macromolecules in aqueous solutions under membrane mimetic environments.
- Molecular dynamics: The most powerful technique for quantifying motional properties of bio-macromolecules.
- Protein folding: The most powerful tool for determining the residual structures of unfolded proteins and the structures of folding intermediates.
- Ionization state: The most powerful tool for determining the chemical properties of functional groups in bio-macromolecules, such as the ionization states of ionisable groups at the active sites of enzymes.
- Weak intermolecular interactions: Allowing weak functional interactions between macro biomolecules (e.g., those with dissociation constants in the micro molar to mill molar range) to be studied, which is not possible with other technologies.
- Protein hydration: A power tool for the detection of interior water and its interaction with bio-macromolecules.
- Hydrogen bonding: A unique technique for the DIRECT detection of hydrogen bonding interactions.
- Drug screening and design: Particularly useful for identifying drug leads and determining the conformations of the compounds bound to enzymes, receptors, and other proteins.
- Native membrane protein: Solid state NMR has the potential for determining atomic resolution structures of domains of membrane proteins in their native membrane environments, including those with bound ligands.
- Metabolite analysis: A very powerful technology for metabolite analysis.
- Chemical analysis: A matured technique for chemical identification and conformational analysis of chemicals whether synthetic or natural.
- Material science: A powerful tool in the research of polymer chemistry and physics.

2.4 Scanning Electron Microscope (SEM)

SEM is a type of electron microscope that produces images of as ample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition.

Principle Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons, backscattered electrons (BSE), diffracted backscattered electrons, photons, visible light and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples. X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete orbitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength. SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyse the same materials repeatedly.

Working

Components of all SEMs include electron source, electron lenses, sample stage, detectors for all signals, output devices, power supply, infrastructure requirements, vacuum system, cooling system, vibration-free floor & a room free of electric & magnetic fields.

SEMs have at least one detector and most have additional detectors. The specific capabilities of a particular instrument are critically dependent on which detectors it accommodates.

Applications

The SEM is routinely used to generate high-resolution images of shapes of objects (SEI) and to show spatial variations in chemical compositions:

1. Acquiring elemental maps or spot chemical analyses using EDS,
2. Discrimination of phases based on mean atomic number using BSE, and
3. Compositional maps based on differences in trace element "activitors" using CL.

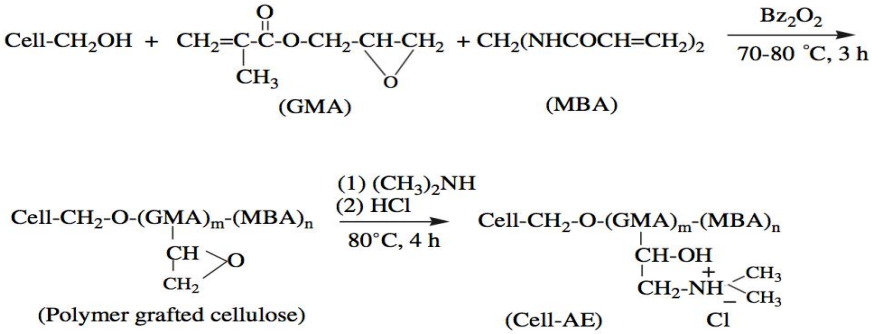
SEM is used to identify phases based on qualitative chemical analysis and/or crystalline structure. Precise measurement of very small features and objects down to 50 nm in size is also accomplished using the SEM. Backscattered electron images (BSE) can be used for rapid discrimination of phases in multiphase samples. SEMs equipped with diffracted backscattered electron detectors can be used to examine micro-fabric and crystallographic orientation in many materials.

3. Materials & Methods

3.1 Materials

Cellulose, Urea and Sodium hydroxide (used for making templates), Glycidyl methacrylate, N,N'-methylene-bi-acrylamide, Benzoyl peroxide, Isopropyl Alcohol, Cyclohexane, Poly(vinyl alcohol), 02.N HCl and 40% Di-methylamine (used to prepare Grafted Cellulose).Deuterium Oxide was used as solvent for NMR.

3.2 Methods
A) Synthesis



Scheme 1: Preparation of Cell-AE

Derivatization

The general procedure adopted for the preparation of a cellulose-based anion exchanger (Cell-AE) is given in Scheme.1. A mixture of 9.9 g GMA, and 0.1 g MBA and 1.0 gBz2O2 (initiator) was added to a suspension of 10 g of cellulose in 100 mL distilled water followed by a mixture of 1 mL isopropyl alcohol and 12 mL cyclohexane. All the contents were then poured into a flask containing 75 mL 1% polyvinyl alcohol. Graft polymerization in aqueous alcohol mixtures is associated with a high extent of graft copolymer formation.

The contents were heated in a water bath at 70-80°C with continuous stirring. A heavy white precipitate of polymer grafted cellulose was obtained. The product, after washing & drying, was subjected to amination reaction with 150 mL, 40% di-methylamine solution at 80°C for 4 hours. The product was washed with distilled water till the filtrate was free from amine. Then it was treated with 200 mL, 0.2 N HCl and the final product was washed well to remove excess chloride ion. It was dried, ground and then sieved to get 80-230 mesh size particles.

Solubilisation Three samples were prepared. First sample contained cellulose solvent which was prepared by mixing NaOH, urea and distilled water directly. 0.287gm of NaOH, 0.5gm of urea and 3.375 ml of distilled water was frozen for 2-3 hours and then 0.125gm of Cellulose was dissolved in the pre-cooled solvent and then was kept in ice-bath for 1 hour.

Second sample contained methyl cellulose solvent which contained 0.287gm of NaOH, 0.5gm of urea, 3.375 ml of distilled water and 0.125gm Methyl cellulose. Methyl cellulose was added to the pre-cooled Urea/NaOH solution and then kept in ice-bath for 4 hours.

Third sample contained grafted cellulose solvent which contained 0.287gm of NaOH, 0.5gm of urea, 3.375 ml of distilled water and 0.125gm Grafted cellulose. Grafted cellulose was added to the pre-cooled Urea/NaOH solution and then kept in ice-bath for 4 hours.

All the three solvents were refrigerated for 30 hours and then were taken in polar packs for NMR, SEM, FTIR and XRD recordings.

B) Characterisation

FTIR

A pinch of sample was mashed with Potassium Bromide (KBr). A ratio of 1:100 of Sample: KBr was taken. Then the pellets were formed in hydrolytic press. Then FTIR was recorded. NICOLE 380FTIR Spectrometer was used for recording the observations from a range of 400 cm^{-1} to 4000 cm^{-1}.

XRD

Powder X-ray diffraction experiments were performed with a Bruker D4 X-ray diffractometer operating at 30 kV and 15 mA using with Cu K-alpha radiation. Additionally, XRD patterns were collected in the 2θ range of 10 to 70 with step sizes of 0.02 (2θ) and a counting time of 4 second per step.

NMR

Each sample was dissolved in 200 micro-litre of Deuterium Oxide. Then it was put in NMR tube and then the NMR tube was placed in tube holder in the NMR instrument.

SEM

Place a pinch of sample on double adhesive carbon tape. The tape is attached on the stub. Place the stub in the SEM instrument for recordings. Zeiss EVO 40 was used.

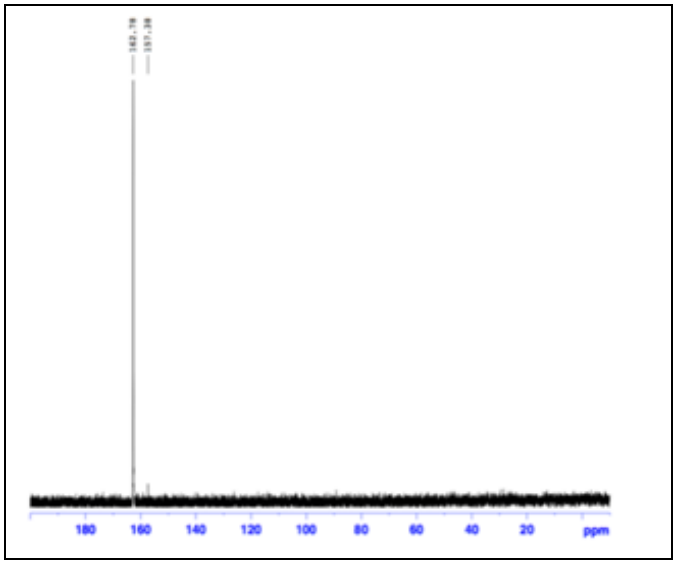
4. Results and Discussion

4.1 Study of Urea, Cellulose and NaOH interaction through NMR

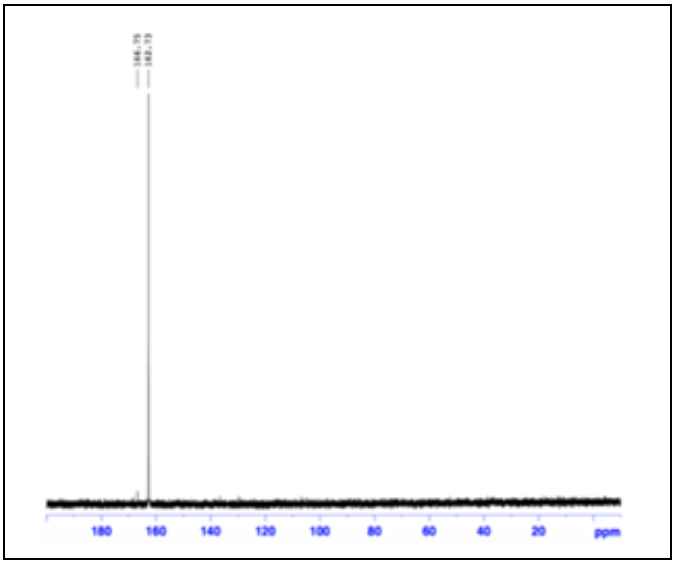
To detect the interaction between urea and cellulose, 13C NMR spectra of five different solutions were measured. Only about 0.010 ppm downfield shift of urea was observed when cellulose & its derivatives were added to NaOH/urea system. The unchanged resonances of urea& cellulose in solution suggest that there is no relatively strong interaction such as hydrogen bonding between urea & cellulose, and no particular structure formed. There is relative weak interaction between urea & cellulose &water. In urea/ D_2Osolution, the chemical shift of urea is 162.78 ppm; when NaOH added, little change of only0.03ppm downfield takes place. This indicates that NaOH has not changed the interactions of urea, or there is no strong interaction between urea and NaOH.

Cellulose has both equatorial hydroxyl groups and axial C–H bonds. It is amphiphilic in nature with the intra-sheet region hydrophilic and the inter-sheet region hydrophobic. When cellulose is dissolved in solvent, the intra- and inter-molecular hydrogen bonds should be mostly broken, the hydroxyl groups should be stabilized by the solvent molecules and the hydrophobic region should be separated

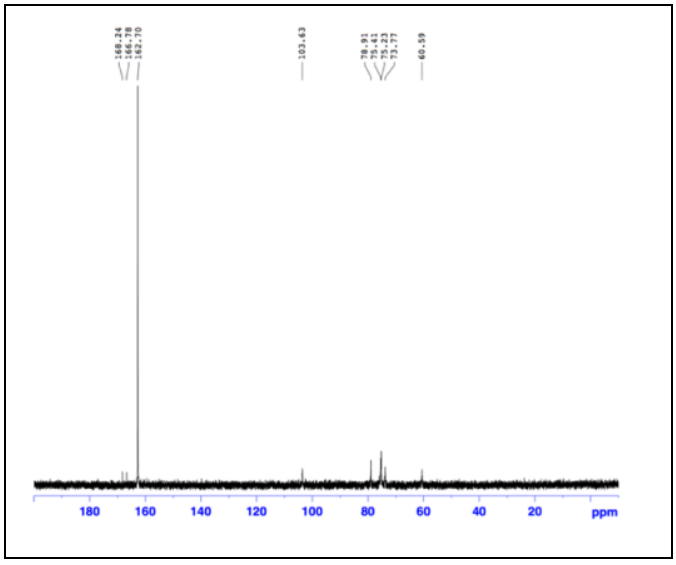
from each other. NaOH solution can dissolve cellulose with low DP, but the solution is not stable and easy to form gel. When dissolved, the hydrophobic parts of cellulose, which suffer from great repulsion from surrounding, tend to aggregate together. NaOH is strong enough to break the cellulose hydrogen bonds but has limited capacity to dissolve cellulose. Urea may play its role through interacting with the hydrophobic part of cellulose. In aqueous solutions, OH- is the strongest base and most capable of forming hydrogen bonds. The hydrogen bond of water molecules is stronger than that of urea molecules. When cellulose is dissolved in NaOH/ urea aqueous solutions, cellulose hydroxyl groups prefer to interact with OH- and water molecules than urea. Urea is more likely to stabilize the solution rather than to break hydrogen bonds and dissolve cellulose directly. When cellulose is dissolved in NaOH/urea solution, NaOH breaks the hydrogen bond between chains and interacts with the hydrophilic hydroxyl groups of cellulose; while urea interact with the hydrophobic parts of cellulose. The addition of urea reduces the hydrophobic effect of cellulose backbone and improves the dissolution of cellulose in alkali solutions and can keep the solution more stable. NaOH/ additive solvent system works better at a lower temperature. In this solvent, amore orientated arrangement of solvent molecules is preferred, since both hydroxyl groups & nonpolar backbone should be stabilized by solvent molecules. At low temperature, the small solvent molecules are less mobile and tend to form larger clusters.



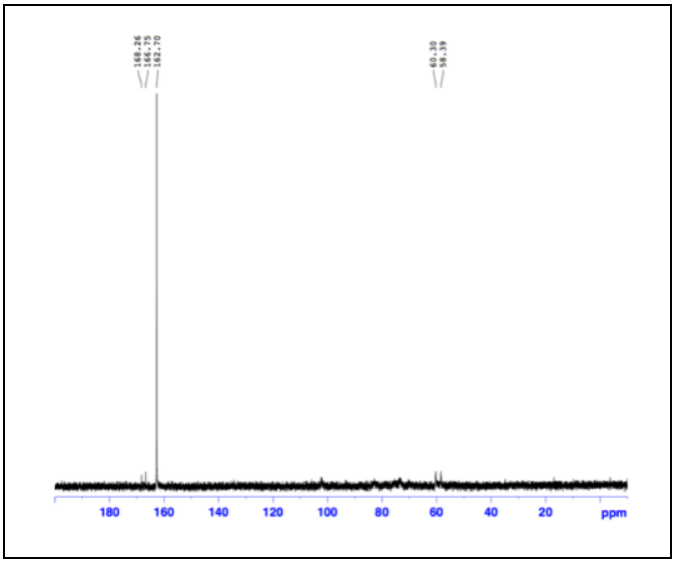
a) NMR Spectra of Urea Peak at 162.78ppm



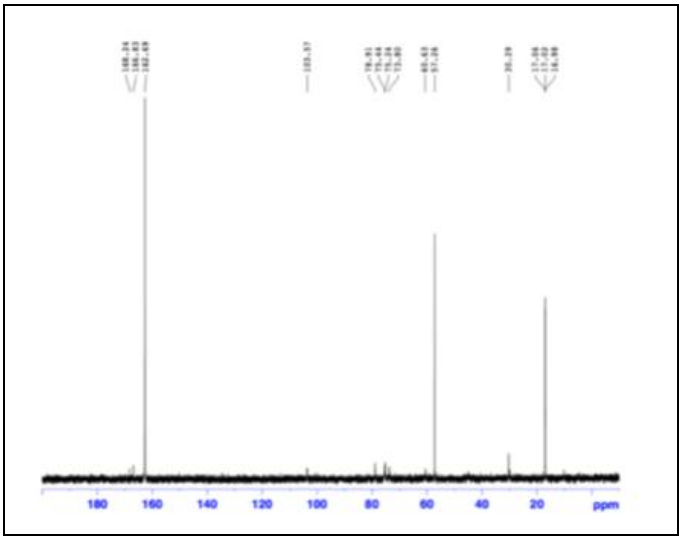
b) NMR Spectra of Urea +NaOH Peak at 162.73ppm



c) NMR Spectra of Cellulose template Peak at 162.70ppm



d) NMR Spectra of Methylcellulose template Peak at 162.70ppm

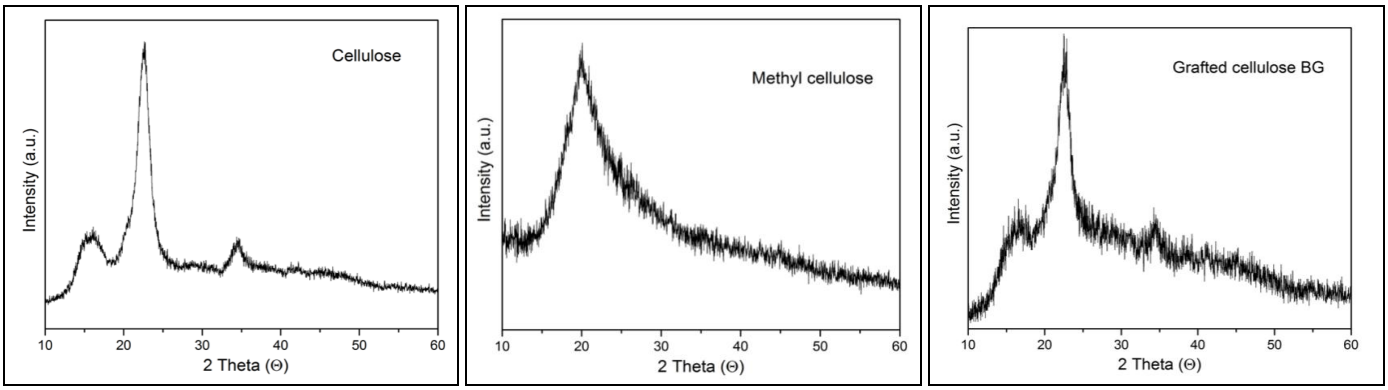


e) NMR Spectra of Grafted cellulose template Major Peak at 162.69ppm

The insolubility of cellulose in common solvents is considered due to difficulty of breaking hydrogen bonds; while cellulose cannot dissolve in some solvents such as aqueous KOH and ammonia which can also provide sufficient OH- to break hydrogen bond. But when some co solvents such as cup ammonium added, cellulose dissolves. Both hydrogen-bond breaking and stabilization of separated cellulose chains are of same importance in cellulose dissolution. OH- is the strongest hydrogen-bond breaker.

In the NaOH/ urea solvent system, association with hydrated Na prevents hydroxyl groups of cellulose to interact with each other; urea separates hydrophobic parts of cellulose to aggregate, stabilizing the solution. Addition of urea in the NaOH solvent system can improve the dissolving capability of the solvent significantly. The NMR Spectra shows that all Cellulose & its derivatives have completely dissolved in Urea and NaOH.

4.2 Study of *crystallinity* through XRD results

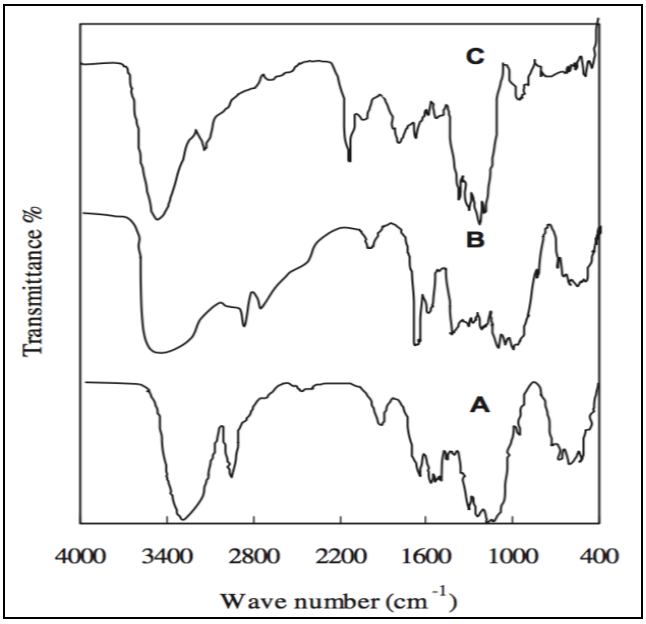


(a) Cellulose template (b) Methyl cellulose template (c) Grafted Cellulose template

Bio-glass formed with cellulose template shows semi-crystalline structure. Bio-glass formed with Methylcellulose template shows amorphous structure. Bio-glass formed with Grafted cellulose template shows crystalline structure.

4.3 Results obtained from FTIR

The FTIR spectra of Cell, Cell-AE and As (V) loaded Cell-AE [As (V)-Cell-AE] is presented. As shown in figure, the peak at 3344 cm^{-1} has been attributed to hydrogen bonded –OH group. The C-H stretching from the -CH2 group can be observed at 2900 cm^{-1}. A band at 1725 cm^{-1} in the IR spectrum of Cell-AE provides evidence that GMA has been incorporated on the cellulose backbone. The most important peaks on this spectrum are at 2714 and 1480 cm^{-1} indicating the presence of -CH2-NHR2 type nitrogen and aliphatic C-N group, respectively. These peaks confirm the presence of tertiary amine functionality. Two different characteristic bands at 836 and 470 cm^{-1} were observed in the spectrum of arsenic-loaded Cell-AE for the arsenate adsorption by Cell-AE.



FTIR Spectra of: (a) Cellulose,(b) Grafted Cellulose,(c) As Cellulose Anion Exchange

4.4 Results obtained from SEM

SEM micrograph of cellulose shows agglomerate of bio-glass formed without any definite structure. SEM micrograph ofbioactive glass with Methyl Cellulose is nearlyspherical with varied particle size distribution. SEM micrograph of Bio-glass with grafted cellulose as template material has well defined spherical shaped bio-glass particles spread on the surface with uniform sized particles can clearly be seen. Grafted Cellulosehas got a distinct fluffy appearance & it has a greatersurface area than Cellulose.

5. Conclusion and Future Scope

The templates of Cellulose & its derivatives namely, Methyl cellulose and Grafted Cellulose for theBio-Glass were successfully synthesised and characterised under NMR. The interaction of Cellulose& its derivatives with Urea/NaOH was proved. The crystalline and amorphous structures oftemplates were shown through XRD. Cellulose & its derivatives were differentiated using SEM. Itwas also studied why Cellulose/Urea/NaOH system has to be kept in cold conditions every time. Itwas shown that Cellulose & its derivatives were completely soluble in Urea/NAOH system.
In future, we can check the sample for bioactivity.Mechanical properties of Bio-Glass can also be studied.Anti-Bacterial properties of Bio-Glass can be studied.Bioactive Glass Scaffolds can be made for bone regeneration.It can be used in bone tissue engineering.It can be used in dental applications.

6. References

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