INVESTIGATIONS ON THE PREPARATION OF COMPOSITE POLYSACCHARIDE FILMS AND MICRO / NANOPARTICLES FOR NOVEL APPLICATIONS

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To my loving parents and husband for their sincere prayers……

DECLARATION

I, Simi C.K., do hereby declare that the thesis entitled "**Investigations on the preparation of composite polysaccharide films and micro / nanoparticles for novel applications**" is a bonafide record of the investigation carried out by me in the Chemical Sciences and Technology Division of National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram, under the supervision of Dr. T. Emilia Abraham and no part of this thesis has been submitted elsewhere for the award of any other degree or diploma.

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled "**Investigations on the preparation of composite polysaccharide films and micro / nanoparticles for novel applications**" is a record of bonafide research carried out by Ms. Simi C.K. under my supervision at chemical Sciences and Technology Division, National Institute for Interdisciplinary Science and Technology, CSIR, Thiruvananthapuram, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry, under the faculty of science, Cochin University of Science and Technology, Kochi and the same has not been submitted elsewhere for any other degree or diploma.

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Symbols and Abbreviations

AgCl	Silver chloride
AOT	Aerosol OT
AFM	Atomic force microscopy
CAN	Cerium ammonium nitrate
CH_3CN	Acetonitrile
DCM	Dichloromethane
DHB	2, 5-dihydroxybenzoic acid
DLS	dynamic light scattering
DMF	Dimethyl formamide
DMSO	Dimethyl sulphoxide
DNS	3, 5-Dinitrosalicylic acid
DS	Degree of substitution
DSC	Differential scanning calorimetry
DTA	Differential Thermal Analysis
EGCG	Epigallocatechin gallate
FT-IR	Fourier- Transform Infrared Spectroscopy
G′	Storage modulus
G″	Loss modulus
GPC	Gel permeation Chromatography
HACS	High amylose content starch
HRTEM	High-resolution transmission electron microscopy
HNO_3	Nitric acid
KBr	Potassium bromide
KCl	Potassium chloride
KOH	Potassium hydroxide
MALDI-TOF	Matrix-assisted laser desorption/ionization- time of flight

$NaBH_4$	Sodium borohydride
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance Spectroscopy
PAN	Poly acrylonitrile
PEC	Polysaccharide nanoparticles by polyelectrolyte complexation
PVA	Poly vinyl alcohol
SAXS	Small-angle X-ray scattering
ST-Ol	Starch grafted with oleic acid
ST-St	Starch grafted with stearic acid
T_5	Temperature at 5 % weight loss
T_{10}	Temperature at 10 % weight loss
TEM	Transmission electron microscope
TFA	Trifluoro acetic acid
TGA	Thermo Gravimetric Analysis
THF	Tetrahydrofuran
T_f	Final temperature
T_i	Initial temperature
T_p	Maximum peak temperature
TKP	Tamarind kernel powder
TMS	Tetra methyl silane
TPP	Tripolyphosphate
XG	Xyloglucan
XG-CH	Xyloglucan chitosan composite film
$XG\text{-}NH_2$	Aminated xyloglucan
XG-ST	xyloglucan starch composite films
XRD	X-Ray Diffraction
η^*	Complex viscosity
ΔH	Enthalpy of gelatinization

PREFACE

Environmental awareness and the demand for green technology have led to a rapidly increasing interest in the application of natural and renewable polymers especially polysaccharides for industrial applications in areas of foods, textiles, paints, cosmetics and pharmaceuticals. Biocompatibility, biodegradability, non toxicity, water solubility and a broad range of functional properties makes these polysaccharides extremely useful. Moreover polysaccharide particles, gels and edible films are of great significance in today's scenario, particularly in medical, cosmetic and food industries. Considering these bright prospects, we selected two important abundantly available plant derived glucans, an α-glucan, starch and a β- glucan, xyloglucan for our investigations.

The thesis comprises of eight chapters. The first chapter presents a brief over view about polysaccharides, polysaccharide micro/nano particles, films, gels etc.

The second chapter deals with the materials and the main methods used in the investigations.

The third chapter deals with the morphological, physicochemical, and thermal properties of medicinally important njavara rice starch and the properties were compared with native chamba rice starch. It was proved that the inherent high thermal and pasting properties, makes Njavara rice suitable as poultice for the body massage in the panchakarma treatment.

Polysaccharide nanoparticles have wide range of application. The Synthesis of modified hydrophobic starch nanoparticles using long chain fatty acids was accomplished and the results and its drug delivery efficiency is studied in chapter four.

The fifth chapter reports the synthesis of aminated xyloglucan and its properties as a highly versatile and unique blue fluorescent hydrogel. Amination of xyloglucan with ethylene diamine in aqueous medium is found to be a good strategy to get versatile xyloglucan gels. By this facile synthetic strategy, xyloglucan is functionalised with amino group which leads to the formation of *insitu* irreversible hydrogels without using any cross linking agents with blue emission characteristics in the fluorescence spectra.

The sixth chapter deals with the oxidation of xyloglucan to dialdehyde and subsequent formation of a composite hydrogel (Chitam) which is clear, transparent and stable. The xyloglucan was oxidized by using periodate and the oxidized xyloglucan was blended with chitosan.

Chapter seven deals with the preparation, characterization of xyloglucan and its composites with chitosan and starch as transparent films. The thermal and mechanical properties, the swelling, wettability and controlled drug release properties of the films were studied.

The Summary and Conclusion are given at the end of the thesis.

CHAPTER 1

Introduction

This chapter is devoted to the literature survey of polysaccharide. Scope and objectives of the present thesis are also described.

1.1. Carbohydrates

Carbohydrates or saccharides are the most abundant among the four major classes of biomolecules. The general stoichiometric formula of an unmodified monosaccharide is $(CH_2O)_n$, where n is any number equal to three or greater. All carbohydrates have C=O and –OH functional groups. According to the number of sugar unit they are classified as

1.2. Monosaccharides

Monosaccharides are the simplest carbohydrates. Many carbohydrates contain one or more modified monosaccharide units that have had one or more groups replaced or removed.

1.3. Disaccharides

Disaccharide is the carbohydrate formed by the condensation reaction between two monosaccharides which involves the elimination of a small molecule of water, from the functional groups.

1.4. Polysaccharides

Polysaccharides are ubiquitous can be either homopolysaccharides or hetero polysaccharide and occur in algae (e.g.alginate), plants (e.g. pectin, guar gum), microbes (e.g.dextran, xanthan gum), and animals (chitosan, chondroitin) [Sinha *et al.*, 2001]. They are highly stable, safe, non toxic, hydrophilic and biodegradable.

Polysaccharides having large number of reactive groups, a wide range of molecular weight, varying chemical composition etc. contribute to their diversity in structure and in property. Hydrophilic groups in polysaccharide form non-covalent bonds with biological tissues causes bioadhesion [Lee *et al.*, 2000]. Polysaccharides can be divided into polyelectrolytes and non polyelectrolytes and the former can be further divided into positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, heparin, hyaluronic acid, pectin, etc.). Due to the presence of various derivable groups on molecular chains, polysaccharides can be easily modified chemically and biochemically having different functional properties [Almond, 2005].

The following section briefly describes the major classification of polysaccharides.

1.4.1. Storage polysaccharides

They are energy storage polysaccharides which are present in seeds, stems, tubers, rhizomes (starch) and liver (glycogen) etc.

1.4.2. Structural polysaccharides

Structural polysaccharides are used to provide protective walls or lubricative coating to cells. Cellulose, chondroitin sulphates, hyaluronic acids and chitin are examples.

1.4.3. Hetero polysaccharides

Hetero polysaccharides contain two or more different types of monosaccharide unit and occur in both straight chain and branched chain forms. The major hetero

polysaccharides include the connective tissue polysaccharides, the blood group substances, glycol proteins and glycol lipids, particularly those formed in the central nervous system of animals and a wide variety of plant gums like pectin, lignin, and mucopolysaccharides etc.

1.4.4. Homo polysaccharides

Homo polysaccharide has one type of monosaccharide and subdivided into straight chain and branched chain, depending upon the arrangement of the monosaccharide units. And this homopolysaccharides are further classified into plant polysaccharide, animal polysaccharide (e.g. chitin, glycogen), microbial polysaccharide [fungal (e.g. pullulan) and bacterial polysaccharide (e.g. xanthan gum, gellan gum, dextran, and curdlan)], and seaweed polysaccharide (e.g. agar, carrageenan, furcellaran and alginate). Here in our work we have studied the two plant derived polysaccharide.

1.4.4.1. Plant polysaccharides

These polysaccharides are classified as according to their origin.

1.4.4.1.1. Plant seed polysaccharides

These polysaccharides are extracted from seeds. Examples are starch, glucomannan, galactomannan, xyloglucan etc. Galactomannans are polysaccharides consisting of a mannose backbone with galactose side groups, more specifically, α-(1, 4)-linked β-D-mannopyranose backbone with branch points from their 6^{th} positions linked to α-D-galactose. Different types of galactomannans according to mannose-to-galactose ratio are

Fenugreek gum,	mannose:galactose ~1:1
Guar gum,	mannose:galactose ~2:1
Tara gum,	mannose:galactose ~3:1
Locust bean gum or carob gum	mannose:galactose ~4:1

1.4.4.1.2. Tuber polysaccharides

Starch is the major polysaccharide of tubers and rhizomes apart from glucomannan and inulin. In glucomannan the component sugars are β-(1, 4)-linked D-mannose and D-glucose in a ratio of 1.6:1. The basic polymeric repeating unit has the pattern: GGMMGMMMMMGGM. Inulins are mainly comprised of fructose units joined by a β-(2, 1) glycosidic bond.

1.4.4.1.3. Exudates gums

Exudates gums mainly contain arabinogalactan. Some of the exudates gums are Gum Arabic, Tragacanth, and Gum karaya.

1.4.4.1.4. Cell wall polysaccharides

The major carbohydrates making up the primary cell wall are cellulose, hemicellulose and pectin. The cellulose microfibrils are linked via hemicellulosic tethers to form the cellulose-hemicellulose network, which is embedded in the pectin matrix. Approximately equal amounts of pectin and hemicellulose are present in dicot primary walls whereas hemicellulose is more abundant in grasses (e.g., switch grass). The secondary walls of woody tissue and grasses are composed predominantly of cellulose,

lignin, and hemicellulose (xylan, glucuronoxylan, arabinoxylan, or glucomannan). The cellulose fibrils are embedded in a network of hemicellulose and lignin. The major polysaccharides in the primary cell walls are:

a) Cellulose

Cellulose is a linear chain of several hundred to over nine thousand β-(1, 4) linked D-glucose units. The multiple hydroxyl groups on the glucose residues from one chain form hydrogen bonds with oxygen molecules on another chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength which is important in cell walls. Cellulose esters and cellulose ethers are the most important commercial materials. Ether derivatives include ethylcellulose, methylcellulose, hydroxypropyl cellulose and carboxymethyl cellulose.

b) Hemicellulose

Hemicelluloses are complex polysaccharides have a backbone of 1, 4-linked β-D-pyranosyl residues. In contrast to cellulose, hemicellulose is derived from several sugars in addition to glucose, such as xylose, mannose, galactose, rhamnose, and arabinose. The predominant hemicellulose in many primary walls is xyloglucan. Other hemicelluloses found in primary and secondary walls include glucuronoxylan, arabinoxylan, glucomannan, and galactomannan. Hemicelluloses (10 to 30%) can be extracted from cell walls by alkaline solutions (eg: 15% KOH). Neutralization of the alkaline extract causes precipitation of major component of the hemicelluloses, the xylans or hemicelluloses A. The remaining in the neutralized extract solution is a

group of low molecular weight, acidic polysaccharides known as hemicelluloses B which can be precipitated by adding ethanol.

1.5. Glucans

Glucan molecule is a polysaccharide of D-glucose monomers linked by glycosidic bonds. According to the type of glycosidic bond they are classified into alpha and beta glucans. Dextran, glycogen, pullulan and starch are some examples for alpha glucans. Cellulose, curdlan, laminarins etc are some example for beta glucans [Wasser *et al.*, 1999]. Alpha glucans are mainly (1, 4) and (1, 6) linked but in beta glucan mainly (1, 3), (1, 6) and (1, 4) glycosidic linkages are present. The most active form of β-(1, 3)-D glucans are those that contain 1, 6 side-chains branching off from the longer beta (1, 3) glucan backbone. These are referred to as beta (1, 3)/ (1, 6) glucan. In some cases, proteins linked to the beta (1, 3) glucan backbones may also be involved in imparting therapeutic activity. Because of its exciting potential for enhancement of the immune system, and other properties like resistant to oral acids/enzyme and insoluble in water it is widely used in the medical field [Gelderman *et al.*, 2004].

1.6. Nanotechnology

Nanotechnology focuses on the characterization, fabrication, and manipulation of biological and non biological structures smaller than 100 nm. Structures on this scale have been shown to have unique and novel functional properties. The potential benefits of nanotechnology have been recognized by many industries, and commercial products are already being manufactured, such as in the microelectronics, aerospace,

pharmaceutical, food and cosmetic industries. Nanoparticles have been prepared mainly by methods like salting out, spontaneous emulsification/diffusion, solvent evaporation, polymerization, and nanoprecipitation [Ibrahim *et al.*, 1992]. In addition, electrospraying or the supercritical technology has shown to be capable of producing uniform particles of less than 100 nm. Nanoparticles can be prepared from a variety of materials such as protein, polysaccharide and synthetic polymers. The selection of matrix depends on the size of nanoparticle required, surface characteristics, degree of biodegradability, biocompatibility and toxicity. Among biologically inspired nanocomposites, polysaccharides are probably among the most promising sources for the production of nanoparticles.

1.6.1. Polysaccharide nano/microparticles

Nanotechnology has emerged as one of the most fascinating area in the biopolymer research which find exciting enhancement of applications in drug delivery systems, food technology and cosmetic field. This following discussion is focused on the polysaccharide nanoscience, emphasises on basic methodologies for preparing biopolymer based nanomaterials. Polysaccharide nanocrystals, films and gels are continuously attracting the researchers and hence a non-exhaustive review on all these topics is necessary to corroborate the works done in this thesis.

Polysaccharide or biopolymer particles may be formed by promoting self association or aggregation of single biopolymers or by inducing phase separation in mixed biopolymer systems. As time goes on, more polysaccharide based nanoparticles

emerge, which greatly enriches the versatility of nanoparticle carriers in terms of category and function [Janes *et al.*, 2001; Prabaharan *et al.*, 2005; Liu *et al.*, 2008]. Research on the nanoparticles of polysaccharides such as chitosan, alginate, and glucomannan, which are biocompatible, nontoxic, hence used for different applications in medical, food and cosmetic industries growing very fast [Alonso-Sande *et al.*, 2006; Zhang *et al.*, 2007]. Natural polysaccharides, due to their outstanding merits, have received more and more attention in the field of drug delivery systems. For the application of these naturally occurring polysaccharides for drug carriers, issues of safety, toxicity and availability are greatly simplified [Yi *et al.*, 1999; Cascone *et al.*, 2002; Vandervoort *et al.*, 2004; Pasparakis *et al.*, 2006]. All these merits endow polysaccharides a promising future as biomaterials.

According to structural characteristics, the polysaccharide nanoparticles are prepared mainly by four mechanisms, namely covalent crosslinking, ionic crosslinking, polyelectrolyte complexation, and self-assembly of hydrophobically modified polysaccharide

1.6.1.1. Covalent crosslinking

The early preparation of polysaccharide nanoparticles was by means of covalent crosslinking. Chitosan is used frequently to prepare nanoparticles using glutaraldehyde as the crosslinker. The toxicity of glutaraldehyde on cell viability limits its utility in the field of drug delivery. Along with the use of biocompatible crosslinkers, biocompatible covalent crosslinking is promising. With the aid of water-soluble

condensation agent of carbodiimide, natural di- and tricarboxylic acids, including succinic acid, malic acid, tartaric acid and citric acid, were used for intermolecular crosslinking [Bodnar *et al.*, 2005; 2005].

1.6.1.2. Ionic crosslinking

Compared with covalent crosslinking, ionic crosslinking has more advantages such as mild preparation conditions and simple procedures. For charged polysaccharides, low molecules of polyanions and polycations could act as ionic crosslinkers for polycationic and polyanionic polysaccharides, respectively. Nowadays, the most widely used polyanion crosslinker is tripolyphosphate (TPP) which is non-toxic and has multivalent phosphate anions. Chitosan nanoparticles are prepared by this method.

1.6.1.3. Polysaccharide nanoparticles by polyelectrolyte complexation (PEC)

Polyelectrolyte polysaccharides can form polyelectrolyte complexation with oppositely charged polymers by intermolecular electrostatic interaction. Polysaccharide based polyelectrolyte complexation nanoparticles can be obtained by means of adjusting the molecular weight of component polymers in a certain range. In theory, any polyelectrolyte could interact with polysaccharides to fabricate polyelectrolyte complexation nanoparticles. But in practice, these polyelectrolytes are restricted to those water-soluble and biocompatible polymers in view of safety purpose [Douglas *et al.*, 2005; Sarmento *et al.*, 2006] .

1.6.1.4. Self-assembly of hydrophobically modified polysaccharides

In recent years, numerous studies have been carried out to investigate the synthesis and the application of polysaccharide based self aggregate nanoparticles as drug delivery systems. When hydrophilic polymeric chains are grafted with hydrophobic segments, amphiphilic copolymers are formed. Upon contact with an aqueous environment, polymeric amphiphiles spontaneously form micelles or micelle like aggregates via intra or intermolecular associations between hydrophobic moieties, primarily to minimize interfacial free energy. These polymeric micelles exhibit unique characteristics, such as small hydrodynamic radius (less than microsize) with core-shell structure, unusual rheology, thermodynamic stability, depending on the hydrophilic/hydrophobic constituents. In particular, polymeric micelles have been recognized as a promising drug carrier, since their hydrophobic domain, surrounded by a hydrophilic outer shell, can serve as a reservoir for various hydrophobic drugs [Letchford *et al.*, 2007].

1.6.2. Polysaccharide Nanocrystals

During the past decade, many attempts have been reported to mimic natural bionanocomposites by blending polysaccharide nanocrystals from different sources with polymeric matrices [Berglund, 2005; Angellier *et al.*, 2006; Dufresne, 2006; Oksman *et al.*, 2006; Kristo *et al.*, 2007; Wu *et al.*, 2007]. The resulting nanocomposite materials display outstanding properties, in terms of both stiffness and thermal stability. Formation of a rigid percolating network, resulting from strong

interactions between them was the basis of this phenomenon. Any factor that affects the formation of this percolating network or interferes with it, changes the mechanical performances of the composite media. The chemical modification of the surface of the nanocrystals constitutes another alternate and sound approach to broaden the number of possible polymeric matrices [Roman *et al.*, 2006]. However, a severe loss of mechanical performances was reported for nanocomposites processed from these modified nanocrystals because of the coating of the nanoparticles and the resulting damage of the percolating network of the nanoparticles. Starch, cellulose and chitin nanocrystals are promising candidates for the different applications [Dong *et al.*, 2007; Goodrich *et al.*, 2007; Fan *et al.*, 2008; Habibi *et al.*, 2008]. Stable suspensions of polysaccharide nanocrystals can be prepared by slow acid hydrolysis of the amorphous part of the native polysaccharide with mineral acids such as sulphuric acid and hydrochloric acid [Marchessault *et al.*, 1959; Pusey *et al.*, 1986; Folda *et al.*, 1988; Revol *et al.*, 1992; Revol *et al.*, 1994; Dong *et al.*, 1996; Gabriel *et al.*, 2000; Beck-Candanedo *et al.*, 2005].

1.7. Polysaccharide films

In the past 50–60 years, synthetic polymeric food packaging materials are used widely due to various advantages such as high strength, elongation, gas barrier properties, low cost, lightness and water resistance [Mali *et al.*, 2002]. These plastic materials are convenient, safe, strong and economical but not biodegradable and pollute the earth. In order to solve the problems, polysaccharides such as starch, cellulose derivatives and

plant gums are being studied for edible films and coatings as potential alternatives by the plastic industries [Kester *et al.*, 1986; Debeaufort *et al.*, 1994; Baldwin *et al.*, 1995; Garcia *et al.*, 2000]. Several studies have been conducted investigating the properties of protein, polysaccharide, and lipid based films [Gennadios *et al.*, 1993; Gontard *et al.*, 1993; McHugh *et al.*, 1993; Park *et al.*, 1993; McHugh *et al.*, 1994; Chen, 1995; Lourdin *et al.*, 1995; Park *et al.*, 1995; Gontard *et al.*, 1996; Lawton, 1996; Arvanitoyannis *et al.*, 1998; Kim *et al.*, 2002; Rhim, 2003; Habibi *et al.*, 2008]. These edible/biodegradable films were reported to have been successfully utilized in a number of commercial applications such as the encapsulation of supplements, drugs, and flavour. Starch films are mainly used for coatings for drug tablets, confections and dried fruits etc [Krochta, 2002]. Reports indicated that consumers who are vegetarian and those whose religion prohibits the consumption of animal derived products, may prefer polysaccharide based biodegradable films and capsules than gelatin based products [Myllarinen *et al.*, 2002].

High water vapor permeability of polysaccharide film was reduced by the incorporation nano-clay in to the films. Moreover, introduction of the dispersed clay layers into the biopolymer matrix structure has been shown to greatly improve the overall mechanical strength of the film, making the use of these films industrially practicable. Mathew and Dufresne [Mathew *et al.*, 2002] examined the nanocomposites from starch and amorphous poly (beta-hydroxyoctanoate), and tucinin whiskers. Nanocomposites have also been developed using plant oil-clay hybrid materials [Park *et al.*, 2003; Uyama *et al.*, 2003].

1.8. Gels and hydrogels of polysaccharides

Gels are defined as a substantially dilute crosslinked system, which exhibits no flow when in the steady-state. By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional crosslinked network within the liquid. This internal network structure may result from physical or chemical bonds. Physical bonds may be electrostatic, hydrogen or van der Waals interactions and the chemical bond is mainly of covalent in nature. They are classified into organogel, xerogel and hydrogel. Organogel is a non-crystalline, non-glassy thermoreversible solid material composed of a liquid organic phase entrapped in a three-dimensionally cross-linked network and used in pharmaceuticals, cosmetics, and food industries [Visintin *et al.*, 2005; Pernetti *et al.*, 2007]. A xerogel is a solid formed from a gel by drying with unhindered shrinkage. Xerogels usually retain high porosity and enormous surface area along with a very small pore size.

1.8.1. Hydrogels

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids [Peppas *et al.*, 2000]. These networks can be classified into two main categories according to the type of cross-linking among the macromolecules, whether it is chemically or physically based [Clark *et al.*, 1987]. Because of their ability to retain a significant amount of water, hydrogels are quite similar to natural living tissues, rendering them useful for a wide variety of biomedical applications [Hoffman, 2002]. Among the numerous polymers that have been proposed

for the preparation of hydrogels, polysaccharides have a number of advantages over the synthetic polymers which were initially employed in the field of pharmaceutics [Wichterle *et al.*, 1960].

A number of pioneering studies have greatly contributed to our present understanding of polysaccharide hydrogel networks. The physically cross-linked gels are of great interest, particularly because the gel formation can be often carried out under mild conditions and in the absence of organic solvents. This peculiarity allows a very wide range of applications, and derivatizations which further increases their versitality. As a consequence, an ever increasing number of publications and patents concerning hydrogels prepared from native and derivatised polysaccharidese are there [Nishinari *et al.*, 2000]. These hydrogels have wide applications in different areas like encapsulation of living cells, biologically friendly scaffolds in tissue engineering, sustained-release delivery systems, biosensors and so on [Langer *et al.*, 1993; Rowley *et al.*, 1999; Peppas *et al.*, 2006].

Environmentally sensitive hydrogels have the ability to sense changes of pH, temperature, or the concentration of metabolite and release their load as a result of such a change. Thus, hydrogels that undergo physicochemical changes in response to applied stimuli, such as biomolecular binding, are promising materials for drug delivery and tissue engineering. Recent research has proved that hydrogels with such additional functionalities offer highly specific bioresponsiveness [Lin *et al.*, 2004;

Ehrick *et al.*, 2005; Murakami *et al.*, 2005; Li *et al.*, 2006; Famulok *et al.*, 2007; Murphy *et al.*, 2007; Oh *et al.*, 2007; Thornton *et al.*, 2007; Wei *et al.*, 2008] .

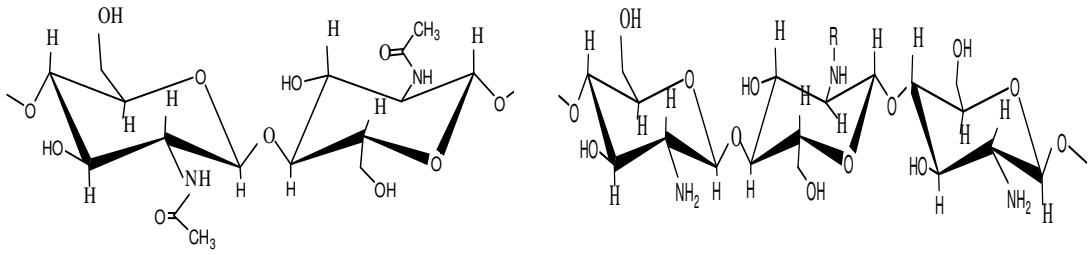
Nanosize hydrogels (nanogels), which are composed of nanoscale gel particles, have attracted growing interest with respect to their potential application in drug delivery systems [Akiyoshi *et al.*, 1993; Vinogradov *et al.*, 2002; Hayashi *et al.*, 2004; Missirlis *et al.*, 2005; Nayak *et al.*, 2005; Kim *et al.*, 2006; Oh *et al.*, 2007; Thornton *et al.*, 2007; Wei *et al.*, 2008]. Ayame et al recently developed a self-assembly method for preparing physically cross-linked nanogels (<50 nm) through the controlled association of hydrophobically modified polymers in water [Ayame *et al.*, 2008]. Microscale hydrogels of controlled sizes and shapes are useful for cell-based screening, in vitro diagnostics, tissue engineering, and drug delivery in a sustained manner [Uludag *et al.*, 2000; Kim *et al.*, 2008].

This thesis is focused on the investigations on three biopolymers mainly, viz: starch, xyloglucan and chitin/chitosan. Attempts are made to modify the biopolymers and study the chemical, thermal, optical, rheological, and biological properties. Hence, a comprehensive review on the recent developments in the polymers is needed.

1.9. Chitin / chitosan

Chitin is a long-chain polymer of N-acetylglucosamine (Scheme 1.1), a derivative of glucose, and it is the main component of the cell walls of fungi, the exoskeletons of arthropods etc. It is the second abundant biopolymer in the nature. It is a highly insoluble material and is soluble in hexafluoroisopropanol, hexafluoroacetone,

chloroalcohols in conjugation with aqueous solutions of mineral acids and dimethylacetamide containing 5% lithium chloride [Ravikumar, 1999].



a b

Scheme 1.1. Structure of a) chitin, b) chitosan

Like cellulose, it functions naturally as a structural polysaccharide. Chitin is a white, hard, inelastic, nitrogenous polysaccharide classified into α, β and γ chitins [Cabib *et al.*, 1988]. α-chitin has a structure of anti-parallel chains whereas β-chitin has intra hydrogen-bonding sheets by parallel chains. As a result, β-chitin has weaker intermolecular hydrogen bonding and so has a more open structure that is more facile to chemical modification [Minke *et al.*, 1978]. γ-chitin has a parallel and anti-parallel structure, which is a combination of α-chitin and β-chitin [Jang *et al.*, 2004].

Chitosan derived by partial *N*-deacetylation of chitin is also a straight-chain polymer of glucosamine and *N*-acetylglucosamine [Kumar, 2000]. Industrially, chitosan is more useful than chitin, because of its solublity in most of the organic solvents. Most of the naturally occurring polysaccharides are neutral or acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides.

Their unique properties include polyoxy salt formation, ability to form films, chelate metal ions and optical structural characteristics and gel forming ability made it as a versatile material in different areas [Ravikumar *et al.*, 1999]. There are numerous work was carried out on the chemical modification of chitin and chitosan to various derivatives with improved properties [Kurita, 2001; Sato *et al.*, 2001; Gingras *et al.*, 2003; Khor *et al.*, 2003; Kweon *et al.*, 2003; Mao *et al.*, 2003; Wang *et al.*, 2003] .

Chitosan can be moulded in to different forms. One form is chitosan nanoparticles of size in the range of 200-1000 nm. Many researchers concentrated in the area of chitosan nanoparticle for different applications mainly in the medical field as drug carrier [Wu *et al.*, 2005; Maestrelli *et al.*, 2006]. Chitosan nanoparticles can be prepared by different methods like freeze drying, preparation of multiple emulsion/solvent evaporation and coacervation and ionic gelation method. From these methods ionic gelation is the best one for chitosan.

Chitosan possesses good film forming properties. This chemical combination of natural and synthetic polymers yields new materials which could have desirable properties including biodegradability. The chitin and chitosan can be formed into fiber shape also.

1.9.1. Applications

The poor solubility of chitin is the major limiting factor in its utilization. Despite this limitation, various applications of chitin and modified chitins have been reported. But chitosan having properties like biocompatibility, solubility, antimicrobial and

antifungal activities and gel-forming ability at low pH, makes it a favorable option for biomedical applications. A wide variety of medical applications for chitin, chitosan and its derivatives have been reported over the last three decades [Pariser *et al.*, 1980; Whistler, 1983; Yalpani *et al.*, 1992; Shigemasa *et al.*, 1996]. Chitosan may be used to inhibit fibroplasia in wound healing and to promote tissue growth and differentiation in tissue culture [Yannas *et al.*, 1982; Muzzarelli *et al.*, 1999]. Swelling power in acid medium makes it a good candidate for controlled drug release formulations. Fibres made of chitin and chitosan are useful as absorbable sutures and wound-dressing materials and this have applications in wastewater treatment too [Malettas *et al.*, 1986]. Chitosan's strong positive charge allows it to bind to negatively charged surfaces such as hair and skin which makes it a useful ingredient in hair and skin products like creams, lotions and permanent waving lotions and several derivatives have also been reported [Mark *et al.*, 1985].

1.10. Starch

Starch is an important naturally occurring polymer of glucose, with diverse applications in food and polymer science, found in roots, rhizomes, seeds, stems, tubers and corms of plants, as microscopic granules having characteristic shapes and sizes [Buleon *et al.*, 1998]. Each starch typically contains several million amylopectin molecules accompanied by a much larger number of smaller amylose molecules. The largest source of starch is corn (maize) with other commonly used sources being cereals (e.g. corn, wheat, rice, oat, barley) contain 60% to 80%, legumes (e.g.

chickpea, bean, pea) 25% to 50%, tubers (e.g. potato, cassava, cocoyam, arrowroot) 60% to 90% and some green or immature fruit (e.g. banana, mango) contain 70% starch in dry base [Bello-Perez *et al.*, 1999]. Genetic modification of starch crops has recently led to the development of starches with improved and targeted functionality. Annual worldwide starch production is 66.5 million tons and the growing demand for starches has created interest in identifying new sources and modifications or derivatives of this polysaccharide [Thomas *et al.*, 1999].

1.10.1. Structural Unit

Starch is tightly and radially packed into dehydrated granules with origin-specific shape and size. Granules contain both crystalline and amorphous areas, consists of two types of molecules, amylose (normally 20-30%) and amylopectin (normally 70-80%). The granules are insoluble in cold water, but grinding or swelling them in warm water causes them to burst. Both amylose and amylopectin consist of polymers of α-D-glucose units in the conformation. The relative proportions of amylose to amylopectin and α - (1, 6) branch-points both depend on the source of the starch. The starch granule absorbs water; they swell, lose crystallinity and leach out amylose. The higher the amylose content, the lower is the swelling power and the smaller is the gel strength for the same starch concentration. Of the two components of starch, amylose has the most useful functions as a hydrocolloid. Its extended conformation causes the high viscosity of water soluble starch and varies relatively little with temperature. The extended loosely helical chains possess a relatively hydrophobic inner surface that is not able to

hold water well and more hydrophobic molecules such as lipids and aroma compounds can easily replace this. Amylose forms useful gels and films. Its association and crystallization (retrogradation) on cooling decreases its storage stability, causing shrinkage and the release of water (syneresis).

According to X-ray studies starch can be classified to A, B and C forms. In the native granular forms, the A pattern is associated mainly with cereal starches, while the B form is usually obtained from tuber starches.

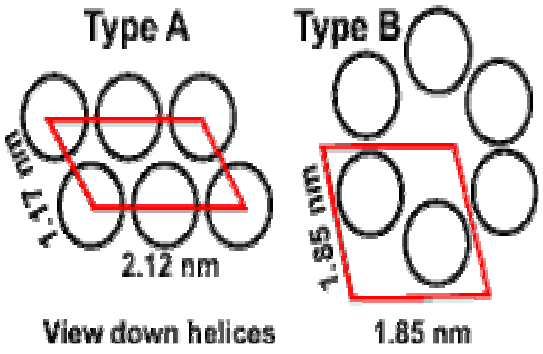


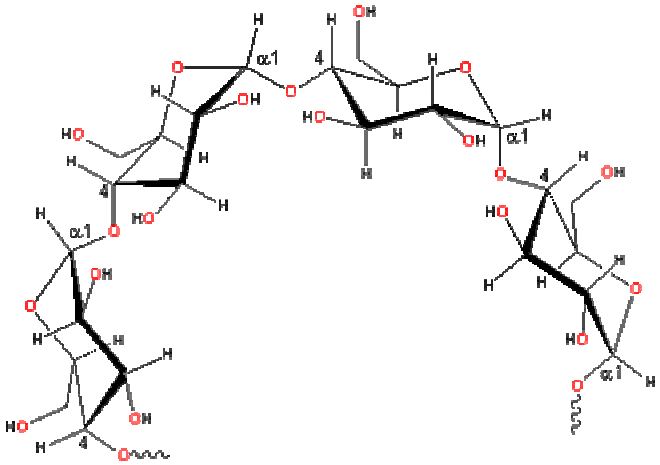
Figure 1.1. Crystalline nature of starch

The C pattern is a mixture of both A and B types, but also occurs naturally, e.g. smooth-seeded pea starch and various bean starches. The V-type conformation is a result of amylose being complexed with substances such as aliphatic fatty acids, emulsifiers, butanol and iodine.The main difference between A and B types is that the former adopt a close-packed arrangement with water molecules between each double helical structure, while the B-type is more open, there being more water molecules,

essentially all of which are located in a central cavity surrounded by six double helices (Figure 1.1) [Cheetham *et al.*, 1998].

1.10.2. Amylose

Amylose molecules consist of single mostly unbranched chains with 500-20,000 α - (1, 4)-D-glucose units dependent on source (Scheme 1.2).



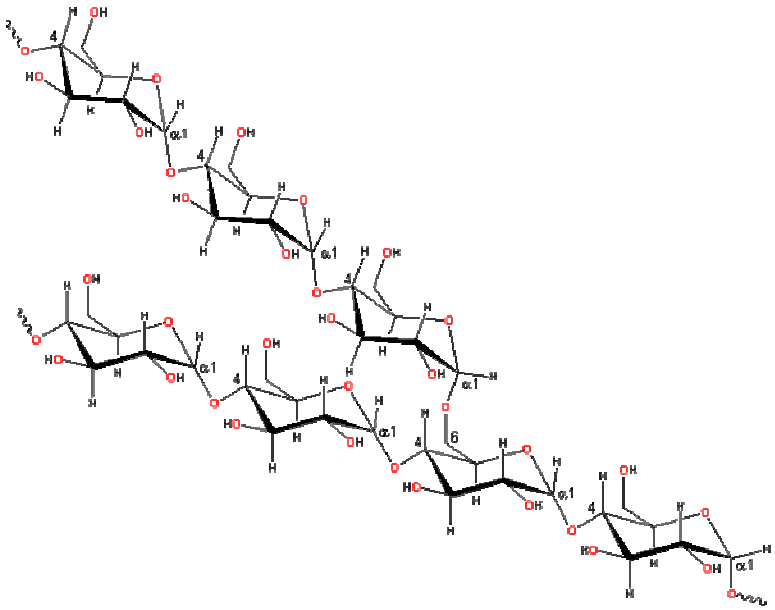
Scheme 1.2. Structure of amylose

Amylose can form an extended shape (hydrodynamic radius 7-22 nm) [Bertoft *et al.*, 2008] but generally tends to wind up into a rather stiff left-handed single helix or form even stiffer parallel left-handed double helical junction zones. Single helical amylose has hydrogen-bonding O_2 and O_6 atoms on outside surface of the helix with only the ring oxygen pointing inwards. Hydrogen bonding between aligned chains causes retrogradation and releases some of the bound water (syneresis). The aligned chains may then form double stranded crystallites that are resistant to amylases. These possess extensive inter- and intra-strand hydrogen bonding, resulting in a fairly

hydrophobic structure of low solubility. Single helix amylose behaves similar to the cyclodextrins, by possessing a relatively hydrophobic inner surface that holds a spiral of water molecules, which are relatively easily to be replaced by hydrophobic lipid or aroma molecules. It is also responsible for the characteristic binding of amylose to chains of charged iodine molecules.

1.10.3. Amylopectin

Amylopectin is formed by non-random α -1, 6 branching of the amylose-type α - (1, 4)-D-glucose structure (Scheme 1.3). Each amylopectin molecule contains a million or so residues, about 5% of which form the branch points. There are usually slightly more outer unbranched chains (called A-chains) than inner branched chains (called B-chains). There is only one chain (called the C-chain) containing the single reducing group. A-chains generally consist of residues between 13 and 23. There are two main fractions of long and short internal B-chains with the longer chains (greater than about 23-35 residues) connecting between clusters and the shorter chains similar in length to the terminal A-chains [Tang *et al.*, 2006]. Each amylopectin molecule contains up to two million glucose residues in a compact structure with hydrodynamic radius of 21-75 nm. The molecules are oriented radially in the starch granule and as the radius increases so does the number of branches required in filling up the space, and the consequent formation of concentric regions of alternating amorphous and crystalline structures.



Scheme 1.3. Structure of amylopectin

Amylopectin double-helical chains can either form the more open hydrated type B hexagonal crystallites or the denser type A crystallites, with staggered monoclinic packing, dependent on the plant source of the granules [Betancur-Ancona *et al.*, 2001; Tang *et al.*, 2006].

1.10.4. Modification of starch

Current starch research has focused on the search for non-conventional starch sources with diverse physicochemical, structural and functional characteristics that provide them with a broad range of potential industrial uses. Physicochemical and functional properties must be identified before determining the potential uses of starches in food systems and other industrial applications [Li *et al.*, 2008]. A fundamental characteristic of native starches from different vegetable sources is that their granule size distribution and molecular structures. These properties then influence a starch's usefulness in

different applications [Nabeshima *et al.*, 2001; Kaur *et al.*, 2007; Ahmed *et al.*, 2008; De la Torre Gutierrez *et al.*, 2008; Xue *et al.*, 2008].

Native and modified starches are used widely in food processing operations in order to impart viscosity and texture [Rosalina *et al.*, 2002; Wattanachant *et al.*, 2003]. In their native form, gels or pastes of starches tend to breakdown either from prolonged heating, high shear or acidic conditions and also they have the tendency to retrograde and undergo syneresis [Ruan *et al.*, 2009]. Starch derivatisations like etherification, esterification, acetylation, and cross-linking have been used to improve the gelatinisation and cooking characteristics and to prevent retrogradation [Garcia-Alonso *et al.*, 1999; Morikawa *et al.*, 2000; Mali *et al.*, 2001; Li *et al.*, 2005; Huang *et al.*, 2007; Onofre *et al.*, 2009]. Each anhydroglucose unit of starch contains two secondary hydroxyls and a primary hydroxyl group. These hydroxyls potentially are able to react with any chemical capable of reacting with alcoholic hydroxyls. This would include a wide range of compounds such as acid anhydrides, organic chloro compounds, aldehydes, epoxy, ethylenic compounds etc. where the specific chemical contains two or more moieties capable of reacting with hydroxyl groups. There is a possibility of reacting at two different hydroxyls resulting in cross linking between hydroxyls on the same molecule or on different molecules. The most common modification to starches to impart structural integrity is chemical cross-linking. These chemically cross-linked starches are usually resistant to shear, pH, temperature during food processing conditions. The most widely used cross-linking reagents for modifying food starches are mixtures of adipic/acetic anhydride, and phosphorus oxychloride or sodium

trimetaphosphate, which yield distarch adipates, distarch phosphates, respectively [Luo *et al.*, 2009]. Cross linking reinforces the hydrogen bonds in the granule with chemical bonds which act as bridge between the molecules [Achayuthakan *et al.*, 2008]. Several research groups have studied the rheological characteristics of starches mixed with other hydrocolloids to improve their rheological properties. Commonly used hydrocolloids are gum arabic, guar, carboxymethylcellulose, carrageenan, xanthan, xyloglucan etc.[Chaisawang *et al.*, 2006; Rodriguez-Hernandez *et al.*, 2006; Chaudemanche *et al.*, 2008; Pongsawatmanit *et al.*, 2008; Savary *et al.*, 2008; Ptaszek *et al.*, 2009]. The literature regarding the rheology of starch-hydrocolloid systems has attracted more attention than other systems. Blends of native starches and other polysaccharide hydrocolloids have been used in the modern food industry to modify and control the texture, improve moisture retension, control water mobility and eating quality of food products. Polysaccharide blending affected the pasting, and rheological properties of cationic, native, and anionic starches differently. Components compatibility, coupled with their individual properties like gelation, ageing etc. leads to a large variety of structures and properties of prepared materials. The interactions between starch and hydrocolloid make possible enhancing of viscosity and it has been explained in different ways. Some authors [Eidam *et al.*, 1995; Fama *et al.*, 2005] have simplified the system neglecting the granular phase and have explained the enhancement of blend viscosity on the basis of complexation between soluble starch and the added hydrocolloid, while others based on the two-phase model, have suggested that the increase observed in starch–hydrocolloid mixtures can be attributed

to an artificial increase of hydrocolloid concentration in the continuous phase due to the reduction of its volume by swelling of the starch granules during gelatinization.

Starch and starch derivative films have been widely studied from 1950 due to their great molding and film forming properties, high oxygen barrier and good mechanical strength [Lawton, 1996; Lourdin *et al.*, 1997; Lee *et al.*, 2000; Forssell *et al.*, 2002; Mali *et al.*, 2002; Parra *et al.*, 2004; Liu *et al.*, 2005; Zhang *et al.*, 2006]. Even though there have been numerous studies conducted on the properties of starch based films, few studies have related starches from different sources with the resulting film forming characteristics, mechanical and physical properties. Among the starch films, potato, sweet potato, mungbean and waterchestnut were selected due to their superior film-forming properties when compared with synthetic films. Forssell et al [Forssell *et al.*, 2002] reported that starch based polymer films, plasticized with water only had good oxygen barrier properties under ambient humidity. Since water acts as a plasticizer for these materials and their mechanical and barrier properties strongly depend on water content [Bader *et al.*, 1994; Mehyar *et al.*, 2004]. HACS forms a strong and flexible films probably due to amylose crystallization [Koskinen *et al.*, 1996; VanSoest *et al.*, 1996]. Amylose is responsible for the film-forming capacity of starch based films. The addition of plasticizing agent to edible films is required to overcome film brittleness caused by extensive intermolecular forces, thereby improving flexibility and extensibility of films [Gontard *et al.*, 1992]. Plasticizers extend, dilute and soften the structure and increasing the chain mobility.

1.10.5. Starch micro/ nanoparticles

Starch microspheres have been found to be effective in the systemic delivery of peptides after nasal administration and of vaccine given orally and intramuscularly [Rothman *et al.*, 1977; Bjork *et al.*, 1990; Illum *et al.*, 1990; Edman *et al.*, 1992; Mao *et al.*, 2004]. Pharmaceutical applications of starch microspheres necessitate controlled particle size, generally narrow size distribution, because the localization and distribution of the particles in the body depend on these parameters. Most studies based on the use of starch microspheres have been made with Spherex microspheres which have been commercialized since 1994 by pharmacia. These particles were prepared by the action of epichlorohydrin on partially hydrolysed starch. Hamidi *et.al* prepared starch-based microparticles by a water-in-water (w/w) emulsification-crosslinking method for different application [Hamdi *et al.*, 2001]. The main part of the starch particles has been produced by polymerization of acryloylated starch in water in oil emulsion or by crosslinking soluble starch with epichlorohydrin. The emulsion method is considered as best method for control the size of starch particles. In these cases, the amount of surfactant, epichliorohydrin/starch molar ratio etc are very important [Tuovinen *et al.*, 2004; Elfstrand *et al.*, 2006; Elfstrand *et al.*, 2009; Li *et al.*, 2009]. Starch acetate microparticles are also used widely for targeted drug delivery application [Jain *et al.*, 2008].

Inspite of great potential as a bioadhesive carrier, starch microspheres are not normally sufficient to provide clinically relevant plasma levels of large polypeptides. To address

above limitations, extensive studies were carried out on microsphere formulations comprising permeation enhancers and few studies dealt with nanoparticles too [Chakraborty *et al.*, 2005]. Using a new approach developed at ATO-DLO, it was shown that a novel type of starch-based micro or nanoparticles could be prepared which behaved as colloids in aqueous solution. The synthesis of the particles was based on a unique combination of gelatinization and crosslinking, performed in water-oil emulsions. Starch-based nanoparticles with variations in sizes, charge, density and suspension properties were prepared by varying starch source, crosslinker, pH, emulsion type, energy, temperature, and other parameters. These materials are biodegradable and expected to be applicable in both the food area and the non-food area.

Chakraborty et al studied the solution properties of starch nanoparticle in water and DMSO using DLS and selective esterification of starch nanoparticles was performed using as catalyst *Candida antartica* Lipase B in its immobilized and free forms. The starch nanoparticles were made accessible for acylation reactions by formation of AOT stabilized microemulsions. Starch nanoparticles in microemulsions were reacted with vinyl stearate, ε-caprolactone, and maleic anhydride at 40 °C for 48 h to give esterified starch nanoparticles [Chakraborty *et al.*, 2005].

1.10.6. Starch nanocrystals

Starch nanocrystals can be obtained by an acid hydrolysis on starch native granules [Dufresne *et al.*, 1996; Putaux *et al.*, 2003]. They consist of crystalline nanoplatelets

about 6-8 nm thick with a length of 20-40 nm and a width of 15-30 nm. These starch nanocrystals displayed interesting reinforcing properties when dispersed in different mediums [Dufresne *et al.*, 1998; Angellier *et al.*, 2005; Angellier *et al.*, 2005; Angellier *et al.*, 2006; Angellier *et al.*, 2006]. Interesting reinforcing capability was also obtained for nanocrystals of reinforced starch plasticized by glycerol [Kristo *et al.*, 2007]. Grafting of larger chains on the surfaces of starch nanocrystals enhance the nonpolar nature of original nanoparticles and dispersion in organic media. The mechanical properties of nanocomposite materials processed from the modified nanocrystals and natural rubber were found to be lower than those for unmodified nanoparticles, because the length of the grafted chains is high enough, entanglements are expected to occur with the polymeric matrix [Thielemans *et al.*, 2006; Labet *et al.*, 2007; Habibi *et al.*, 2008].

1.10.7. Njavara rice starch

Njavara is a rice variety widespread to Kerala, mainly seen in the northern parts (Figure 1.2). The cultivation of this rice variety is recorded from 2500 years back. Njavara is a unique grain plant in the Oryza group and widely used in the Ayurvedic system of medicine, especially in Panchakarma treatment. Njavara as a special cereal, have the properties to rectify the basic ills affecting our circulatory, respiratory and the digestive systems. This variety is highly resistant to drought conditions and is generally resistant to diseases. Dehusked Njavara rice has 73% carbohydrates, 9.5% protein, 2.5% fat, 1.4% ash and 1628 kJ per 100 g of energy. Higher amounts of

thiamine (27–32%), riboflavin (4–25%) and niacin (2–36%) and the total dietary fibre content in njavara was found to be 34–44% higher than compared to the other rice varieties.

Figure 1.2. Njavara rice

Significantly higher phosphorus, potassium, magnesium, sodium and calcium levels were found in Njavara rice [Deepa *et al.*, 2008]. Two types of Njavara are recognized, the black and golden yellow glumed. In the case of black glumed variety, the seed color is red.

1.10.8. Applications of starch

Starch is a versatile and economical, and has many uses as thickener, water binder, emulsion stabilizer and gelling agent. Starch is often used as an inherent natural ingredient but it is also added for its functionality. Mixing with hydrocolloids and low molecular weight sugars can also reduce retrogradation. At high concentrations, starch gels are both pseudoplastic and thixotropic with greater storage stability. Their water binding ability can provide body and texture to food stuffs and can be used as a fat replacement. Many functional derivatives of starch are marketed including cross-

linked, oxidized, acetylated, hydroxypropylated and partially hydrolyzed material. Hydrolysis of starch, usually by enzymatic reactions, produces a syrupy liquid consisting largely of glucose. It is widely used to soften texture, add volume, inhibit crystallization and enhance the flavor of foods. Nowadays starch based films are used for food packging, a coating for tablets or capsules. Starch nanoparticles and microparticles were used for the release of drugs, cosmetics and aromas etc. [Thomas *et al.*, 1999]

1.11. Xyloglucan

Xyloglucans are members of a group of polysaccharides typically referred to as hemicelluloses [Waldrona *et al.*, 2007]. Xyloglucan is found in the plant cell wall that is cross-linked with load-bearing cellulose microfibrils and affect wall mechanical properties and cell wall enlargement [Hayashi, 1989; Wang *et al.*, 1997; Chanliaud *et al.*, 2004; Cosgrove, 2005; Najmudin *et al.*, 2006]. It was first found as amyloids in the cell walls of plant seeds [Kooiman, 1957]. These are also present as storage products in some seeds as a resource for the embryo after germination. Xyloglucan oligomers, which have been shown to exert signaling effects on plant tissues, have been widely studied to define their biological activity. The flow behavior of the Xyloglucan solution is nearly Newtonian, and very stable against heat, pH, and shear [Nishinari *et al.*, 2000]. Xyloglucan is expected to have new applications in food, serving as a thickener and stabilizer, gelling agent, ice crystal stabilizer, and starch modifier [Yoshimura *et al.*, 1999].

Xyloglucan is extracted from different sources like tamarind seed, seed from African tree Afzelia africana Se. Pers, fruit cell wall of apple, seed from Hymenaea courbaril, grape berry cell walls, Detarium senegalense, white-mustard seeds, rapeseed hulls, rice hull, cotton fiber, azuki beans, pine hypocotyls etc. [Gould *et al.*, 1971; Aspinall *et al.*, 1977; Hayashi *et al.*, 1988; Acebes *et al.*, 1993; Vierhuis *et al.*, 2001; Docoa *et al.*, 2003; Ren *et al.*, 2004; Ren *et al.*, 2005; Fu *et al.*, 2006; Tine *et al.*, 2006; Hilz *et al.*, 2007; Soga *et al.*, 2007]. Among these, xyloglucan derived from tamarind seed was highly studied for different applications.

General structure of Tamarind xyloglucan consists of a β (1, 4) glucan backbone variously substituted with xylosyl and galactosyl, residues [Fry, 1989]. In the seed, this exists in thickened cell walls of the cotyledonary cells. Nowadays, research group at Brazil studied some modifications and properties of xyloglucan from Hymenaea courbaril [Busato *et al.*, 2001; Lima *et al.*, 2003; Busato *et al.*, 2009]. Xyloglucan from seeds of Hymenaea courbaril was first detected by Buckeridge, Dietrich and Kooiman [Kooiman, 1960; Buckeridge *et al.*, 1990]. In Brazil, xyloglucans have been studied since 1993, when Lima, et al [Lima *et al.*, 1993] isolated one from crushed seeds of Hymenaea courbaril, known locally as jatoba [Lima *et al.*, 1995] and studied some oligosaccharides obtained by enzymatic hydrolysis, which had a composition similar to that of Tamarindus indica seeds. In the case of bilberry xyloglucans, more than 20 different building blocks were found to make up the xyloglucan polymer which contains XXXG-type and some XXG type oligomers. The building blocks contain

galactose–xylose (L) and fucose–galactose–xylose (F) side chains. Seed storage xyloglucans of Hymenaea courbaril possess a structure composed of xylocellopentaosyl and xylocellohexaosyl backbone units in addition to the more common xylocellotetraosyl units [Martin *et al.*, 2003; Freitas *et al.*, 2005]. Apple fruit xyloglucan was composed of XXXG, XXFG, XLXG and XLFG type oligomers [Watt *et al.*, 1999]. Detarium senegalense Gmelin, the seed flour of an African leguminous plant traditionally used in Nigeria for its food thickening properties in soups and stews was also found to contain a high proportion of xyloglucans. Its structural composition is very similar to tamarind xyloglucan, the main difference in terms of simple composition being in the proportion of galactose, relative to xylose and glucose [Wang *et al.*, 1996]. White mustard xyloglucan have the amyloid type of structure in which chains of (1, 4)-linked β-D-glucopyranose residues carry D-xylose-rich side chains through position 6.

1.11.1. Cellulose-xylglucan network

The cellulose-xylglucan network is believed to be the major load-bearing structure in the primary wall (Figure 1.3). Xyloglucan coats the surface of the cellulose microfibrils, limiting their aggregation and connecting them via tethers that directly or indirectly regulate the mechanical properties of the wall [Hanus *et al.*, 2006]. Many studies have shown that xyloglucan adsorbs strongly to cellulose [Hayashi *et al.*, 1984; Levy *et al.*, 1991; Hayashi *et al.*, 1994; Vincken *et al.*, 1995; Whitney *et al.*, 1995; Stiernstedt *et al.*, 2006], and recently, this specific interaction has been harnessed in

the development of a new versatile method to functionalize cellulosic surfaces [Brumer *et al.*, 2004; Zhou *et al.*, 2005].

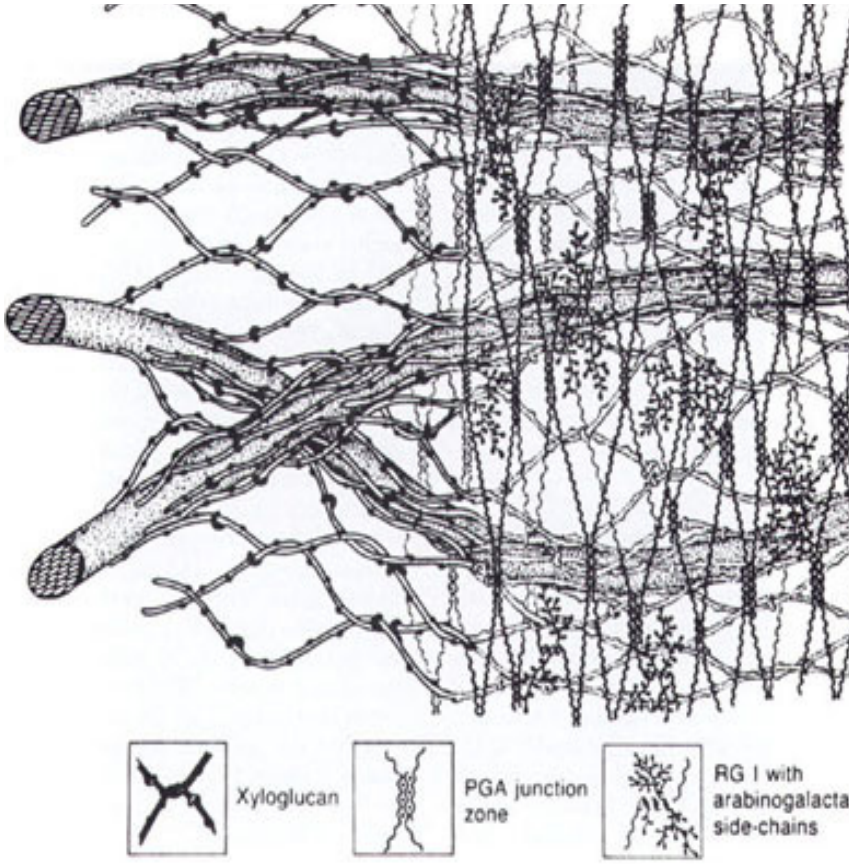


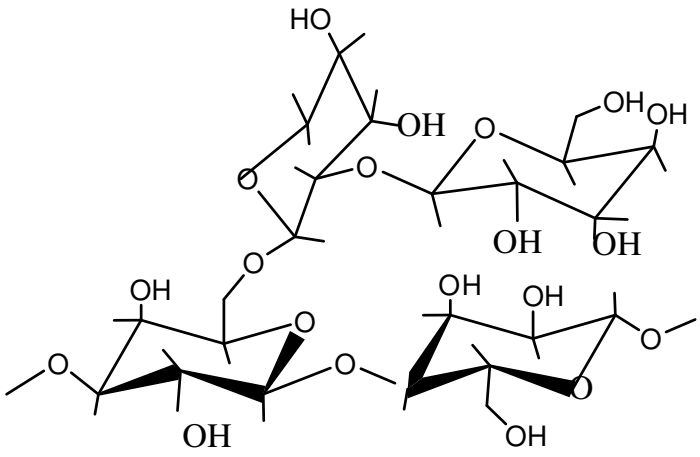
Figure 1.3. cellulose-xylglucan network

An important effect of the xyloglucan adsorption is that the very small adhesion between cellulose surfaces in water is significantly increased due to the bridging and formation of specific bonds of xyloglucan to both surfaces. This provides a possible mechanistic explanation for the recent observation that the tensile strength of paper increases by about 30% by the addition of xyloglucan [Lima *et al.*, 2001; Christiernin *et al.*, 2003]. Within the cellulose–xyloglucan network three xyloglucan domains are

described: the first domain includes the parts of xyloglucans that bridge the space between cellulose microfibrils or that form free loops. This domain can be degraded by endo-glucanases. The major part of xyloglucans belongs to the second domain that covers the cellulose microfibrils and can be extracted with concentrated alkali. In this, xyloglucans are not in direct contact with the cellulose. These regions are the crosslinking tethers. The third domain of the xyloglucans is entrapped within the amorphous cellulose microfibrils, which have to be degraded before the xyloglucan is accessible for enzymatic degradation or extraction [Hayashi *et al.*, 1987; Pauly *et al.*, 1999; Bootten *et al.*, 2004; Lima *et al.*, 2004; Zykwinska *et al.*, 2008].

1.11.2. Structure of Xyloglucan

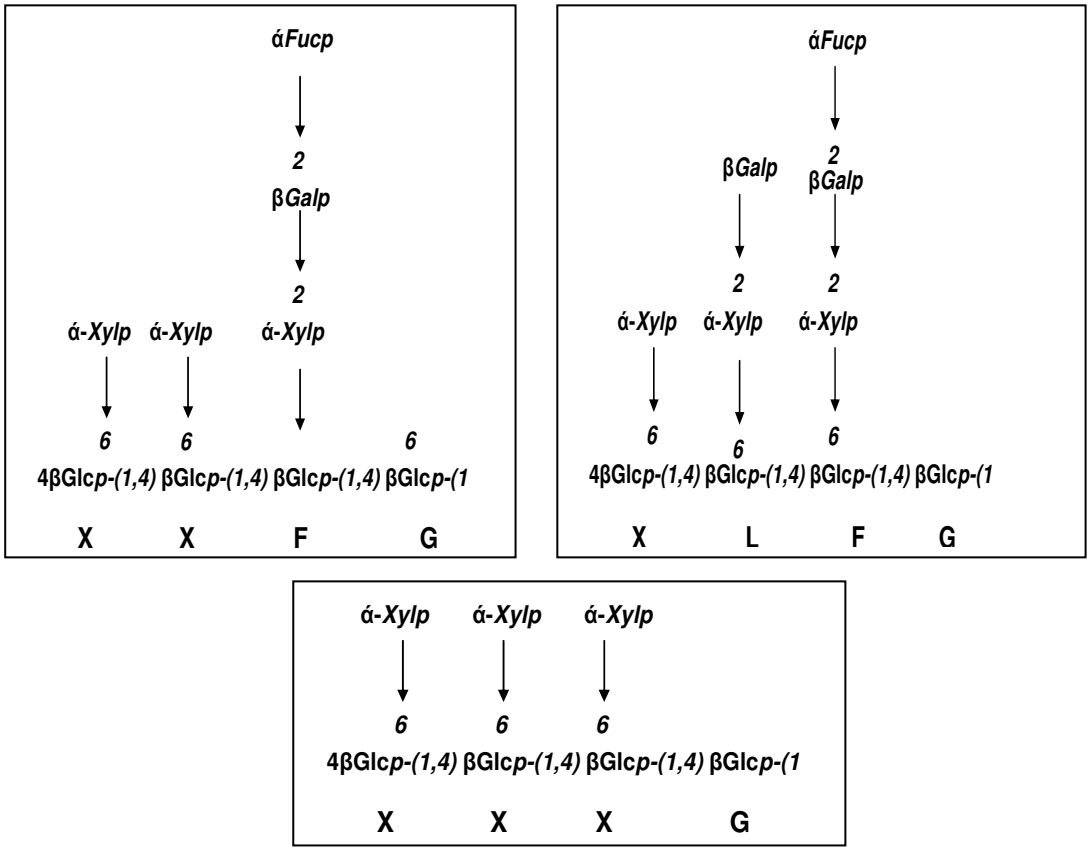
The backbone β-(1, 4) linked glucose residue of xyloglucan is partially substituted by ά-(1, 6) linked xylose units (Figure 1.4).



Scheme 1.4. Structure of xyloglucan

Some of the xylose residues are β-D-galactosylated at O-2 (Scheme 1.4). It is known that the distribution of side chain residues is different in the xyloglucans extracted from different species [Picout *et al.*, 2003]. Up to 75% of these residues are substituted at O-6 with mono-, di-, or triglycosyl side chains.

A single letter nomenclature is used to simplify the naming of xyloglucan side chain structures (Scheme 1.5).



Scheme 1.5. Single letter nomenclature of Xyloglucan

For example, G represents an unbranched Glc*p* residue, F represents a Glc*p* residue that is substituted with a fucose-containing trisaccharide, X represents xylose substituted Glcp residue and L represents galactose substituted Glcp residue. The main

chain is identical to the cellulose chain, which has ribbon-like conformation composed of each glycosidic ring face. The experimental data from X-ray fiber diffraction have indicated a two fold helical conformation for the main chain in the crystalline state, which is similar to the flat ribbon-like conformation of cellulose chains in the crystal (Figure 1.4). Picard et al reported a twisted main-chain conformation of xyloglucan heptamer in aqueous solution from NMR and molecular mechanics studies. Their study has revealed that the twisted conformation is significantly populated in solution, although the main chain includes only four glucose residues [Picard *et al.*, 2000]. The orientation of xyloglucan side chains in aqueous solution has not been experimentally deduced with the exception of the report mentioned by Picard et al.

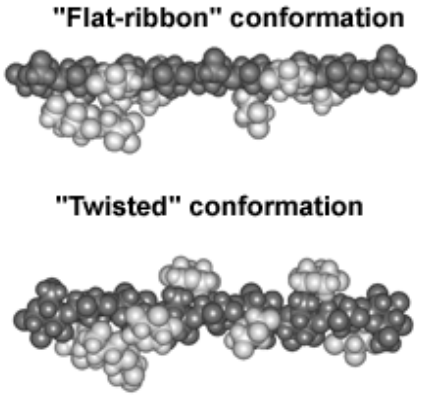


Figure 1.4. Conformation of xyloglucan

The xyloglucan heptamer in their study has only xylose side chains, while most xyloglucans in plants include not only xyloses, but also galactoses and fucoses [Umemura *et al.*, 2005].

1.11.3. Tamarind Xyloglucan

Tamarind seed polysaccharide is the major constituent of seeds from the tree *Tamarindus indica*. In India approximately 3-5 lakhs tonn tamarind kernel powder was produced per year. Tamarind cultivation was concentrated in the southern states of India. The seeds contain xyloglucans and are used extensively as food thickeners, stabilizers and gelling agents and it is used as a wet end additive in the paper industry as a replacement for starches and galactomannans. Tamarind xyloglucan prevents suppresion of delayed-type hypersensitivity responses and reduced interleukin production in UV-irradiated murine epidermis and also prevents suppresion of immune responses to alloantigen in mice exposed to 30kJ/m2 UVB radiation. The chemical structure of the tamarind seed xyloglucan backbone is α-(1,4)-linked D-glucan and is partially substituted at the O-6 position xylopyranose. Some of the xylose residues are α-D-galactosylated at O-2. There are three different structures for the repeating units of tamarind seed xyloglucan: heptasaccharide (Glu4Xy3), octasaccharide (Glu4Xy3Gal), and nonasaccharide (Glu4Xy3 Gal2) [Marry *et al.*, 2003; Kim *et al.*, 2006].

Heptasaccharide	XXXG	Glc:Xyl = 4:3
Octasaccharide	XLXG	Glc:Gal:xyl= 4:1:3
	XXLG	
Nonasachharide	XLLG	Glc:Gal:xyl=4:2:3

The ratio of oligosaccharides XXXG : XLXG : XXLG: XLLG- is 1: 0.42 : 2.07: 6.20 [Gidley *et al.*, 1991; Picout *et al.*, 2003]. Tamarind xyloglucan is water-

soluble,and flat ribbon like two-fold helical models [Kumar *et al.*, 2008]. The side-chains of xyloglucan play an important role in determining its structure and make them water-soluble and impart various rheological and biological functions. To improve the solubilization of tamarind seed xyloglucans, a pressure cell heating method has been used. The architecture of tamarind seed xyloglucan, has been investigated by DLS, SAXS and synchrotron radiation [Lang *et al.*, 1993]. The data appeared to show that tamarind xyloglucan in aqueous solution consisted of multistranded aggregates, with a high degree of particle stiffness but no reproducibility of the molar mass was achieved. Various *M*Ws values for tamarind seed xyloglucan [115 0007 or 650 0008 (GPC) or 880 0009 (light scattering) or even 2 500 0006] were reported in the literature.

1.11.4. Gelation of xyloglucan

Xyloglucan normally does not form a gel, however it forms thermo responsive gels in water, under certain conditions. When it is partially degraded by beta-galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solutions. A detailed study of the sol-gel transition temperature as a function of of the degree of galactose elimination has been reported by Yuguchi et al [Yuguchi *et al.*, 1997]. Gelation is only possible when the galactose removal ratio exceeds 35% [Shirakawa *et al.*, 1998]. The transition temperature is reported to be inversely related to polymer concentration [Miyazaki *et al.*, 1998] and the galactose removal ratio. These thermally reversible gels have been tested in several medical applications, such as rectal delivery of indomethacin, intraperitoneal administration of mitomycin C, vehicles for oral

delivery of indomethacin, paracetamol, cimetidine, and theophylline, ocular delivery of pilocarpine and timolol, and also percutaneous administration of non-steroidal anti-inflammatory drugs [Suisha *et al.*, 1998; Kawasaki *et al.*, 1999; Burgalassi *et al.*, 2000; Burgalassi *et al.*, 2000; Miyazaki *et al.*, 2001; Miyazaki *et al.*, 2001; Miyazaki *et al.*, 2001; Takahashi *et al.*, 2002; Miyazaki *et al.*, 2003; Ruel-Gariepy *et al.*, 2004; Nisbet *et al.*, 2006; Itoh *et al.*, 2008; Busato *et al.*, 2009]. Time-resolved small angle x-ray scattering studies have shown that enzyme-degraded xyloglucan forms gels by the lateral stacking of the rod-like chains. In this respect these gels differ from block copolymer gels which are formed by packing of micelles behaving effectively as hard spheres [Yamanaka *et al.*, 2000; Yuguchi, 2002]. Enzymatically degraded xyloglucan forms gels at higher temperatures [Shirakawa *et al.*, 1998], while the original xyloglucan forms gels at a lower temperature in the presence of ethanol. Here the gelation mechanism is different, and is mainly due to the structure of the crosslinking domains. In the case of enzymatically degraded xyloglucan, the cross-linking domains are composed of aligned xyloglucan chains in the shape of flat plates, whereas no ordered structure was found for the crosslinking domains in the xyloglucan/ethanol system at lower temperatures. The cross-linking domain seems to be formed by random aggregation of xyloglucan chains due to poor solubility in ethanol [Yuguchi *et al.*, 2004]. It has also been reported that xyloglucan interacts with polyphenols or dyes like iodine and Congo red forms gel [Yuguchi *et al.*, 2005; Yuguchi *et al.*, 2005]. The thermoreversibilty of the gel indicates the network formation through a noncovalent

bond. Epigallocatechin Gallate was found to bind to xyloglucan chains in this gel network [Nitta *et al.*, 2004].

Different polysaccharides are used together in order to meet increasing demands for product having finely tuned properties. Yoshimura, et al [Yoshimura *et al.*, 1999] have investigated effects of xyloglucan on gelatinization and retrogradation of corn starch and concluded that xyloglucan does not synergistically interact with corn starch. Synergistic interactions are often observed between a helix-forming polysaccharide and non-gelling polysaccharide [Doublier, 1994]. Ikeda et al and Nitta et al studied the synergetic effect of xyloglucan and gellan mixtures using dynamic rheometry, DSC, AFM [Nitta *et al.*, 2003; Ikeda *et al.*, 2004]. Tapioca starch paste found to have good rheological, thermal properties and storage stability when mixed with Xyloglucan [Freitas *et al.*, 2003; Temsiripong *et al.*, 2005; Pongsawatmanit *et al.*, 2006; Pongsawatmanit *et al.*, 2007]. The mixing of xyloglucan and xanthan results in an increase in the elastic moduli, and this is a synergistic interaction, which is also the combination of the helix-forming polysaccharide and α-D-(1,4)-linked cellulosic polysaccharide [Sims *et al.*, 1998; Kim *et al.*, 2006].

1.11.5. Modifications of xyloglucan

Studies on the modification of xyloglucan is a growing area of research. Many researchers studied the grafting of poly acrylonitrile (PAN) and acrylamide onto xyloglucan and have been done successfully by using the CAN/HNO_3 redox initiator system to improve properties of Xyloglucan [Goyal *et al.*, 2008; Mishra *et al.*, 2008].

XG has been used as a new synthetic extracellular matrix for primary mouse hepatocyte attachment in Ca-alginate capsules [Seo *et al.*, 2005]. Thin xyloglucan films were prepared by spin-coating and drop deposition under pH=3, 5 and 12, on silicon and mica substrates in order to study the dewetting pattern on silicon [Lubambo *et al.*, 2009]. Films from xyloglucans might be suitable substrates for adsorption of BSA, especially close to its isoelectric point, making them potential materials for biomedical and biotechnological devices [Jo *et al.*, 2009]. The oxidized product of xyloglucan at the C-6 position of galactose or glucose units, using 2, 3, 6, 6-tetramethylpiperidine-1-oxyl, to form uronic acid-containning polysaccharides, with different degrees of oxidation can be useful and can be applied this hydrophilic polyelectrolyte in drug encapsulation [Lucyszyn *et al.*, 2009].

1.11.6. Applications of xyloglucan

The noncarcinogenicity mucoadhesivity, biocompatibility and high drug holding capacity of xyloglucan led to its application as excipient in hydrophilic drug delivery system [Burgalassi *et al.*, 1996; Kulkarni *et al.*, 2008]. It is an important excipient, having good release kinetics of both water-soluble and water insoluble drugs from this matrix and has high thermal stability. It is used as binder in tablets, gelling agent, thickening agent, as emulsifier and as stabilizer in food, and pharmaceutical industries. Due to its hydrophilic and mucoadhesive property, it can be used in mucoadhesive drug delivery system and it is a suitable candidate for addition to ophthalmic solutions

of β adrenergic blockers to increase the residence time on the cornea [Burgalassi *et al.*, 2000].

Tamarind seed xyloglucan is expected to find new food applications, serving as a thickener and stabilizer, gelling agent, ice crystal stabilizer and starch modifier, etc. [Takagi *et al.*, 2008]. Because of it's ability to form gels in presence of sugar over a wide pH range it can be used in the production of jams, jellies and marmalades and as stabilizer for ice cream and mayonnaise. Xyloglucan shows a hypolipidemic effect and may be important for the treatment and prevention of geriatric diseases, including diabetes and cardiac disorders [Yamatoya *et al.*, 1997].

Xyloglucan is used as a ultraviolet protective agent and also in combination with other ultraviolet protective agent in cosmetic industries [Tadashi *et al.*, 1997; Koji *et al.*, 1998; Koji *et al.*, 1999; Yokoyama *et al.*, 2008]. It is used as an agent for permanent waving/curling of hairs and as oxidized hair dye mixed with primary dyeing agent [Hajime *et al.*, 2004; Hajime *et al.*, 2004]. Xyloglucan polymers and oligomers, and derivative compounds, are used as phytosanitary products and biofertilizers [Lienart, 2002]. New xyloglucan oligosugars are also useful as intermediates for preparation of plant protective agents inhibiting plant cell growth [Tomoya *et al.*, 1991].

In this respect, natural polymers are recommended as suitable functional materials, because they have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption properties, etc.

1.12. Scope and objective of the present work

Environmental awareness and the demand for green technology have led to a rapidly increasing interest in the application of natural and renewable polymers especially polysaccharides in industrial applications in areas of foods, textiles, paints, cosmetics and pharmaceuticals. Biopolymers remain a hot topic, with major medical and pharmaceutical industries turning to natural materials and their unique properties with regard to biodegradability, non toxicity, water solubility etc. which are based on the broad range of functional properties. Today's consumers in the cosmetic, food and beverage market are increasingly interested in healthy life styles, a trend which has produced a rising demand for health oriented products. Cosmetic and personal care products manufacturers claim that there is promising trend for transparent products for instance those which use clear formulation techniques in their gels and emulsions. In recent years, there has been considerable interest in the development of conjugates of non-starch polysaccharide molecules. Growing environmental concerns have created an urgent need to develop biodegradable materials that have comparable properties with today's polymeric materials at an equivalent cost. Research has been growing on producing biodegradable polymeric films. Natural biopolymers have advantage over synthetic biodegradable polymers in that they are biodegradable and renewable raw materials.

In view of this situation, we have selected abundantly available storage glucans such as starch and xyloglucan as preferred material having green properties, for our studies.

Starch is an alpha glucan and has many properties which can be utilized in the medicinal food and cosmetic field. Njavara is the unique rice, short duration cultivar grown only in certain areas in Kerala state, south India, traditionally used effectively in the Ayurvedic system of medicine in certain specific treatments like *Panchakarma* and this treatment is now getting more and more popular, not only in this region of the country but also in other parts of the nation and even in other countries. A detailed study has to be undertaken on the unique physico chemical properties of starch from the njavara rice. So we investigated the njavara rice starch and compared chamba rice starch which is our staple food.

Starch is a neutral polysaccharide and is degraded completely to glucose by the amylases in the human body. Cassava Starch is abundantly available at a low cost and an also made into nanoparticles for use in controlled release of drugs. Nanoparticles loaded with drugs show drug release at right rate and dose at specific sites in the body for certain duration to realize the accurate delivery, which enhances the therapeutic effect and reduces the toxicity and side effects of the drug. In view of this the hydrophilic starch will be partially modified to hydrophobic and made into nanoparticles.

Xyloglucan is a beta glucan present in the seeds of the tamarind tree (*Tamarindus indica*) which is abundantly available in South India and have applications in food, serving as a thickener and stabilizer, gelling agent, ice crystal stabilizer, and starch modifier, in cosmetic and pharmaceutical industries. Chemical derivatisation methods

will be employed to improve the properties of these intractable, inexpensive polysaccharides material. It was found that cationic polysaccharides such as chitosan have wide applications in drug and gene delivery. Hence attempts will be made to make a plant derived cationic polysaccharide from xyloglucan.

The compatibility of one or more polysaccharides to make gels and edible films is of great significance in today's scenario. Development of conjugates of non-starch polysaccharide such as xyloglucan with, chitosan or starch will have good compatibility for food, feed and cosmetic applications.

Hence the present research work is an attempt to study the properties of the starch and non starch polysaccharide and to improve the properties by chemical modifications and to develop new product that can be used in biomedical, food and cosmetic area. Specifically the objectives of the present work are

1. Studies on the unique properties of njavara rice starch compared to other rice starch.
2. Preparation and studies of hydrophobically modified starch nanoparticles for controlled drug release applications.
3. Investigations on the modification of xyloglucan by different chemical methods and the physico-chemical evaluation of the same.
4. Preparation and properties of composite xyloglucan-chitosan gel for food and cosmetic applications
5. Preparation of composite edible films of xyloglucan and xyloglucan -chitosan / starch blends and study the properties.

CHAPTER 2

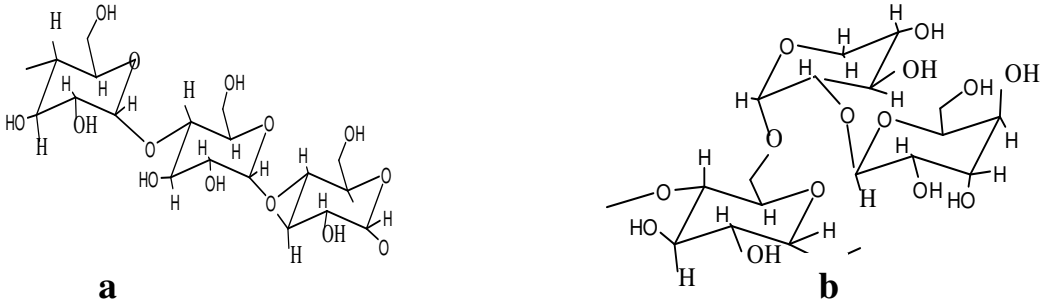
Materials and Characterisation Techniques

This chapter gives a brief description of the various chemicals used for the extraction and modification of polysaccharide especially for starch and xyloglucan, and the analytical techniques employed for the characterisation of modified polysaccharide gels and films prepared in this work.

2.1. Materials

2.1.1. Glucans

Mainly two type plant derived glucans or storage polysaccharides were used throughout the work. One is an alpha linked glucan, starch extracted from two different sources like cassava (tuber) and medicinally important njavara rice (cereal). Second one is a beta linked xyloglucan polysaccharide extracted from tamarind kernel powder. The structures are given below.



Scheme 2.1. Structure of a) Starch, b) Xyloglucan

These are purchased from local market, Trivandrum, Kerala having same maturity and glucan content and from the same cultivar harvested from the same seasonal crop. Extracted and purified by different wet methods.

2.1.2. General wet extraction and purification method

Polysaccharide or glucan extraction can do by several ways, with different methods showing characteristic extraction efficiencies and functional properties. Ideal

extraction conditions cause little or no structural changes in the extracted components. Wet-milling is an alternative extraction method, commonly used for extraction of starch from flour. In the case of starch isolated from dry milling, has poorer functional properties than has starch from wet milling. General procedure for wet extraction method for starch is given as follows [Whistler, 1964]. First the source material was homogenized into a fine paste and made into a suspension in water and filtered through muslin cloth to remove the fibers. The starch suspension was allowed to settle and then centrifuged at 200 xg. Crude impurities were removed from the starch layer by repeated gentle washing and settling, followed by resuspension of the starch in water and centrifugation. Lipids present were removed by washing with hexane. Hexane was added to the starch (0.25% w/v), and stirred for 30 min at 30 ± 2 °C. Filtered and air dried at first and then oven dried at 50 ± 2 °C and stored in air tight containers.

In the case of other polysaccharides especially hemicellulose such as xyloglucan from Tamarind kernel powder, the extraction procedure is different. Here first the coarse Tamarind kernel powder was agitated and homogenated in a blender and the ground samples were extracted with hot distilled water. The extraction time, temperature and pH of the solutions were depend on the type of the glucans to be extracted. After centrifuging to remove the debris fragments, the solution was concentrated and precipitated with four volumes of 95 % ethanol at 4 °C. Precipitate was then freeze dried. Lipids present in the xyloglucan were removed by soxhlet extraction method using hexane as solvent.

Protein extraction method was similar for all glucans. The powdered glucan was deproteinated with protease enzyme (Protease from *Bacillus Licheniformis*). 500µl (>1.2 U/500µl protein) enzyme was added to the glucan suspension in phosphate buffer at pH 7 and kept for 30 min at 30±2 °C with stirring. The slurry was centrifuged at 704 x g for 5 min and the supernatant was discarded. Air dried (room temperature 25°C – 28 °C) the deproteinated glucan and the oven dried at 50 °C.

2.1.3. Extraction of xyloglucan

In our work xyloglucan was extracted from tamarind seed powder reported elsewhere [Lima *et al.*, 2003; Ribeiro *et al.*, 2009]. A short description is given below. Tamarind seed powder was deproteinated with protease from *Bacillus licheniformis* (conditions: 3U enzyme per 1 g of tamarind powder, 30 ± 2 °C, 30 min., pH = 6). The defatting was completed in a soxhlet extractor using hexane as solvent (10 ml hexane per 1 g of tamarind powder) and dried in an oven at 60 °C for 6 h. The slurry of dried powder in distilled water was boiled with acidified water (using citric acid, pH=3) for 30 min. The solution was kept overnight for settling and the supernatent liquid was evaporated to half the volume. The solution was cooled and a fibrous precipitate was formed by adding 95% (v/v) ethanol, which was filtered and freeze dried. The freeze dried material was ground to a fine powder and stored in air tight container for use.

2.1.4. Chemicals

Different chemicals were used for the modifications and extraction of these polysaccharides. All these chemicals used were of analytical reagent grade. Oleic acid,

stearic acid, ethylene diamine, polyethyleneimine, dimethyl sulphoxide-*d,* aspartame, potassium bromide, 2, 5-dihydroxy benzoic acid, chitosan, indomethacin, streptomycine, protease enzyme (from *Bacillus Licheniformis)* and alpha amylase enzyme (from *Bacillus amyloliquifaciens)* were purchased from Sigma Aldrich, USA. Ninhydrin, nutrient agar, Calcium chloride, Sodium tripolyphosphate, potassium persulphate, vanillin, sucrose and ethanol were obtained from Central Drug House, Mumbai, India. Analytical grade citric acid, sodium hydroxide, sodium borohydride, sodium per iodide, ammonium acetate, acetic anhydride, ethylene glycol, glycerol, acetic acid, sulphuric acid, hydrochloric acid, nitric acid, and also number of polar and non polar organic solvents like DMSO, THF, DCM, benzene, toluene, carbon tetra chloride, hexane, DMF, TFA, CH_3CN, was procured from M/s Sisco Research Laboratory, Mumbai, India.

The modifications and structure of the polysaccharides are described in their respective chapters. The chemical structure and property evaluation of polysaccharide were performed by various analytical techniques, which are elaborated below.

2.2. Characterization techniques

2.2.1. Fourier- Transform Infrared Spectroscopy (FT-IR)

The use of infrared spectroscopy for the characterization of the polymeric materials has experienced tremendous growth in recent years, primarily because a variety of sampling techniques and experimentations are now available [Colthup *et al.*, 1964; Griffiths *et al.*, 1986; Garton, 1992]. Interaction of electromagnetic radiation with

molecular vibrations gives rise to absorption bands throughout most of the IR region of the spectrum. Identification of composition and morphology of polymers and their blends, curing studies, diffusion and oxidation studies, degradation of polymers, orientation in polymers etc are some of the areas where it finds extensive use. In this research work, FT-IR spectra were obtained with a Perkin Elmer spectrophotometer in the range of 4000-400 cm^{-1}. The sampling techniques viz: KBr pellets and smearing of sample in chloroform solution on AgCl/KBr crystals have been used for recording IR spectra. Each interferogram was generated by signal averaging 32 scans and the spectra were obtained as percentage transmittance versus wave number.

2.2.2. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectroscopy is an important analytical tool, which is extensively used to study the structure and purity of compounds [Purcell *et al.*, 1946; Alpert, 1947; Bloch *et al.*, 1964]. The solution NMR has emerged as one of the premier methods for characterization because of high resolution and sensitivity. The chemical shifts are sensitive to polymer microstructure, including polymer stereochemistry, regio isomerism and the presence of branches and defects. Proton (^1H) was recorded with a Bruker DPX 300 MHz spectrometer, USA (300 MHz). TMS is the internal standard used for comparing the NMR signals. The samples are prepared in DMSO d_6.

2.2.3. Fluorescent Spectroscopy

Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light,

that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy, typically, but not necessarily, visible light. In fluorescence spectroscopy, the species is first excited, by absorbing a photon, from its ground electronic state to one of the various vibrational states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state. Photoluminescence spectra were recorded on a spectrofluorimeter, Spex-Fluorolog DM3000F, with a double grating 0.22 m Spex 1680 monochromators and a 450 W Xe lamp as the excitation source using the front face mode [Sharma *et al.*, 1999; Gauglitz *et al.*, 2003; Lakowicz, 2006] .

2.2.4. UV-Visible spectroscopy

Ultraviolet-visible spectrophotometry involves the spectroscopy of photons in the UV-visible region. The absorption in the visible ranges directly affects the color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer-Lambert law:

$$A = -\log 10\ (I/I0) = \in CL \qquad (2.1)$$

where A is the measured absorbance, I_0 is the intensity of the incident light at a given wavelength, I is the transmitted intensity, L the path length through the sample, and c the concentration of the absorbing species. For each species and wavelength, ε is a constant known as the molar absorptivity or extinction coefficient. UV –Visible studies on the different samples throughout the work was carried out by using Shimadzu UV –Visible spectrometer model UV 2100, Kyoto, Japan.

2.2.5. X-Ray Diffraction analysis (XRD)

X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined. 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 (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda = 2d \sin\theta$). X-ray powder diffraction is most widely used for the identification of unknown crystalline materials. In polymer science X-ray diffraction expertise is a key tool for understanding the properties of polymers and composites in relation to their solid-state structures. Crystalline materials give rise to the most obvious applications, but there is also important information to be obtained from semi-crystalline and even amorphous components. It is used to determine the

crystalline form, crystallinity, crystalline perfection, orientation etc. X-ray diffraction patterns were record on X-ray diffractometer, XPERT, Philips, Eindhoven, Netherlands, with Nickel filtered Cu K radiation ($\lambda = 0.154$ nm) at a voltage of 40 kV and current of 30 mA. The Degree of crystallinity of samples was quantitatively estimated by the following method. A smooth curve which connected peak baselines was plotted on the diffractograms. The area above the smooth curve was taken as the crystalline portion, and the lower area between smooth curve and the linear baseline in the samples was taken as the amorphous portion. The equation used for calculating degree of crystallinity from XRD is as follows:

$$\mathrm{Xc} = \frac{\mathrm{Ac}}{(Ac + Aa)} \times 100 \qquad (2.2)$$

where X_c refers to the degree of crystallinity, A_c is the crystallized area; A_a refers to the amorphous area on the X-ray diffractogram [Klug *et al.*, 1974; Aigbodion *et al.*, 2003].

2.2.6. Thermo Gravimetric and Differential Thermal Analysis (TGA/DTA)

This technique employs a thermo-balance, which continuously measures the change in sample weight with respect to temperature (at a controlled rate of heating or cooling) and time (in isothermal mode) in a specified environment. The observed weight loss in the polymer can be the result of volatile products formed by thermal degradation. The gravimetric estimation of moisture, volatile ingredients and inert or thermally stable additives in a polymer can be easily made by this technique. TGA was done on a

DTA-TG apparatus, Shimadzu DTG –60 simultaneous Japan, at a heating rate of 20 °C/min under nitrogen atmosphere.

2.2.7. Differential Scanning Calorimetry (DSC)

DSC is based on the principle of measuring the energy necessary to establish zero temperature difference between the test sample and reference material against either time or temperature under specified environment. Constant energy input is provided to heat both the sample and reference material at a constant rate. The sample may evolve or absorb energy as against the reference material, depending on the type of change, which can be exothermic and endothermic. The heat flow vs temperature (thermogram) is recorded for the run. DSC analysis was done by using a differential scanning calorimeter, model Perkin Elmer, Japan. Samples were placed in sealed aluminium cells and heated at a rate of 10 °C/min. under nitrogen. In this work Indium is used as the internal standard. Characteristic phase transition or melting temperature was determined from DSC curves [Pungor, 1994; Dean, 1995] .

In the case of starch DSC analysis was done as follows starch (about 3mg) was weighed in to an aluminium pan and moisture level was adjusted to 70-80 % by adding deionised water. The pan was hermetically sealed and left to equilibrate for 2h at room temperature. The samples were scanned at temperature from 0 to 130 °C at a rate of 10 °C/min. Gelatinization temperature was determined by automatically computing the initial temperature (T_i), maximum peak temperature (T_p), final temperature (T_f) and gelatinization enthalpy, ΔH from the resulting thermogram [Sandhu *et al.*, 2007] .

2.2.8. Rheological analysis

Rheology is the study of the flow of matter mainly liquids but also soft solids or solids under conditions in which they flow rather than deform elastically. The shear storage modulus (G'), loss modulus (G'') and complex viscosity (η^*) are the important parameters used for the analysis of gelation and gel strength. In the world of semi-solid material, rheological properties such as yield stress**,** thixotropy, and viscoelasticity have a direct impact on pharmaceuticals, foods, cosmetics, and consumer products. Semi-solid materials exhibit liquid and solid like properties depending on temperature, as well as on being subjected to stress and strain over time. Rheological instrumentation is used for various reasons, for example, to determine yield stress (stress that must be exceeded for flow to occur) to help predict shelf-life and strain sweeps to determine the critical strain (minimum energy needed to disrupt structure, where the higher the critical strain the better the systems is dispersed). Some of the commonly used tests for characterizing rheology of semi-solid materials are flow behavior of non-Newtonian materials (to determine yield stress, shear thinning and/or thixotropy), strain sweep (to determine viscoelasticity), critical strain (to determine how stable a system is), and creep/recovery (to determine relaxation times, zero shear viscosity and viscoelastic properties to determine strength of bonds)

Dynamic viscoelastic and steady flow properties of the freshly prepared gels, were measured at 30 ±1°C using a rheometer, Physica MCR 301, Anton Paar TA Instruments Inc., New Castle, DE, USA, equipped with a cone and plate geometry

sensor with gap width is adjusted to 0.5 mm. Stress sweep measurements were also performed to confirm the data within the linear viscoelastic strain region. After that, a dynamic frequency sweep was conducted by applying a constant strain of 1% which was within the linear region, over a frequency range between 0.1 and 100 rad/s. The storage modulus (G'), loss modulus (G''), and phase angle as a function of frequency (ω) were obtained. Temperature sweep experiments were carried out by changing the temperature from 30 °C to 90 °C and controlled precisely by a peltier system attached with the instrument. The moisture loss was prevented by covering the edge of the plate with a thin layer of light paraffin oil. The strain was taken as constant 0.5% and frequency sweep was at a rate of 1 rad/s.

2.2.9. Pasting Properties

Pasting properties of a material is determined by Rapid Visco Analyzer model RVA-4, Newport Scientific Warriewood, Australia. It is a computer-integrated instrument developed to determine the viscous properties of cooked starch, grain, batter and other foods. This instrument continuously measures apparent viscosity under variable conditions of shear and temperature. A variable heating rate primarily affects the events occurring early within the pasting profile (time to gelatinization, time to peak viscosity, peak viscosity), while changes in peak temperature influences viscosity attributes (trough viscosity, breakdown, final viscosity, total setback). Pasting characteristics were determined at a fixed starch concentration of 10 % (w/v) and at a constant speed 160 rpm using the standard profile. The temperature profile employed

was as follows. Heating from 50 °C to 95 °C at 12 °C/min holding at 95 °C for 2 min and then cooling at the same rate. The viscosity profile recorded by the RVA reflects the peak, trough and final viscosity, pasting temperature and peak time.

2.2.10. Texture Analysis

The Texture Profile Analysis set up is designed to measure the hardness, cohesiveness, springiness, gumminess, chewiness, fracture force, adhesive force, adhesiveness and springiness index of a sample. In our work the textural property of the gel was evaluated on a Food texture Analyser, model TADH stable microsystem, Godalming, Surrey, UK. Texture profile analysis (TPA) was performed at 25 ± 2 °C. Sample was taken in a glass petri dish of 30 mm diameter and 10 mm height. The texture profile was measured by compressing the gel about 4 mm (40% compression) under a cylindrical probe of diameter 25 mm (P/25) at a test speed of 1 mm/s and a control force of 5 g. From the texture profile curve, hardness (HA), cohesiveness (CO), adhesiveness (AD), and springiness (SP) was calculated using the computer software (SAS) accompanying the texture analyser.

The deformation level between 20% and 50% had been applied on starch gel food systems. Because under large deformation, the samples collapsed and invalid parameters were obtained. A crosshead speed (50 mm/min) was chosen, thus avoiding a total destruction of the gel structure in the first compression. This speed was also recommended to get values highly correlating with the sensory responses. Two replicate samples were tested.

2.2.11. Matrix-assisted laser desorption/ionization (MALDI-TOF)

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biopolymers and large organic molecules such as polymers, dendrimers and other macromolecules, which tend to be fragile and fragment when ionized by more conventional ionization methods. The ionization is triggered by a laser beam normally a nitrogen laser. A matrix is used to protect the molecule from being destroyed by direct laser beam and to facilitate vaporization and ionization. The matrix consists of crystallized molecules, of which the three most commonly used are 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), α-cyano-4-hydroxycinnamic acid (alpha-cyano or alpha-matrix) and 2,5-dihydroxybenzoic acid (DHB). A solution of one of these molecules is made, in a mixture of highly purified water and an organic solvent. The matrix solution is mixed with the analyte. The organic solvent allows hydrophobic molecules to dissolve into the solution, while the water allows for water-soluble (hydrophilic) molecules to do the same. This solution is spotted onto a MALDI plate. The solvents vaporize, leaving only the recrystallized matrix. The matrix and the analyte are said to be co-crystallized in a MALDI spot. The matrix absorbs the laser energy and it is ionized. The matrix is then transfer a part of its charge to the analyte molecules, thus ionizing them while still protecting them from the disruptive energy of the laser. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained on MALDI-TOF mass spectrometer, Shimadzu Biotech Axima CFR Plus Japan, equipped with nitrogen laser operating at 337 nm using 2,5-dihydroxy benzoic acid as the matrix

and 0.1% sample in trifluoro acetic acid. Each spectrum represents the accumulated data from 50 laser shots in linear mode at 12 kV accelerating voltage. Sample was mixed with matrix solution (saturated solution of 2, 5-dihydroxy benzoic acid in TFA: CH_3CN) and total 1 µl of this solution was applied to a stainless steel sample slide and dried under vacuum [Hillenkamp *et al.*, 1991; Schrepp *et al.*, 2003; Katalinic *et al.*, 2007] .

2.2.12. Scanning Electron Microscopy (SEM)

The scanning electron microscope is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. During this work the morphological studies were performed by using scanning electron microscope, JEOL make, model JSM 5600 LV, Japan, by sputtering the sample with gold to impart electrical conductivity and reduce charging artifacts and observed the surface morphology at 10 kV accelerating voltage.

2.2.13. Transmission Electron Microscopy (TEM)

Transmission electron microscopy is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected

by a sensor such as a CCD camera. High-resolution transmission electron microscopy (HRTEM) is an imaging mode of the transmission electron microscope (TEM) that allows the imaging of the structure of a sample at an atomic scale [Williams *et al.*, 1996]. Because of its high resolution, it is an invaluable tool to study nanoscale properties of the materials. For this study the morphology of the polysaccharide gel was examined by a high resolution transmission electron microscope, FEI, TECNAI, 30G2s-TWIN microscope. A thin layer of gel was coated on a carbon coated copper grid and dried under vaccum. This sample coated grid was used for the TEM analysis.

2.2.14. Atomic Force Microscopy (AFM)

The atomic force microscope is a very high-resolution type of scanning probe microscope, with demonstrated resolution of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The AFM consists of a microscale cantilever with a sharp tip (probe) at its end that is used to scan the specimen surface. The cantilever is typically silicon or silicon nitride with a tip radius of curvature on the order of nanometers. When the tip is brought into proximity of a sample surface, forces between the tip and the sample lead to a deflection of the cantilever according to Hooke's law. Depending on the situation, forces that are measured in AFM include mechanical contact force, Van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces etc. Typically, the deflection is measured using a laser spot reflected from the top surface of the cantilever into an array of photodiodes. The primary modes of operation are static (contact) mode and dynamic mode. In the

static mode operation, the static tip deflection is used as a feedback signal. Static mode AFM is almost always done in contact where the overall force is repulsive. Consequently, this technique is typically called contact mode. In contact mode, the force between the tip and the surface is kept constant during scanning by maintaining a constant deflection. In this work the surface morphology of polysaccharide gel was examined by atomic force microscopy, NanoScope IIIa from Digital Instrument, USA, operated in the semi contact mode. AFM scans of the surface were performed with a scan rate of 0.5 Hz. A very thin layer of gel was placed on a freshly cleaved mica sheet. Vaccum dried and analysed.

2.2.15. Fluorescent Microscopy

A fluorescence microscope is a light microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption [Rost, 1991; Bradbury *et al.*, 1996]. The specimen is illuminated with light of a specific wavelength which is absorbed by the fluorophores, causing them to emit longer wavelengths of light. The illumination light is separated from the much weaker emitted fluorescence through the use of an emission filter. Typical components of a fluorescence microscope are the light source (xenon arc lamp or mercury-vapor lamp), the excitation filter, the dichroic mirror (or dichromatic beam splitter), and the emission filter. Fluorescent nature of the gel was examined by using a Leica DM 2500 instrument, Germany.

2.2.16. Mechanical properties

Mechanical properties of polysaccharide films include tensile strength and elongation or flexibility of the material. The tensile strength of a material quantifies how much stress the material will endure before it breaks. In general tensile strength increases with polymer chain length and crosslinking of polymer chains [Meyers *et al.*, 1999] . Elongation describes the extent to which the specimen stretched before fracture. All mechanical tests were performed using a Universal Testing Instrument Model H5KS (Tinius Olsen, Horsham, PA, USA) fitted with a 100N static load cell. The films were cut into strips 10 mm wide and 100 mm long and mounted between cardboard grips (200- 300 mm) using adhesive so that the final area exposed was 10- 50 mm. A minimum of four strips were prepared from each film. The tensile properties of the films were measured according to the standard testing method at a crosshead speed of 50 mm/min and extension of 100 mm and the initial grip separation was 50 mm.

2.2.17. Contact Angle measurements

Hydrophobicity/non-wettability is a highly desirable property for materials that are used in a wide range of applications. The contact angle is the angle at which a liquid/vapor interface meets the solid surface. The contact angle is specific for any given system and is determined by the interactions across the three interfaces. Contact angle measurements were conducted with a video-based contact angle measurement device Data Physics OCA15 PLUS (Germany) by sessile drop method using 3µL of

distilled water. The imaging software used for the study was SCA20 software (Germany). The reported contact angles are the average of five measurements.

CHAPTER 3

Physico chemical rheological and thermal properties of njavara rice (Oryza Sativa) starch

An investigation on the morphological, physicochemical, and thermal properties of Njvara rice starch was carried out in this chapter. The properties were compared with native chamba rice starch and were found to be different from the native chamba variety rice starch.

3.1. Introduction

Starch is a reserve carbohydrate in the plant kingdom that world wide 70–80% of the calories consumed by humans, and is generally deposited in the form of minute granules or cells ranging from 1 to 100 μm or more in diameter in the storage organs [Wurzburg, 1986; Zobel *et al.*, 1995; Whistler *et al.*, 1997; Buleon *et al.*, 1998; Freitas *et al.*, 2004]. Rice is one of the most important cereals and more than two thousand varieties of rice are grown commercially throughout the world and is the main food item in Asia. Starch is the main component in rice, and as isolated from endosperm, has been used for food and nonfood applications in many forms. It has also been used to produce modified starch, such as cross-linked, substituted, acid-thinned, bleached, and oxidized starches [Xie *et al.*, 2008]. Rice (*Oryza sativa*) starch has many unique attributes that make it one of the most interesting starches in the food industry. Rice starch is hypoallergenic, bland in taste, white in colour and, as a gel, is smooth in texture. However, rice starch, in common with other cereal starches, has negative aspects, such as gel syneresis, retrogradation, and tendency to exhibit breakdown, either from extended cooking, high shear or acidic conditions, producing weak-bodied, cohesive, rubbery pastes, and undesirable gels [Viturawong *et al.*, 2008]. The Ayurvedic Treatise (Indian Materia Medica) records show the existence of many medicinal rice varieties in India. Njavara is the unique rice, short duration cultivar grown only in certain pockets in Kerala state, south India [Deepa *et al.*, 2008] and belongs to the family Oryza. This is the only cultivar traditionally used effectively in

the Ayurvedic system of medicine in certain specific treatments like Panchakarma. It is interesting to note that this treatment is now getting more and more popular, not only in this region of the country but also in other parts of the nation and even in other countries. Njavara as a special cereal, reported to have properties to rectify the basic ills affecting the circulatory, respiratory as well as the digestive system. Black glumed njavara has been used in Ayurveda treatment from the age of Charaka-ie, BC.600. njavara kizhi and njavara theppu are two major treatments in Ayurveda system of medicine in conditions of arthritis, paralysis, neurological complaints, degeneration of muscles, tuberculosis, for children with Anemia, for women during lactation, in certain ulcers, and skin diseases. Njvara rice endosperm has around 73 % of starch.

Starches exhibit difference in various properties in accordance with the source and genotype. The properties mainly depend on physical and chemical characteristics such as granule size, amylose amylopectin ratio, the shape and size of starch granules which is characteristics of their botanical origin etc [Madsen *et al.*, 1996]. Starch exhibits unique viscosity behaviour with change of temperature, concentration and shear rate [Lii *et al.*, 1996]. Many researchers have used the dynamic rheometer for studying the viscoelastic or rheological properties of starches [Tsai *et al.*, 1997]. The rheological properties of different starches vary to a large extent as a function of granule structure and physico chemical composition. Several rheological changes occur in starch, when starch–water systems are heated above the gelatinization temperature, the starch granules loose their crystallinity, absorb large amounts of water, and leach out

amylose, thereby forming a paste composed of swollen starch granules dispersed in an amylose matrix [Ring, 1985]. Starch gelatinization refers to the disruption of the molecular order within starch granules when they are heated in the presence of water. Evidence for the loss of an organized structure includes irreversible granule swelling, loss of birefringence and crystallinity. Gelatinization is an energy-absorbing process that can be followed by differential scanning calorimetry [Whistler *et al.*, 1997] .

We have investigated into the unique properties of this njavara rice compared to common chamba variety of rice, which is the staple food of kerala and has found out the unique physico chemical, thermal, rheological and textural characteristics of this starch which makes it preferred rice for the pancha karma treatment.

3.2. Experimental

3.2.1. Materials

Njavara (Black glumed) and chamba (Jaya) rice were purchased from the local market, Trivandrum, Kerala, India. Protease and amylase enzyme were purchased from Sigma Aldrich, USA.

3.2.2. Methods

Properties of njavara starch and native chamba starch were studied by scanning electron microscope, TGA and DSC, Rapid visco analyzer, Rheometer, Food texture analyser, X-ray diffraction pattern etc.

3.2.2.1. Starch isolation

Njavara rice and Chamba rice starch was extracted as described in chapter 2.

3.2.2.2. Amylose content

Njavara amylose was separated by alkali leaching, purified and used as a standard. For this 10 g starch was dispersed in 50 ml water. This dispersion is added to the 0.5N 500 ml NaOH solution. The temperature was maintained as 25 ºC – 28 ºC, kept this alkaline solution for 5minutes with magnetic stirring. Then 100 ml 5% (w/v) NaCl solution was added and then the dispersion was neutralized with 1N HCl to pH 6.5 to 7.5. Kept this solution for settling down for 15 h, the gel settles and it occupies about 1/3 rd of the total volume. The supernatant was the amylose which was precipitated by ethanol and purified.

Amylose content of njavara rice was determined according to the procedure of Sowbhagya and Bhattacharya [Sowbhagya *et al.*, 1971]. According to this method accurately weighed 100 mg of the starch was mixed with 1 ml alcohol. 10 ml (0.1N) of sodium hydroxide solution was added, and left overnight at room temperature. Alternatively the mixture may be intermittently heated in a boiling water bath for a few minutes till the starch is dispersed, cooled and diluted to 100 ml. Mixed thoroughly and pipetted 5ml into a 100ml volumetric flask added 3 drops of phenolphthalein and about 50 ml of distilled water. Added dilute hydrochloric acid drop wise with shaking till the colour is just discharged. Added 2 ml 1% (w/v) iodine solution and made up to

volume with boiled water. Read the solutions after 30 min in a spectrophotometer at 600 nm against the blank.

3.2.2.3. Solubility and swelling power

Solubility and swelling power at various temperatures such as 60°C, 70°C, 80°C, 85°C and 90°C were determined [Whistler, 1964]. Starch (0.1 g) was taken in a previously tared bottle and sufficient distilled water was added to give a total volume of water equivalent to 9 g. Heated in a water bath for 30min at any constant temperature (60°, 70°, 80°, 85°, 90 °C) with magnetic stirring. After heating, the bottle was rinsed with distilled water to bring the volume to 10 g. Centrifuged the sample at 313 xg for 15 min. The clear supernatant, 5ml was drawn off into a clean dry dish. The dish was dried in a vaccum oven for 4 h at 120 °C. Cooled in a desicator and weighed. The supernatant was completely decanted and the swollen granules were weighed. Percentage solubility and swelling power were calculated using the formula

$$\% \text{ Solubility} = \frac{\text{Dry weight}}{\text{Sample weight}} \times 100 \tag{3.1}$$

$$\text{Swelling Power} = \frac{\text{weight of swollen granule}}{\text{Sample weight (100 - \% solubility)}} \times 100 \tag{3.2}$$

3.2.2.4. Light transmittance or gel clarity

Starch suspensions (0.6% w/v in DMSO) were placed in a water bath at 90 °C for 30 min and agitated well and then cooled to room temperature. The percentage transmittance of this gelatinized starch was determined at 640nm using a

spectrophotometer. The gel clarity was studied with time duration (2h, 4h, 6h, 24h, 48h, 72h) [Bello-Perez *et al.*, 1999] .

3.2.2.5. Freeze thaw stability

50 ml of 6 % (w/v) starch solution was heated to 95 °C and was held at this temperature for 15 min and the gel was cooled down to room temperature. The gel was frozen at < 0°C over night and thawed to room temperature and then centrifuged at 604 xg for 15 min and measurements was taken for separation of water (syneresis) from the starch gels and the process was repeated for 5 cycles.

3.2.2.6. Retrogradation properties of starch

After conducting the thermal analysis, the samples were stored in the refrigerator at 4 °C for 7 days for retrogradation studies. These samples were left at room temperature, 28 °C for 2 hour before analysis. Reheated at the rate of 10 °C/min from 0 to 130 °C. The enthalpies of retrogradation was calculated automatically and % retrogradation was calculated as

$$\% \, R = \frac{\text{Enthalpy of retrogradation}}{\text{Enthalpy of gelatinization}} \times 100 \qquad (3.3)$$

3.2.2.7. Enzymatic hydrolysis of starch

The digestibility of starches was studied using the enzyme alpha amylase from *Bacillus amyloliquifaciens*. Starch (25% w/v) was gelatinized in 0.01M phosphate buffer of pH-7.0 in a boiling water bath at 100 °C. The starch gel was then cooled to

80 °C. Incubated the gel at 80 °C for 30 min with 200 units of enzyme and 50 ppm of calcium chloride. After the digestion, the reducing sugar was determined from an aliquot of this sample. DNS was used as the reagent and the absorbance was read at 540 nm using a UV-Vis spectrophotometer.

3.3. Results and discussion

3.3.1. Morphology of the starch granules

The rice starch granules are found to be hexagonal in shape, which is typical in many of cereal starch. SEM shows that the njvara starch granule has diameter in the range of 5 – 6 µm, where as rice starch granules was smaller, and had a size of 1-2 µm (Figure 3.1).

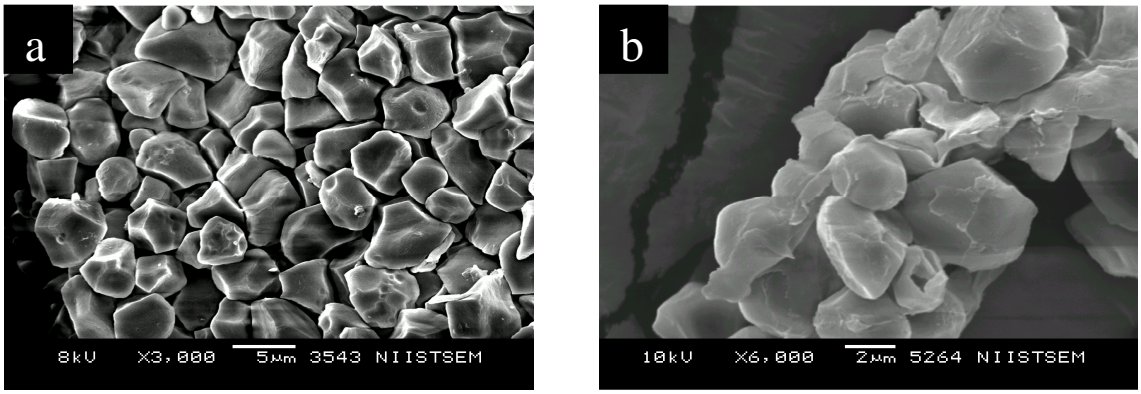


Figure 3.1. SEM image of a) Njavara starch granule, b) Native chamba starch granule.

The variation in starch granule morphology may be due to the biological origin and physiology of the plant and the biochemistry of the amyloplast. This may be also due to the variations in the amylose and amylopectin content and its structure, which in turn play an important role in the control of the starch granule size and shape [Svegmark *et al.*, 1993; Kaur *et al.*, 2007]. Variation in the activity of enzyme, such as

granule bound starch synthase during growth have also been reported to affect the starch granule morphology in potatoes.

3.3.2. Amylose content

Amylose content in njavara rice starch was found to be 20 ± 2 % and was similar to the native rice varieties were the amylose content is in the range of 20-22% [Deepa *et al.*, 2008] .

3.3.3. Swelling power and solubility

Swelling power and solubility of rice starches at different temperatures were studied. Results were shown in Figure 3.2. In the case of njavara starch, the swelling power increases steeply over the range of temperature studied. The pattern shows that njavara starch swelled rapidly at about 76°C and it exceeds the swelling of native rice starch granules and this starch granule has a comparatively sturdy granule structure. The swelling is due to the breaking of intermolecular hydrogen bonds in the amorphous region of the granule that allows irreversible and progressive water absorption [Bello-Perez *et al.*, 1999] . In the case of native rice starch, swelling pattern is less steep and shows a constant increase in swelling power between 60 °C and 76 °C than njavara. After 76 °C, the swelling power value is higher for njavara. The low swelling power of njavara starch below 76°C is mainly due to its higher gelatinization temperature. It is reported that the swelling power is positively correlated to gelatinization temperature, amylopectin chain length and amylose content [Sasaki *et al.*, 1998].

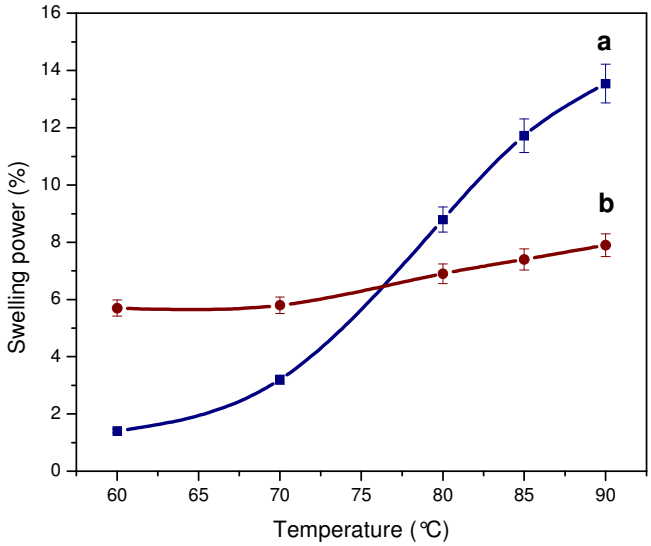


Figure 3.2. Swelling power of (a) njavara starch, (b) native rice starch.

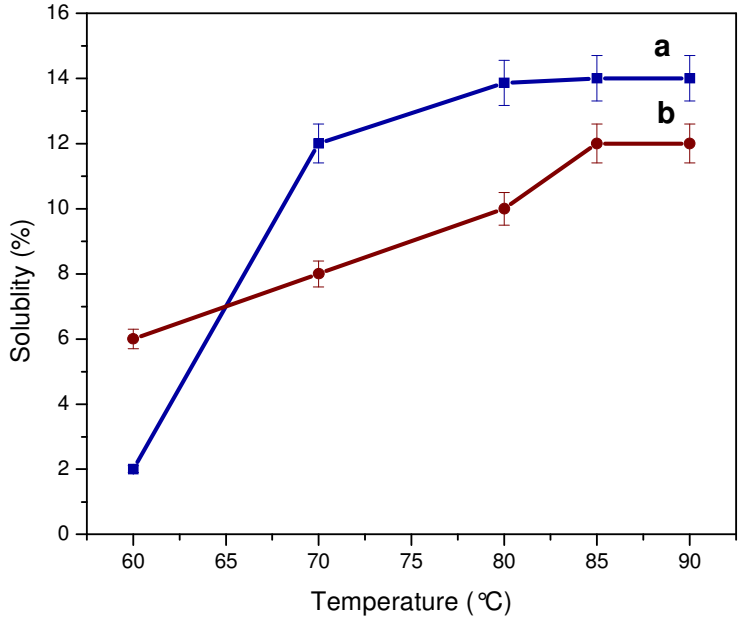


Figure 3.3. Solubility of (a) njavara rice starch and (b) native rice starch.

Solubility of njavara starch shows a sudden increase from 60°C and solubility increases rapidly up to about 68 °C then slowly between 68 °C -78 °C (Figure 3.3).

Then it becomes a constant. Solubility of native rice starch increases rapidly between 70 °C -80 °C. Above 76 °C it shows a slow increase. The solubility is due to the leaching of amylose chains from the amorphous part of the swollen granules. Swelling power and the percentage soluble are higher in njavara rice at 76 °C. Below 76 °C, it shows lower values than native starch. The higher swelling power at a higher temperature makes this starch unique and provides application for panchakarma in Ayurveda system of medical treatment.

3.3.4. Starch gel clarity

Njavara rice starch gel had less transmittance value (87.45 %) compared to native rice (95.30 %) (Figure 3.4), because of the relatively higher granule size of the starch. The % transmittance of gel decreases with an increase in storage time, due to the retrogradation of starch. In the case of njavara starch gel, the decrease in transmittance was sharp up to 6 h then it remained almost constant. However the native rice starch lost its clarity significantly after 2 h. The degree of transmittance is directly correlated to the water absorption capacity. The njavara rice starch has low water absorption capacity at lower temperatures compared to the native rice starch and this may be the reason for the lower clarity of starch gel. In njavara, clarity of the gel is not much affected by the storage time, where as in the case of native rice starch, the gel becomes opaque after 2 h due to rapid retrogradation.

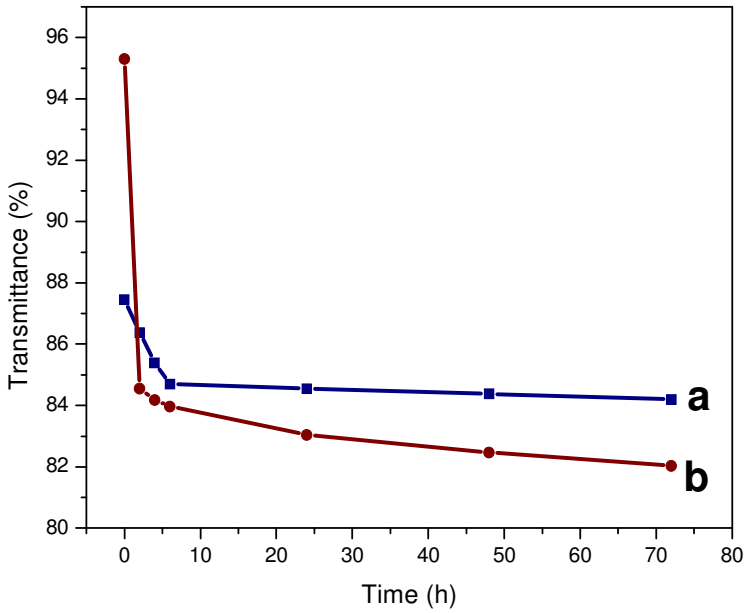


Figure 3.4. Gel clarity of (a) njavara rice starch and (b) native rice starch.

From the studies it was seen that the clarity of gel from njavara starch is more stable than rice starch and hence can be used with advantage in food industry.

3.3.5. Freeze Thaw stability

Freeze–thaw stability is an important property that is used to evaluate the ability of starch to withstand the undesirable physical changes that may occur during freezing and thawing. This property may be simply evaluated by gravimetric measurement of the water of syneresis that separates from starch pastes or gels and the multiple freeze–thaw cycles that involve subjecting samples to repeated freezing and intermittent thawing to room temperature over a period of 2–4 h, are known to drastically accelerate retrogradation and syneresis. In the freezing process, when starch pastes or gels are frozen, phase separation occurs upon formation of ice crystals. Upon thawing,

a phenomenon known as syneresis occurs, the water can be easily expressed out from the dense gel network. Repeating the cycle of freezing and thawing enforces the phase separation and ice growth. As the ice crystals become larger, the syneresis and sponge formation occurs more readily. Syneresis in freeze–thawed gel is due to the increase of molecular association between starch chains, in particular retrogradation of amylose, expelling water from the gel structure.

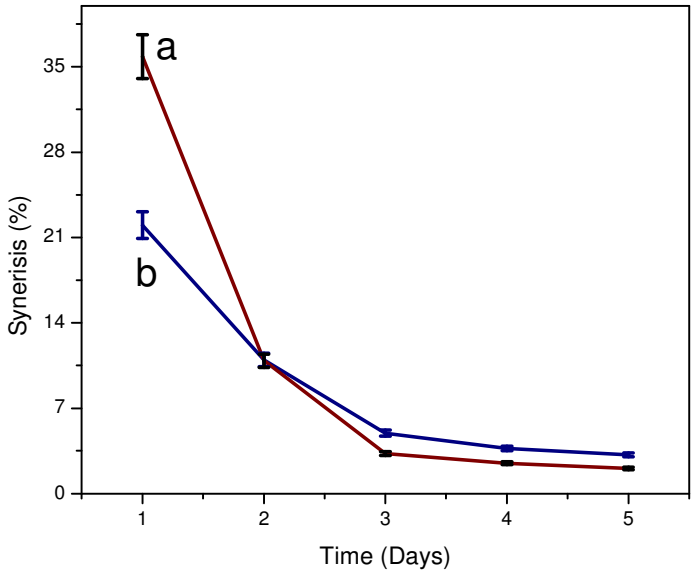


Figure 3.5. Freeze thaw stability of (a) njavara rice starch and (b) native rice starch.

Thus the amount of water due to syneresis of the gel is a useful indicator for the retrogradation tendency of starch [Saartrat *et al.*, 2005]. It is well known that when a starch gel is frozen, starch-rich regions are created in the matrix, where water remains partially unfrozen..

High solid concentration in the regions facilitates the starch chains to associate forming thick filaments, whereas water molecules coagulate into ice crystals forming a

separated phase. These effects contribute to a spongy structure of the gel [Ferrero *et al.*, 2000; Lee *et al.*, 2002; Arunyanart *et al.*, 2008]Freeze thaw stability of both the starch gels has same pattern as shown in Figure 3.5. Njavara starch gel had low syneresis compared to the native rice starch gel and the % syneresis is inversely related to the stability of the gel. Starch with high syneresis, readily absorb and eliminate water like a sponge. Njavara rice starch separate less water at the first cycle than the native rice starch. Maximum water was separated in the first cycle then it was decreased over a time. Compared to other starches, rice starch shows low freeze thaw stability. Low synerisis rate of njavara starch indicates its superior quality for its possible application in food industry and panchakarma in Ayurveda system of medicine.

3.3.6. Differential scanning calorimetry

The gelatinization properties of the two starches were measured by differential scanning calorimetry, and the DSC thermogram of njavara and native rice starches were depicted in Figure 3.6. Compared with native rice starch, njavara starch exhibited higher gelatinization temperatures. The starch gelatinization temperature depends on the particle size distribution, where small granules usually have lower gelatinization temperature values than the large granules. However, there are conflicting reports about gelatinization enthalpy for wheat starch A and B type granules.

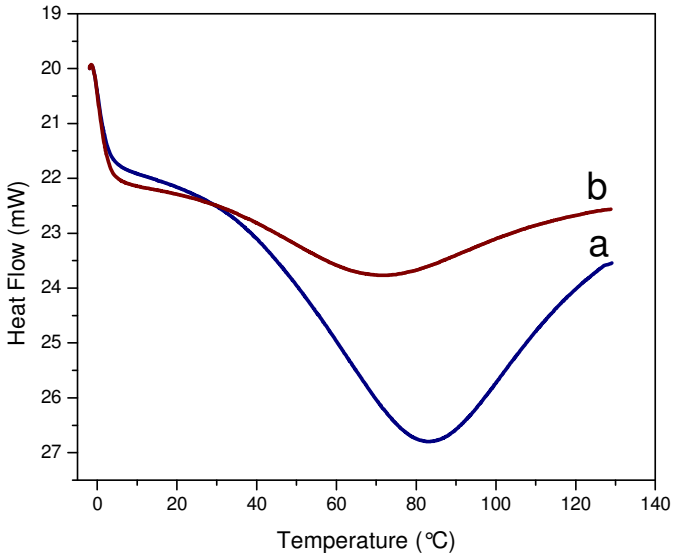


Figure 3.6. Differential scanning calorimetry of (a) njavara starch, (b) native rice starch.

Some studies claimed that A-type starch possess higher gelatinization enthalpy than B-type granules [Peng *et al.*, 1999; Chiotelli *et al.*, 2002], whereas, Wong and Lelievre reported that A-type granules possess a lower gelatinization enthalpy [Wong *et al.*, 1982; Xie *et al.*, 2008]. Here we studied an A type rice starch. Gelatinization endotherm of native rice starch was broader than njavara. The melting temperature range gives an indication of the quality and heterogeneity of the recystallized amylopectin. Thus, a wide melting range might imply a large amount of crystals of varying stability, whereas a narrow range could suggest crystals of a more homogeneous quality and similar stability [Fredriksson *et al.*, 1998]. Njavara rice had high gelatinization temperature (85 °C) than the native starch (70 °C). Njavara starch required more energy to gelatinize since its enthalpy of gelatinization was very high (ΔH=366.17j/g). The enthalpy of gelatinization of native rice starch was lower and is

61.82j/g. The difference in gelatinization temperature among the rice starch may be influenced by the factors like granular architecture and molecular structure of amylopectin [Gunaratne *et al.*, 2002]. The rice starch particles have crystalline region within a starch granule that is composed of small crystallites with different crystal melting temperature which mainly consists of amylopectin [Vasanthan *et al.*, 1996].

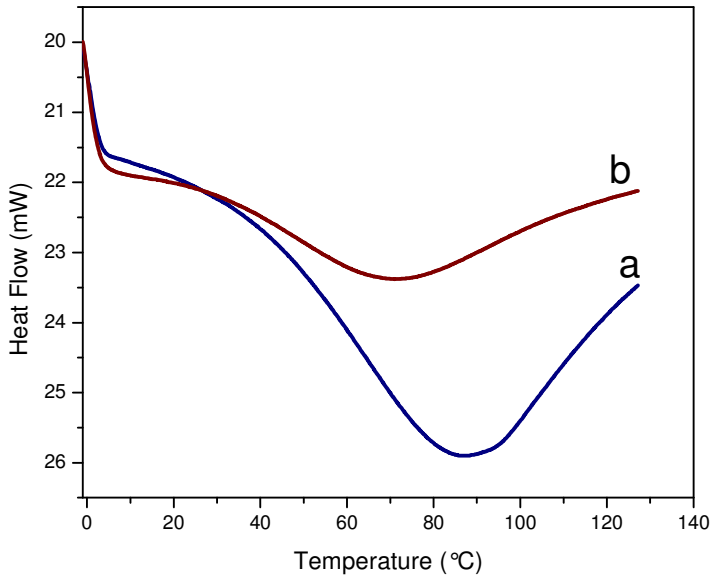


Figure 3.7. Differential scanning calorimetry of (a) njavara starch, (b) native rice starch after retrogradation.

The retrogradation properties of njavara and native rice starches were studied after storage of gelatinized starches at 4 °C for 7 days. Starch retrogradation is caused by the molecular interaction after cooling of the gelatinized starch paste. During the retrogradation, amylose forms a double helical association, whereas amylopectin forms a crystalline region by the association of the outermost short branches [Ring, 1985]. The values of enthalpy of retrograded starch provide a quantitative measure of energy

transformation that occurs during the melting of re-crystalline structure. Retrogradation peak of njavara starch was shifted in the higher temperature regions. (87 °C) and native rice starch shows the similar pattern as in the case of gelatinization endotherm (70 °C) (Figure 3.7). The amylopectin and the intermediate materials play a significant role in starch retrogradation during refrigerated storage. Recrystallization of amylopectin branch chains has been reported to occur in less ordered manner in stored starch gels than in native starches [Ward *et al.*, 1994]. The percentage of retrogradation (%R) was less for njavara starch (85 %) as compared to native rice starch (90.56 %).

3.3.7. Thermogravimetric analysis

Njavara and native starch was subjected to thermal degradation. Thermogravimetric plot of njavara starch and native starch were shown in Figure 3.8. In the case of njavara starch, degradation starts at a temperature of 275 °C. But for native starch the degradation temperature starts at a lower temperature of 225 °C. This showed that njavara starch was thermally more stable than native rice starch. Temperature at which 5% weight loss of njavara starch and native rice starch are at 59 °C, and 62 °C respectively. Temperature corresponding to 10% weight loss of njavara and native rice starch was 85 °C (njavara) and 90 °C (native rice).

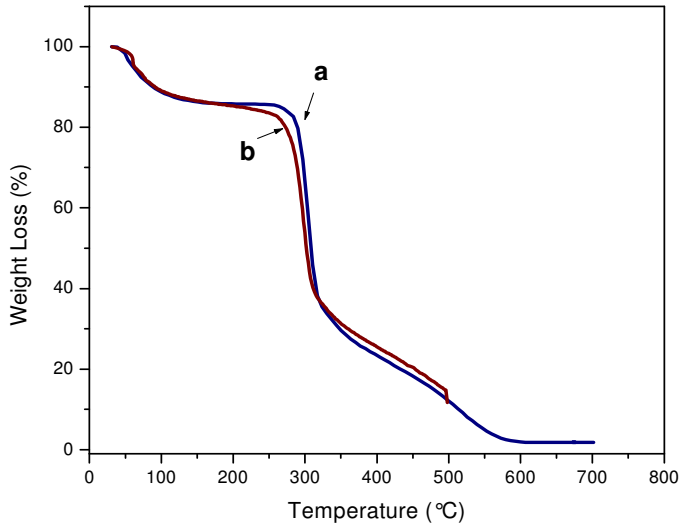


Figure 3.8. Thermogravimetric analysis of (a) njavara starch, (b) native rice starch

3.3.8. Pasting property

Pasting properties are reported to be influenced by granule size, amylose/ amylopectin ratio, starch molecular characteristics and the condition of the thermal process employed to induce gelatinization [Zhou *et al.*, 1998]. Pasting profile of both rice starches are similar and behaves as a typical cereal starch. The results are presented in Figure 3.9. Njavara starch showed higher peak viscosity value (957 cP) than native rice (632 cP), mainly due to the bigger granule size which in turn increases the swelling ratio and viscosity. Final viscosity of these starches followed the same pattern (Final viscosity of njavara is 1054 cP and native chamba rice is 736 cP). Peak viscosity is a measure of the water holding capacity of the starch in terms of the resistance of swollen granules. The breakdown viscosity is regarded as a measure of degree of

disintegration of granules and shows paste stability. During breakdown, the granules are disrupted and amylose molecule will generally leach out in to the solution.

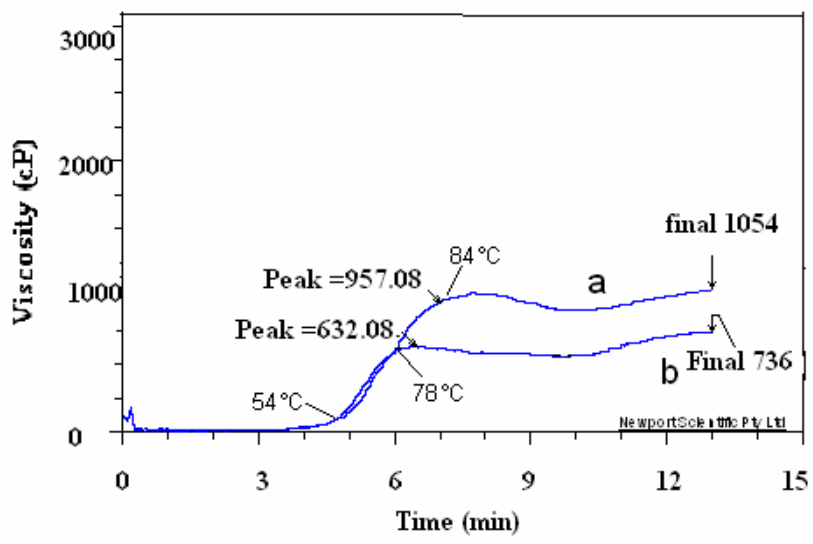


Figure 3.9. Pasting profile of (a) njavara starch, (b) native rice starch

Heating beyond the peak viscosity temperature provides further energy to break down the residual crystalline structure, causing the viscosity to decrease. Njavara starch showed higher breakdown value (324 cP) than native starch (82 cP). The setback viscosity of njavara (421 cP) is higher due to high peak viscosity and amylose content. The set back is the viscosity increase resulting from the rearrangement of amylose molecules that have leached from the swollen starch granules during cooling and is generally used as a measure of the gelling ability or retrogradation tendency of the starch [Abd Karim *et al.*, 2000]. Njavara starch had higher set back viscosity while the native chamba rice starch showed the least. The starting of gelatinization for both njavara and native starch were found to be the same (54 °C). But the pasting

temperature of njavara rice starch was higher and found to be 84 °C, where as native rice starch shows pasting temperature of 78 °C. These values are close to the gelatinization temperature obtained by DSC analysis.

3.3.9. Enzyme digestability

The digestibility of the starch is an important nutritional factor. The gelatinized starches were hydrolysed using bacterial alpha amylase. Both njavara and native starch are not highly digestible and showed the same rate of hydrolysis. Only 14% of starch was hydrolysed in 30 minutes of incubation at pH 7.0, at 80 °C. The enzyme digestibility mainly depends upon the interplay of many factors such as starch source, granule size, amylose amylopectin ratio etc. But here the granule size also did not affect the alpha amylolysis of the starch.

3.3.10. Rheological properties

3.3.10.1. Effect of temperature

Effect of heating of rice starch dispersion at constant rate of temperature on shear modulus is shown in Figure 3.10. Both starches shows same pattern of shear modulus against temperature. Up to a certain temperature both moduli (storage [G′] and loss [G″]) were same. After this storage modulus shows a sharp increase reaches a maximum then decreases. The temperature corresponds to higher storage modulus (G'_{max}) is the gelatinization temperature. In njavara and native chamba rice these gelatinization temperature is different. Njavara rice gelatinizes at higher temperature of 68°C and the gelatinization starts at 59 °C. But in native starch, gelatinization

temperature was found to be 58 °C and gelatinization starts at a lower temperature of 48 °C.

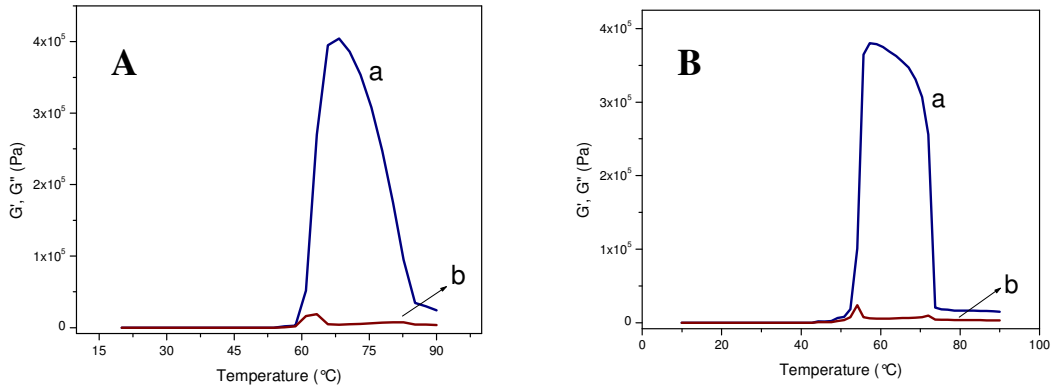


Figure 3.10. Rheological analysis. Temperature dependence of (A) njavara starch, (B) native rice starch against moduli.(a- storage modulus, b- loss modulus)

Higher value of G′ compared to G″ indicates the formation of the gel. The significant increase in G′ value of rice starch on heating is caused by formation of a three dimensional gel network developed by leached out amylose and reinforced by strong interaction among the swollen starch particle [Biliaderis *et al.*, 1980] . Further heating beyond G′ max, the *G′* decreased significantly, indicating damage of gel nature during prolonged heating. The complete loss of gel nature of njavara starch occurs at higher temperature compared to the native rice starch. This indicates the stability of njavara starch gel with temperature. The damage of structure could be due to the 'melting' of the crystalline regions remaining in the swollen starch granule or resulted from the disentanglement of the amylopectin molecules in the swollen particles.

3.3.10.2. Frequency dependence

The dynamic rheological properties G' and G'' are presented as a function of frequency in Figure 3.11. The G', G'' and complex modulus (G^*) decreased in the order $G^* > G' > G''$, as commonly observed for normal starch gels. The frequency dependence of G' and G'' gives valuable information about structure. A material that is frequency independent over a large time scale range is solid-like; a true gel system is such a material. In contrast, strong frequency dependence suggests a material structure with molecular entanglements that behaves more like a solid at higher frequencies and more like a liquid at lower frequencies. When swollen particles are subjected to shear oscillation at a certain angular frequency and amplitude, G' and G″ is proportional to the dissipated or lost energy during the oscillation process. It can be seen from the figure that with increasing frequency G' and G'' gradually increase. G' is higher than the G'' in the whole frequency range, the elastic behavior of the sample predominates over its viscous behavior and the swollen sample exhibits mechanical rigidity. Both rice variety show the same pattern of modulii. But the difference between the G' and G'' value is more in njavara starch compared to the native rice and also in the case of njavara starch, the storage modulus is frequency independent. This indicates that the gel rigidity is higher in the case of njvara starch gel. For a true gel the storage modulus is always higher than loss modulus and is independent throughout the experiment. This property is very useful for the application of gels in food industries, adhesives, and drug delivery etc.

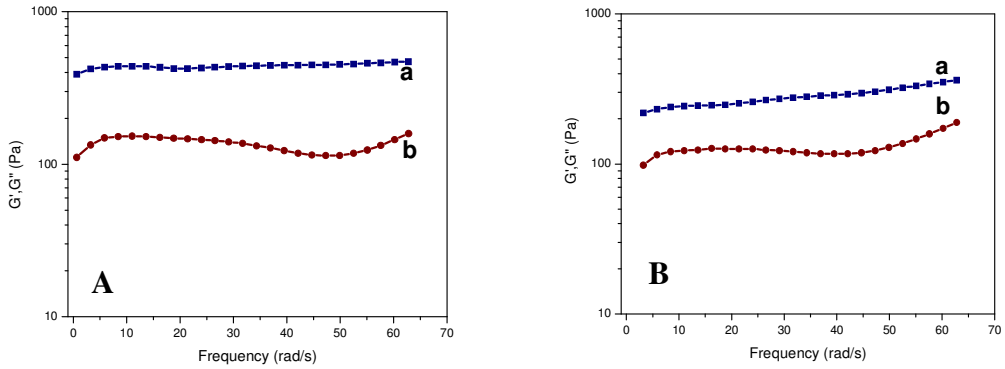


Figure 3.11. Rheological analysis. Frequency dependence of (A) njavara starch, (B) native rice starch against moduli. (a- storage modulus, b- loss modulus)

The ratio of loss and storage moduli (loss tangent or tan δ) or phase angle is a measure of the energy lost compared to energy stored in deformation. Lower tan value of njavara indicates the formation of strong gel compared to native rice starch. The results were shown in Figure 3.12. Torque is the force required to rotate the sample. Figure 3.13 shows the variation of torque with frequency. Both rice shows similar pattern. Torque increases with frequency to a certain limit then it decreases. This may be due to the distortion gel structure at higher frequency. Njavara rice required more force to rotate the sample compared to native rice starch. Complex viscosity or the frequency dependent viscosity decreases with increase in frequency (Figure 3.14).

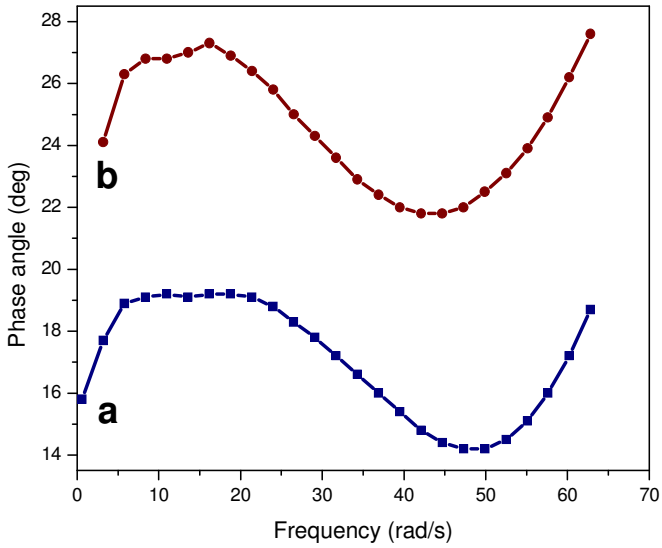


Figure 3.12. Rheological analysis. Phase angle against frequency (a) njavara rice starch, (b) native rice starch.

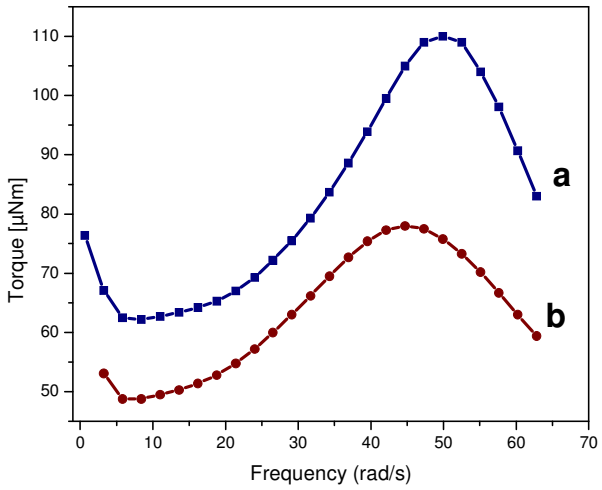


Figure 3.13. Rheological analysis. Torque against frequency of (a) njavara starch, (b) native rice starch.

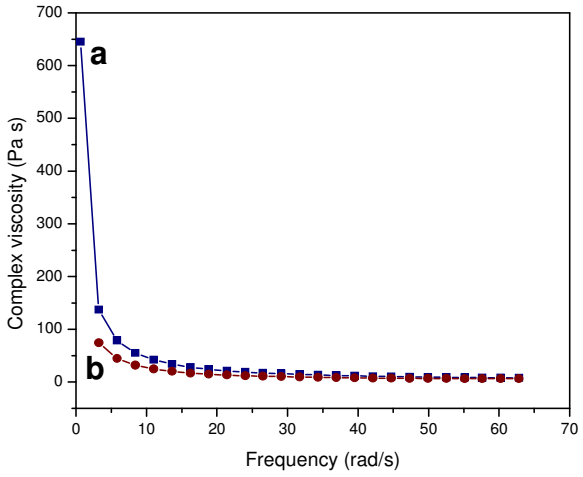


Figure 3.14. Rheological analysis. Complex viscosity against frequency (a) njavara starch, (b) native rice starch.

Both njavara and native rice starch showed decrease in viscosity with shear. These results showed that njavara rice behaves as a non Newtonian fluid with pseudoplastic nature as in the case other starches (Figure 3.15). Yield stress is the stress at which a material begins to deform plastically. Above the yield stress the material act as elastic and will return to its original shape when the applied stress is removed. Once the yield stress point is crossed, the deformation will be permanent and non-reversible. The yield stress of njavara rice starch was found to be 18.384 Pa. But in the case of native rice starch yield stress was low (13.762 Pa), Figure 3.14 and 3.15. This indicates the high gel strength of njavara starch against stress.

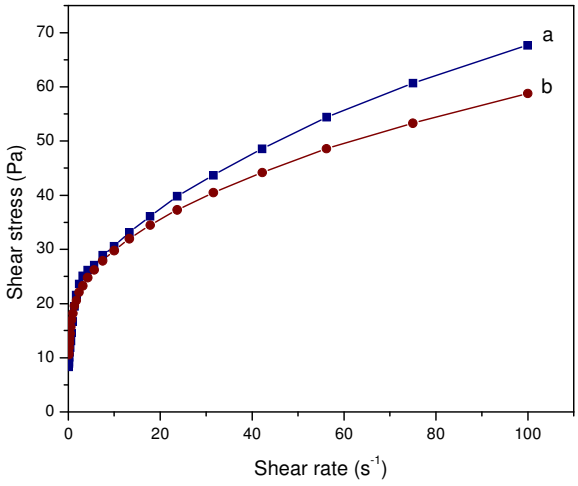


Figure 3.15. Rheological analysis. Newtonian behaviour of (a) njavara starch, (b) native rice starch.

All rheological analysis showed that njavara staches have good properties which can be applicable to food industries, medical field, adhesives, compared to native rice starch. Its high gel strength, heat capacity and high gelatinization temperature may be the reason for its wide application in Ayurveda treatments.

3.3.11. Texture profile analysis

The hardness (HA), adhesiveness (AD) springiness (SP), cohesiveness, and chewiness of gels from njavara and native rice starches were studied. The textural properties were influenced by starch granule size. The hardness of gelatinized starch gel has related to the amylose matrix and the filling effect of the swollen granules [Morris, 1990]. Hardness of njavara starch gel (0.289N) is less than the native rice starch gel (0.391N). Adhesiveness is a surface characteristic and depends on a combined effect of adhesive and cohesive forces, and also viscosity and viscoelasticity. The negative value for

adhesiveness of both rice starches were -0.4041 for Njavara and for native rice starch the value is -0.3231. Springiness or elasticity is a sensitivity of gel rubberiness in the mouth, and is a measure of how much the gel structure is broken down by the initial compression. High springiness appears when the gel structure is broken into few large pieces during the first texture profile analyzer compression, whereas low springiness results from the gel breaking into many small pieces. Less springy gels will break down more easily during mastication than a firm and springy gel. Njavara rice starch (6.9368) showed 4 times higher value for springiness compared to the native rice starch (1.7849). Chewiness is the quantity to simulate the energy required for masticating a semi-solid sample to a steady state of swallowing process. It is the product of gumminess and springiness. Chewiness of njavara rich gel (1.2923) was three times higher compared to native rice starch (0.4017). Cohesiveness is an index that how well the product withstands a second deformation relative to its behaviour under the first deformation. Both the starch gel did not have much difference in cohesiveness, even though the starch gel of Njavara rice showed slightly higher value than native rice. Texture profile analysis showed that njavara starch had good springiness nature. These properties may be very useful in food industries.

3.3.12. XRD analysis

Native starches generally exhibit two main crystalline types, namely, the A-type for cereal starches and the B-type for tuber and amylose-rich starches. The X-ray patterns

of A type starches give the stronger diffraction peaks at around d-values (5.9, 5.2, 4.9 & 3.84 Å) [Buleon *et al.*, 1998].

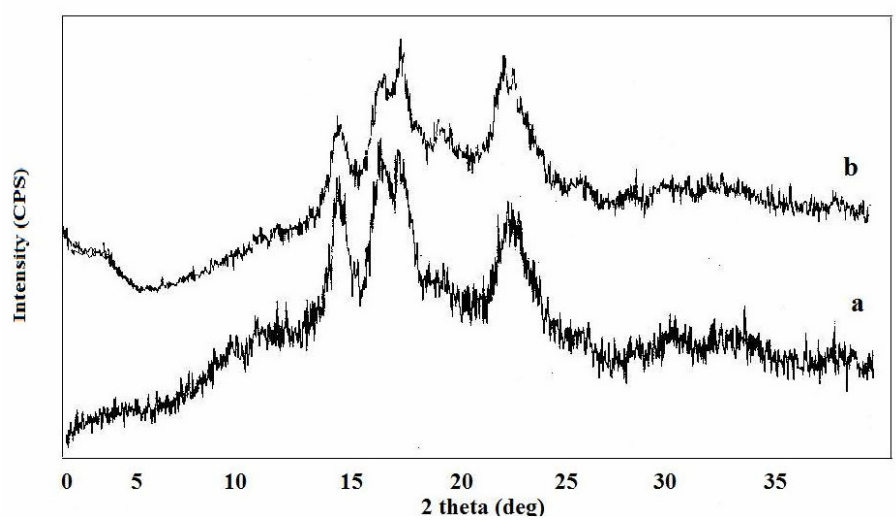


Figure 3.16. Xray diffraction pattern of (a) njavara starch, (b) native rice starch.

These four d values are present in the case of both starches. The XRD pattern of both rice starches are similar. The results are shown in Figure 3.16. The % crystallanity of Njavara and native chamba rice starch was found to be 54.76 % and 44.85 % respectively.

3.4. Conclusion

Physico chemical properties of njavara starch was studied and compared with properties of native chamba rice starch. The njavara starch has high gelatinization temperature, water absorption capacity, solubility and swelling power. It degrades at higher temperature and the enthalpy of gelatinization was very high compared to

native rice starch. Pasting properties showed that it has higher peak viscosity, break down viscosity and set back values. These good thermal properties make it useful in products which need to be processed at a high temperature. Because of its high heat holding capacity, the njavara rice is widely and specifically used in ayurveda treatments. Rheologically also njavara rice starch was superior. Temperature sweep analysis showed that njavara starch gelatinizes at higher temperature (68 °C) compared to the native rice starch (58 °C). Njavara rice starch had high gel strength than the native rice starch, and the difference between the G′ and G″ value is higher in the case of njavara indicating the formation of a strong gel and also lower tan value and higher torque value of njavara also indicates the same. Complex viscosity of both starches decreases with shear rate. Texture properties like hardness, gumminess, adhesiveness, cohesiveness, and chewiness are similar in both the rice starches. But the springiness nature of njavara starch gel is 3 times higher than that of native rice starch. X ray diffraction pattern showed that both starches are A type and are similar in nature.

CHAPTER 4

Hydrophobic grafted and crosslinked starch nano particles for drug delivery

The Synthesis of modified hydrophobic starch nanoparticles using long chain fatty acids was accomplished. The modified starch nanoparticles were crosslinked with sodium tripoly phosphate for better stabilisation. Drug loading and the controlled release of the drug from the nanoparticles was studied using indometacin as model drug.

4.1. Introduction

Starch is a potentially useful polymer for the thermoplastic biodegradable materials because of its low cost, availability and production from renewable resources [Li *et al.*, 2005]. However, the use of starch as a material useful for the industry has been challenged by some limitations, including the low moisture resistance, poor processability, and incompatibility with some hydrophobic polymers. Consequently, several strategies have been created to overcome these problems. Various physical or chemical modification of starch granules have been considered, including blending [Koenig *et al.*, 1995; Lee *et al.*, 1997] and chemical modification [Ramani *et al.*, 1996; 1997; Rouilly *et al.*, 2004] to solve the problems of starch based material produced by conventional melt processing. Chemical grafting is one of the most effective methods of modifying structure and properties of biopolymers. Graft copolymerization of natural polysaccharides is becoming an important resource for developing advanced materials as it can improve the functional properties of natural polysaccharides [Sen *et al.*, 2009]. Starch graft copolymer is one of the modifications of the starch by chemical method. Modified starch has wide application in biodegradable packaging material to components of oil drilling mud [Albertsson *et al.*, 1995]. Modified starches are being used as thickeners and gelling agents. The starch graft copolymer such as starch-g-polystyrene, starch-g-methacrylonitrile, starch-g-PVA, and starch-g-acrylonitrile have been synthesized generally by free radical generation on the surface of the starch granule and the copolymerization of these free radicals with the respective monomers

[Athawale *et al.*, 2000; Cho *et al.*, 2002; Zhai *et al.*, 2002; Combellas *et al.*, 2003; Park *et al.*, 2003]

The hydrophilic nature of the starch due to the abundance of hydroxyl groups, is a major constraint that seriously limits the development of starch based materials. Chemical modification has been studied as a way to solve this problem and to produce water resistant material with varied degree of substitution. Number of reports are there using organic solvents and mixtures to achieve starch solubilisation and then modification [Vazquez *et al.*, 1987; Athawale *et al.*, 1998; Thomas *et al.*, 1999]. Esterification with organic acid is known to result in thermoplastic and hydrophobic starch material.

Nanotechnology is making the most significant advances in biomedical applications, including newer drug delivery techniques. There has been considerable research into the developing biodegradable nanoparticles as effective drug delivery systems [Thakore *et al.*, 1999]. Nanoparticles are solid, colloidal particles consisting of macromolecular substances that vary in size from 10-1000 nanometers. The drug is dissolved, entrapped, adsorbed, attached or encapsulated into the nanoparticle matrix. The nanoparticle matrix can be of biodegradable materials such as polymers or proteins. Depending on the method of preparation, nanoparticles can be obtained with different properties and release characteristics for the encapsulated therapeutic agents [Panyam *et al.*, 2003].

This chapter discusses about the modification of starch using different long chain fatty acids to decrease its hydrophilicity. The graft polymerization reaction of starch with long chain fatty acid and the reaction conditions were optimized. Grafted starch was made into nanoparticle and was subsequently crosslinked with sodium tripolyphosphte for stabilization. Drug release properties of the starch nano particle were studied by using Indomethacin as the model drug.

4.2. Experimental

4.2.1. Materials

Cassava tuber was purchased from local market, Trivandrum, Kerala and starch was extracted as explained in chapter 2.

4.2.2. Methods

Graft polymers and nanoparticles formed were characterized by FTIR spectra, UV-Visible spectra, scanning electron microscope, atomic force microscope, TGA and DSC.

4.2.2.1. Preparation of graft polymer starch-Oleic acid (ST-Ol)

For the graft copolymerization about 1g starch dissolved in 10 ml DMSO was mixed with 5.2 g oleic acid and potassium per sulphate was taken as the catalyst. The reaction mixture was heated at 100 ± 5 °C for 8 h in an oil bath with magnetic stirring. Graft polymer formed was separated by precipitation from ethanol. Filtered, washed thrice with ethanol and then dried the sample at 100 ± 5 °C in a vacuum oven. The above

reaction was repeated by changing the reaction conditions like temperature and reaction time.

$$\text{Graft yield} = \frac{\text{Wg}}{\text{Ws} + \text{Wa}} \times 100 \tag{4.1}$$

Where Wg - weight of graft polymer, Ws - weight of starch, Wa - weight of acid used.

4.2.2.2. Preparation of graft polymer starch-stearic acid (ST-St)

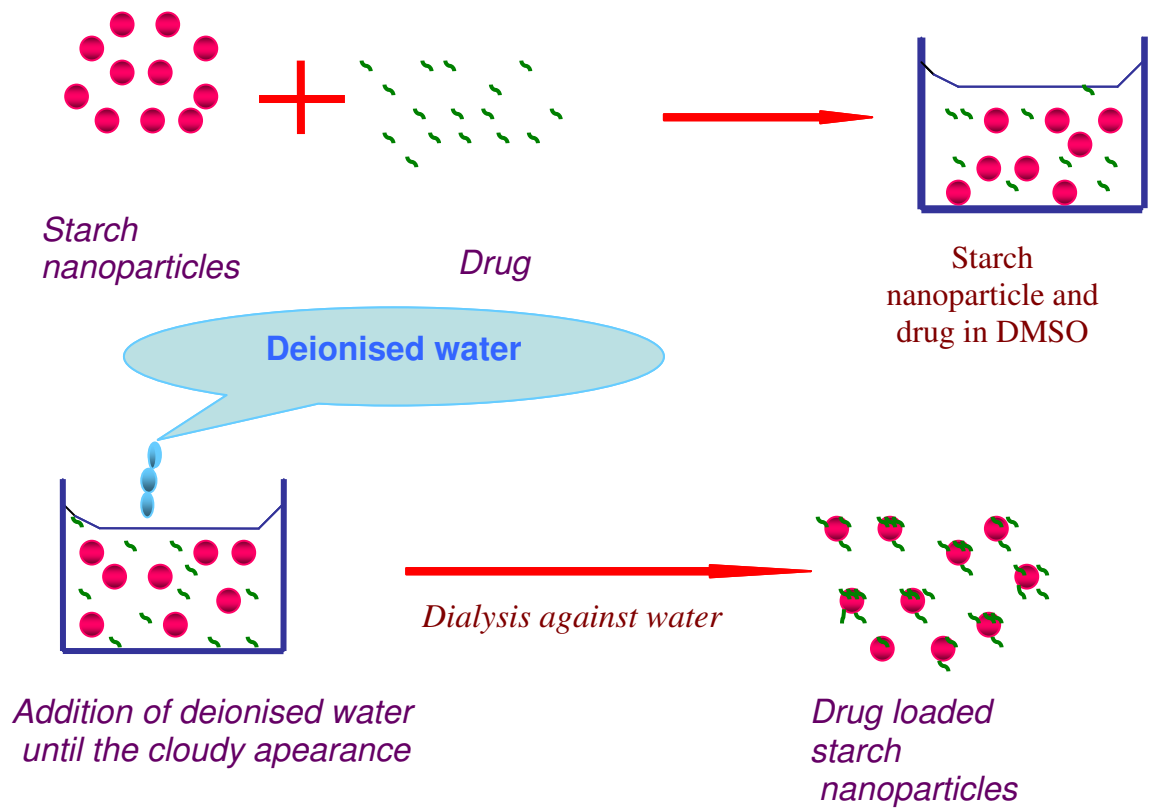
Starch (1 g) was weighed in to a round bottom flask and was dissolved in 10 ml DMSO and mixed with 4.9 g stearic acid. Potassium per sulphate was used as the catalyst. The graft polymerisation reaction was allowed to procede for 8 h at 100 ± 5 °C in an oil bath with magnetic stirring. Graft polymer formed was separated by precipitation from ethanol. Filtered, washed thrice with ethanol and the sample was dried at 100 ± 5 °C in a vacuum oven.

4.2.2.3. Preparation of starch nano particles

Grafted starch nano particles were prepared by the dialysis method [Nah *et al.*, 1998]. Appropriate amount of graft polymer was dissolved in DMSO and the sample was dialysed against water using a dialysis membrane having a molecular cut off of 12000 $gmol^{-1}$. The medium was replaced every hour in the first 3 h and every 5 h for the following 24 h period. The resulting solution was homogenized. Starch nanoparticles were stabilised by crosslinking with sodium tripolyphosphate and an appropriate surfactant.

4.2.2.4. Drug loading and in vitro drug release studies

Drug loading in to the starch nano particles were studied by using indomethacin as the model drug. Appropriate amount of starch nanoparticles and indomethacin were dissolved in DMSO. Deionised water was added dropwise with stirring until the cloud point was reached. Sample was dialyzed against distilled water by a dialysis membrane having a molecular cut off of 12000 $gmol^{-1}$. The medium was replaced every hour in the first 3 h and then every 5 h for the following 24 h period.



Scheme 4.1. Drug loading on the nanoparticles

For determining the drug loading efficiency, an appropriate amount of sample was dissolved in phosphate buffer of pH 7.4. Centrifuged the sample at 704 x g for 15 min.

The supernatant was used for the estimation of the drug at 320 nm using a UV-Visible spectrophotometer (Shimatzu UV 2100). For the study of *in-vitro* drug release, small amount of the drug loaded starch particle in 0.1 M phosphate buffer of pH 7.4 was taken in a dialysis membrane of having a molecular cut off of 12000 $gmol^{-1}$. The sample was dialysed against 0.1M phosphate buffer of pH 7.4. At regular intervals certain amount of medium was replaced by fresh phosphate buffer. The amount of drug released was determined by a UV- visible spectrophotometer at 320 nm. The drug release kinetics was studied by Higuchi and Korsmeyer Peppas method.

4.2.2.5. Swelling power of starch, grafted starch

Solubility and swelling power of starch, oleic acid grafted starch, and cross linked starch were determined at 85 °C [Whistler, 1964]. Starch (0.1 g) was taken in a previously tared bottle and sufficient distilled water was added to give a total volume of water equivalent to 9 g. Heated in a water bath for 30 min at constant temperature of 85 °C with magnetic stirring. After heating, the bottle was rinsed with distilled water to bring the volume to 10 g. Centrifuged the sample at 313 x g for 15 min. The clear supernatant, 5 ml was drawn off into a clean dry dish. The dish was dried in a vaccum oven for 4 h at 120 °C. Cooled in a desiccator and weighed. The supernatant was completely decanted and the swollen granules were weighed. Percentage solubility and swelling power were calculated using the formula

$$\% \text{ Solubility} = \frac{\text{Dry weight}}{\text{Sample weight}} \times 100 \qquad (4.2)$$

$$\text{Swelling Power} = \frac{\text{weight of swollen granule}}{\text{Sample weight (100 - \% solubility)}} \times 100 \qquad (4.3)$$

Solubility of different graft polymers was studied using solvents of different polarity. A 2 % (w/v) concentration of grafted starch was examined in a number of polar and non polar organic solvents to study their solubility.

All the experiments were done in triplicate and the average values are given.

4.3. Results and discussion

4.3.1. Preparation of grafted starch

The results showed that introduction of hydrophobic long chain fatty acid groups into the molecular structure of starch will alter its properties. A starch anhydro glucose unit contains one primary hydroxyl group and two secondary hydroxyl group. The primary hydroxyl group is more reactive towards long chain fatty acids. In all the cases, the reaction products exhibited characteristic ester band signals in their FTIR spectra. The FTIR spectrum of starch and grafted starch was shown in Figure 4.1. In comparison with the spectra of the native starch, the major change is the presence of a carbonyl C=O absorption frequency. The peaks at 1157 cm^{-1}, 1080 cm^{-1}, 1016 cm^{-1}, and 927 cm^{-1} in native starch were due to the CO bond stretching. The peaks at 1080 cm^{-1} and 1016 cm^{-1} are characteristic of the anhydroglucose ring O-C stretching. A characteristic peak occurred at 1645 cm^{-1} is due to the presence of bound water in starch. Strong

absorption band at 1016 cm^{-1} is due to strengthening of the C-OH bond [Goheen *et al.*, 1991]. A broad band due to hydrogen bonded hydroxyl group (O-H) appeared at 3390 cm^{-1} and was attributed to the complex vibrational stretching associated with free, inter and intra molecular bound hydroxyl groups. The band at 2926 cm^{-1} is characteristic of C-H stretching. The C-H stretching absorbance on 2926 cm^{-1} is increased in intensity upon grafting.

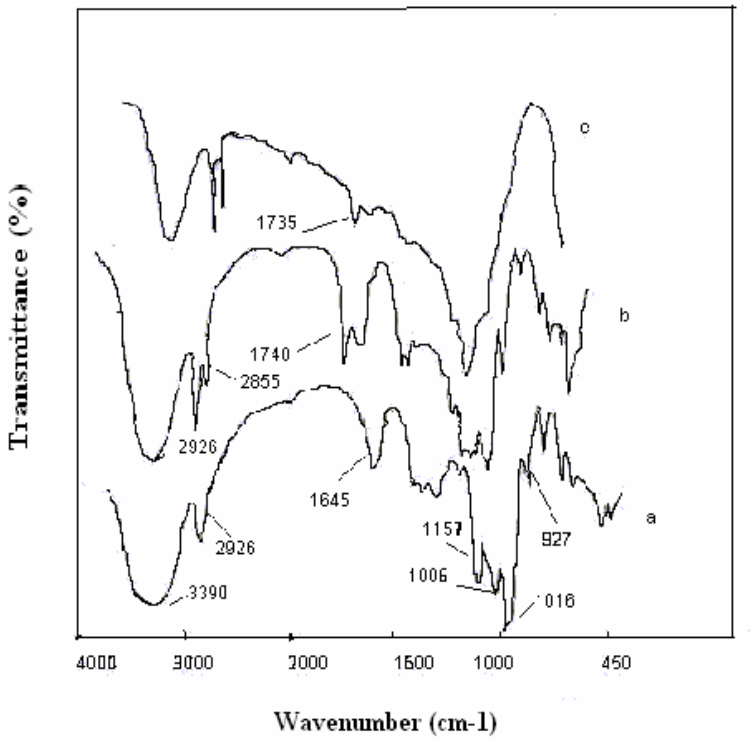


Figure 4.1. FTIR spectra of a) Starch, b) ST-Ol, c) ST-St.

The strong OH stretching band at 3390 cm^{-1} in the native starch decreased in intensity following the grafting reaction. This depends upon the extent of grafting. In the case of grafted starch, the presence of carbonyl peaks at 1740 cm^{-1}, 1735 cm^{-1} for ST-Ol, and ST-St respectively, indicates the grafting. The peaks of strong intensities at 2926 cm^{-1}

and 2855 cm^{-1} in the spectra is attributed to the methyl and methylene C-H stretching associated with the fatty acid substituent. The intensity of C-H stretching increases with increasing carbon chain length, relative to the OH absorbance.

4.3.2. Effect of temperature on grafting

Grafting efficiency against reaction temperature was studied and the results were shown in Figure 4.2. In this experiment duration of the reaction and the starch fatty acid concentration ratio were taken as constant.

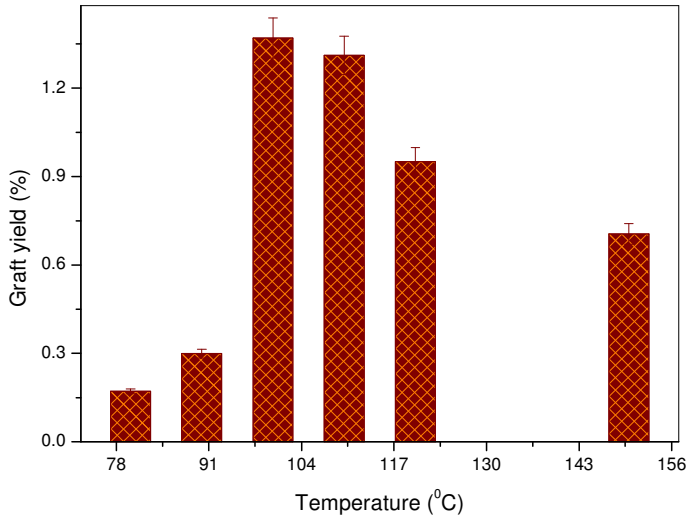


Figure 4.2. Effect of reaction temperature on starch oleic acid grafting

Percentage graft yield increased from temperature 80 °C to 100 °C and thereafter it was decreased. This behavior is mainly due to the fact that, at higher temperature the rate of production of free radical species and grafting sites are generated at a greater rate. Above the critical temperature, the grafting yield decreases due to the faster termination rate or may be due to the occurrence of reverse reaction.

4.3.3. Effect of the duration of reaction

Effect of duration of reaction on % graft yield was shown in Figure 4.3. There is an increase in the graft yield as the reaction time increase from 6 to 8 h. This initial increase is due to the addition of greater number of fatty acid to the hydroxyl groups of the starch. After 8 h, the percentage grafting was almost constant. This leveling of grafting with time could be mainly due to the large termination rate [Kacurakova *et al.*, 2001].

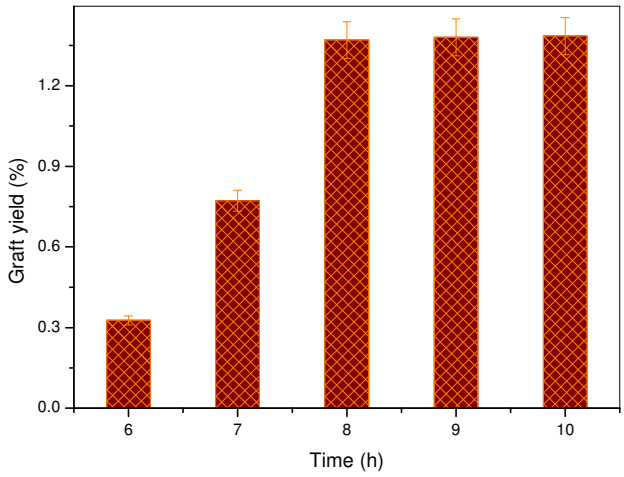


Figure 4.3. Effect of reaction time on starch oleic acid grafting.

Grafting efficiency or the percentage graft yield depends on the temperature, duration of the reaction and the nature of the catalyst. By altering these variables, the percentage graft yield can be improved.

4.3.4. Thermal analysis

Grafted and native starch was subjected to thermal degradation. Thermogravimetric plot of native starch and grafted starch, temperature ranging from 40 ° to 400 °C at a rate of 10 °C/min were shown in Figure 4.4. In the case of native starch, degradation starts at a temperature of 275 °C. But for grafted starches the degradation temperature starts at a lower temperature of 180 °C and 225 °C for ST-Ol and ST-St respectively. Temperature at which 5% weight loss of native starch and ST-Ol, ST-St are 62 °C, and 117 °C respectively. The initial weight loss at about 100 °C for native starch is due to the removal of moisture. Temperature corresponds to 10 % weight loss of native and grafted starch is 88 °C and 217 °C (ST-Ol) and 222 °C (ST-St).

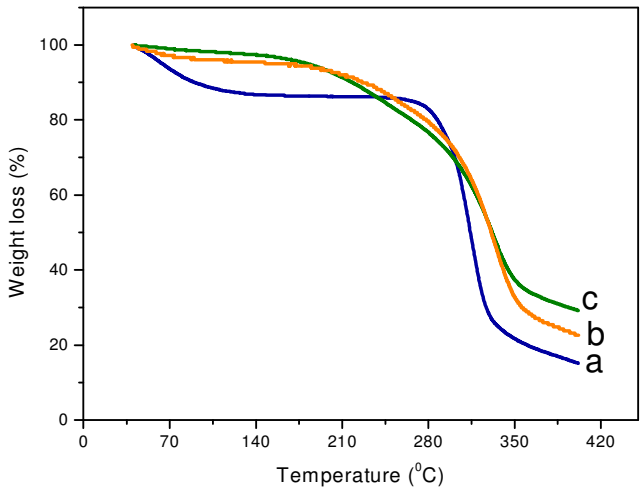


Figure 4.4. TGA thermogram of a) starch, b) ST-Ol, c) ST-St.

Details of decomposition temperature at 5 % weight loss (T_5), 10 % weight loss (T_{10}) and maximum decomposition temperature (T_{max}) are given in Table 4.1.

Table 4.1. Details of thermogravimetric analysis

sample	T_5(°C)	T_{10}(°C)	Tmax(°C)
ST	62	88	350
ST-Ol	178	217	360
ST-St	117	222	360

Gelatinization temperature of grafted and native starch was studied by using DSC at a temperature ranging from 30 °C to 250 °C at a rate of 10 °C/min.

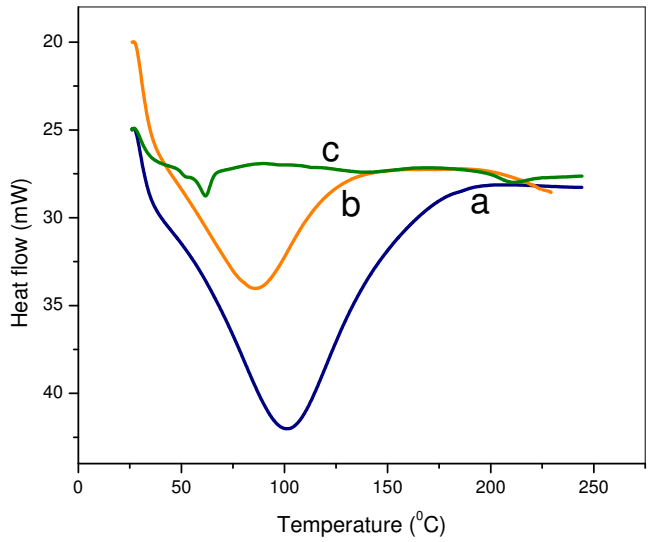


Figure 4.5. Differential scanning calorimetry of a) Starch, b) ST-Ol, c) ST-St.

The DSC thermograms of grafted and native starch were shown in Figure 4.5. These starches give gelatinization endotherm. Native starch showed a peak temperature of 100 °C. Substituted starch granules usually exhibit lower gelatinization temperature [Mostafa *et al.*, 2004], which is also seen from the present study. ST-Ol had a higher

gelatinization temperature of 84 °C whereas ST-St showed a gelatinization temperature of 62 °C. These thermal studies showed that grafted starch was more processable than the native starch.

4.3.5. Swelling power and solubility

The percentage soluble of native starch is low (24 %) compared to grafted starch. But the swelling power of native starch (4.11 %) is higher than that of grafted one. Grafting makes the starch hydrophobic and hence reduces the swelling power in the aqueous medium.

Table 4.2. Details of swelling power studies

Sample	**% soluble**	**Swelling power (%)**
Starch	24	4.11
ST-Ol	40	2.03
ST-St	23	2.9

The percentage soluble was higher in grafted starches because the grafting causes partial degradation of starches to smaller fragments which subsequently leaches out. Results of swelling power studies were shown in Table 4.2. Percentage soluble is higher in ST-Ol (40 %). In the case of ST-St, the percentage soluble is 23 %. Swelling power of ST-Ol, and ST-St are 2.03 % and 2.9 % respectively.

In general, introduction of hydrophobic long chain fatty acid groups into the molecular structure of starch will alter its solubility properties. The solubility of modified starch depends upon a) extend of solubilisation b) nature of substitution c) type of starch d) solvent and temperature [Marcazzan *et al.*, 1999]. All grafted starches were examined in a range of organic solvents to investigate their solubility. All the grafted starches were found to be soluble in warm DMSO. They were partially soluble in DMF and chloroform. The solubility was not significantly enhanced due to the relatively low extent of substitution.

4.3.6. Morphology

Scanning electron micrographs of native and grafted starch at different magnifications were shown in Figure 4.6 and Figure 4.7. SEM of native starch showed typical granules of cassava starch, spheroid with a flat surface on one side having size range of ~5-to 20 μm. After grafting, granular structure of the starch was completely destroyed due to gelatinization and one can see amorphous particles of clumped starch.

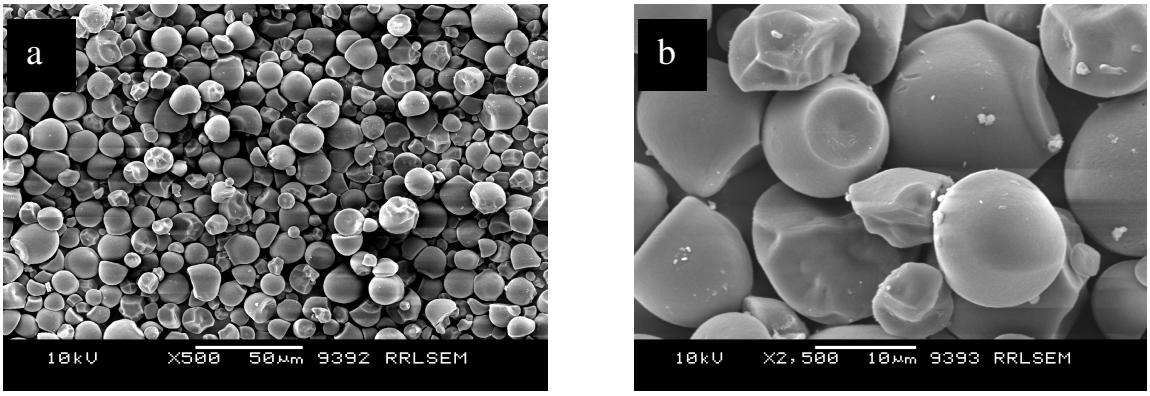


Figure 4.6. Scanning Electron Micrograph of cassava starch granule at different magnifications a) X500, b) X2500.

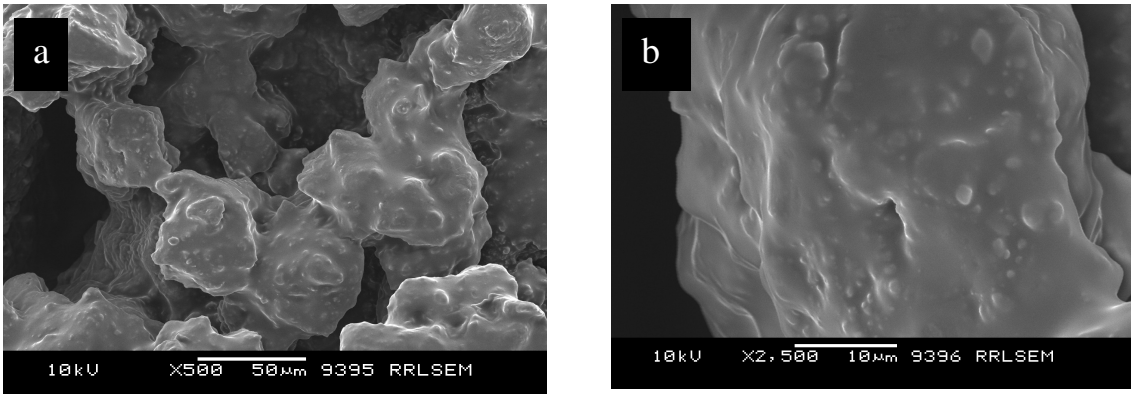


Figure 4.7. Scanning Electron Micrograph of ST-Ol at different magnification a) X500, b) X2500

4.3.7. Grafted starch nanoparticles

Starch nanoparticles were successfully prepared by dialysis method. Shape and size of the particles formed were studied by atomic force microscopy (Figure 4.8 a and 4.8 b).

Nano particles in DMSO water solution were transferred to freshly cleaved mica sheet and analyzed by tapping mode. Size of the particles was found to be in the range of 65nm to 75 nm (diameter), and 17nm to 19 nm (height).

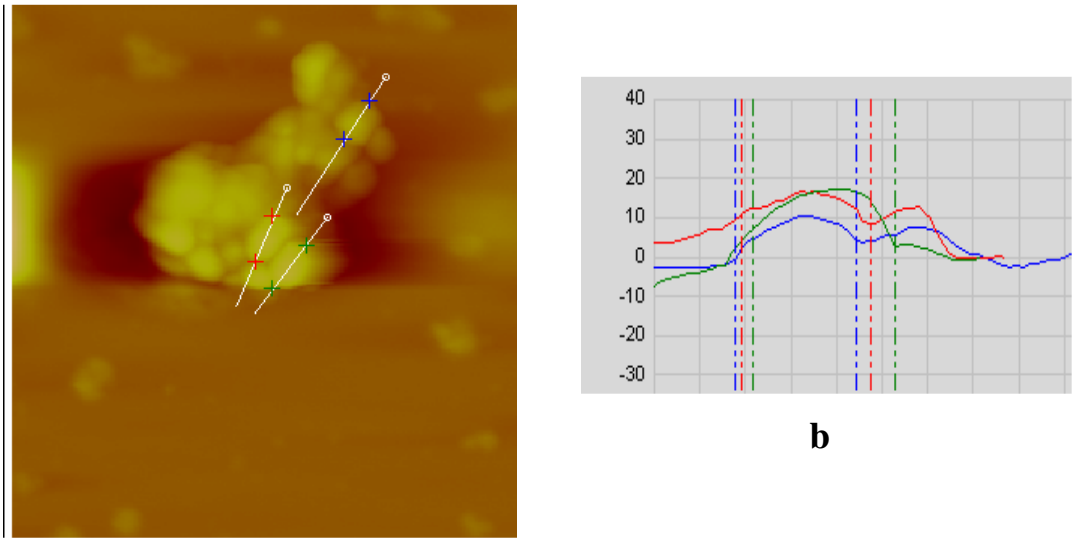


Figure 4.8. AFM image of grafted starch nano particles (a) AFM image of starch nano particle, (b) size distribution graph

Nano particle formed was crosslinked with sodium tripoly phosphate for stabilisation. Formations of crosslinked starch oleic acid grafted nanoparticles were confirmed by FTIR spectroscopy. The typical stretching vibration of P=O and P-O was observed at 1119 and 1109 cm-1 respectively. The FTIR spectra of the ST-Ol nanoparticle were shown in Figure 4.9.

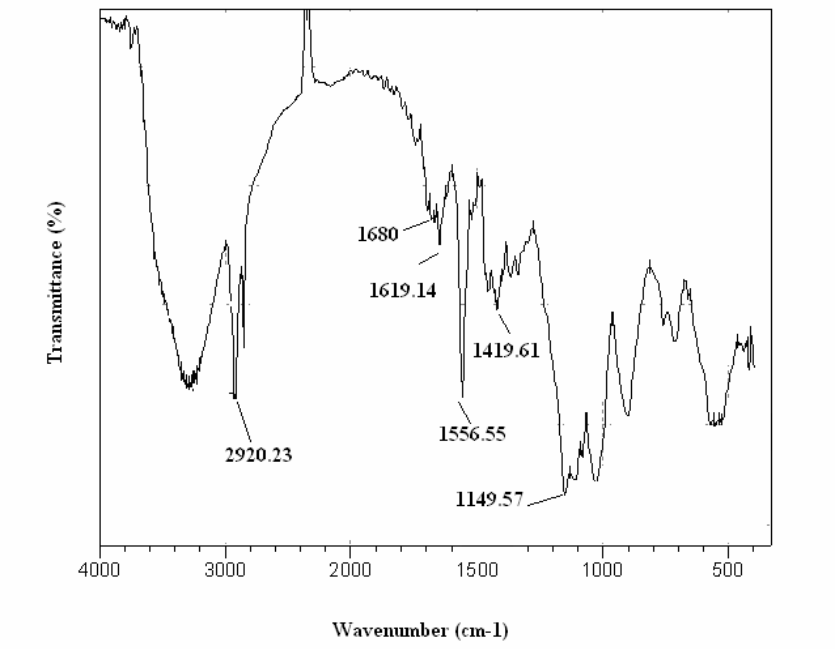


Figure 4.9. FTIR spectra of ST-Ol nanoparticle cross linked with sodium tripoly phosphate

4.3.8. Drug release studies

Maximum loading of drug in nanoparticle was found to be 16 %. Figure 4.10 showed the controlled drug release profile of indomethacin loaded starch nano particles. Results showed that controlled release of drug occurs at a pH 7.4. Surface crosslinks of nano particles slowed down the release of the drug from the nano particle. Slow release of drug in buffer of pH 7.4 has proved that the starch nano particle can be used as a good carrier of drugs.

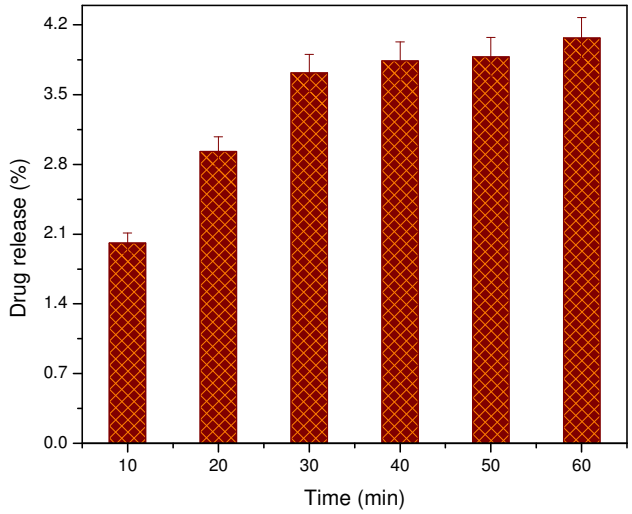


Figure 4.10. Controlled drug release studies

Studied the drug release kinetics by Higuchi and Korsmeyer Peppas model. Higuchi developed theoretical models to study the release of high and low water soluble drugs incorporated in solid and semisolid matrices. According to this model drug release was described as a square root time dependent diffusion process based on Ficks law. Plot of square root of time versus cumulative amount of drug released yields a straight line and slope gives the kinetics of drug release. Under some experimental situation the release mechanism deviates from Fick's equation. In this case Korsmeyer Peppas developed a simple, semi empirical model relating exponentially the drug release to the elapsed time. In our work the release data were analysed on the basis of Korsmeyer Peppas and Higuchi kinetics. The release rates n and k of each model were calculated by linear regression analysis using Microsoft origin 6 software.

Table.4.3. Drug release kinetics

	r	**constants**
Higuchi	0.9427	K =0.43886
Korsmeyer Peppas	0.9639	n =0.3959

Coefficients of correlation (r) were used to evaluate the accuracy of the fit. The r, n and k values were shown in Table.4.3. On calculating and comparing the r values and kinetic constants, the drug release was best fit to Korsmeyer Peppas model and exhibited a Fickian release.

4.4. Conclusions

Chemical modification of starch was successfully carried out by grafting with long chain fatty acids which gave good yields. Grafted products were analysed by FTIR spectroscopy. Morphological studies by SEM shows the changes that occurred during the grafting reaction. Thermogravimetric studies showed the decrease in degradation temperature in the case of grafted starch compared to the native starch. Grafted copolymer was found to be gelatinised at 84 °C and 62 °C, and was confirmed from DSC. Swelling studies shows that grafted starch has high swelling power at neutral pH compared to the native starch. This behavior is useful in drug delivery applications. Starch nanoparticles were prepared and stabilized by crosslinking with sodium tripoly phosphate. AFM studies of the nano particles showed the size and shape of the

particles. Controlled release of the drug previously loaded in nano particle was studied by using indomethacin as the model drug. Fatty acid grafted starch nano particle was found to be a good vehicle for the controlled oral drug delivery. It obeys the Korsmeyer Peppas model and exhibited a Fickian release.

CHAPTER 5

Blue fluorescent self- assembled xyloglucan hydrogels; Synthesis and properties

This chapter describes about the synthesis and properties of cationic xyloglucan. Amination of xyloglucan with ethylene diamine in aqueous medium is found to be a good strategy to get versatile xyloglucan gels. By this facile synthetic strategy, xyloglucan is functionalised with amino group which leads to the formation of *insitu* irreversible hydrogels without using any cross linking agents having blue emission characteristics in the fluorescence spectra.

5. 1. Introduction

Natural, non toxic and water soluble polysaccharides are finding in numerous applications in foods, textiles, paints, cosmetics and pharmaceuticals, which utilize its broad range of functional properties. The utility of these products in many of these applications relies on their ability to confer high viscosities to aqueous media [Yalpani, 1987]. In recent years, there has been considerable interest in the development of conjugates of non-starch polysaccharide molecules. Chemical derivatisation methods are employed in order to use these intractable, but inexpensive polysaccharides. It is widely used as a food additive in Japan [Hayashi, 1989; Whitney *et al.*, 1995] and in USA, it is used as wet-end additive in the paper industry as a replacement for starches and galactomannans [Picout *et al.*, 2003].

Cationic polysaccharides have wide applications in drug and gene delivery. Polycations and negatively charged nucleic acids can spontaneously form nanocomplexes (Polycationic vector) by electrostatic interaction which reduces the electrostatic repulsion between DNA and cell surface by neutralizing the negative charge and also protects it from enzymatic digestion by nucleases in serum and extra cellular fluids.

The major storage polysaccharide present in the seeds of the tamarind tree (*Tamarindus indica*) is xyloglucan. Tamarind kernel powder is the only source of xyloglucan commercially available in large quantities. Xyloglucan is cross-linked with cellulose micro fibrils and endowed with the flexibility necessary for the micro fibrils

to slide. It has a backbone composed of 1, 4- linked β-D-glucopyranose residues. Up to 75% of these residues are substituted at O-6 with α-D-xylopyranose. Some of the xylose residues are *α*-D-galactosylated at O-2 [York *et al.*, 1990; Kim *et al.*, 2006]. Tamarind xyloglucan forms a gel in the presence of alcohol, sugar and polyphenols such as epigallocatechin gallate (EGCG) [Nishinari *et al.*, 2003; Nitta *et al.*, 2004; Yuguchi *et al.*, 2004]. It also forms a synergistic gel even with low concentrations of gellan gum [Nitta *et al.*, 2003]. If a part of the galactose is removed from the xyloglucan it also forms a gel [Dave *et al.*, 1998; Shirakawa *et al.*, 1998; Nishinari *et al.*, 2000; Yamanaka *et al.*, 2000]. However, a simple modification or attachment of amino group (aminated xyloglucan) insitu-gels is not reported till to date. This is a first time report on a polysaccharide hydrogel which is self-assembled with blue fluorescence. It may find use in new areas like biotronics and fluorescent labeling applications in biological area.

5. 2. Experimental

5. 2. 1. Materials

Xyloglucan extracted from tamarind seed, was purchased from the local market at Trivandrum, Kerala, India. The xyloglucan was extracted from tamarind kernel powder as explained in chapter 2. All the other chemicals used are of analytical grade and used without further purification.

5. 2. 2. Methods

Modified xyloglucan and aminated xyloglucan gel was characterized by FTIR spectroscopy, NMR spectroscopy, UV-Visible spectroscopy, scanning electron microscopy, atomic force microscopy, Thermogravimetric analysis, Differential Scanning Calorimetry, MALDI-TOF mass spectrometer, X ray Diffraction pattern, fluorescent spectroscopy, fluorescent microscopy, rheometer and food texture analyser.

5.2.2.1. Synthesis of amino xyloglucan (XG-NH_2)

Xyloglucan was aminated at various conditions by varying temperature (4, 10, 20, 30, 40, 50, 60 and 80 °C), time (1 to 12 h), and concentration of aminating agent (10 to 50 % of ethylene diamine). Xyloglucan was reacted with ethylene diamine in aqueous medium at 30 °C for 6 h. The hydroxyl group of XG in the 2^{nd} 3^{rd} and 6^{th} position was get substituted by $–NHCH_2CH_2NH_2$ which was further reduced to $–NH_2$ using $NaBH_4$ as reducing agent. After the completion of the reaction, the sample was precipitated and washed several times with ethyl alcohol. The precipitate obtained was filtered and dried in a hot air oven at 70 ± 2 °C for 3 h and then powdered to uniform particles [Urreaga *et al.*, 2007]. The conditions were optimized based on their degree of substitution.

5.2.2.2. Degree of substitution

The degree of substitution was determined using UV-Visible spectrophotometer at a wavelength of 570 nm [Zhu *et al.*, 2002; Sun *et al.*, 2006]. The aminated sample (50

mg) was dissolved in 10 ml of 1% (v/v) acetic acid in water. A 5 ml aliquot of this solution was treated with 1ml of 1% (w/v) ninhydrin at 80 ± 5 °C in an oil bath for 5 min under stirring condition. In the presence of ninhydrin, amino group undergoes oxidative deamination and forms a coloured complex. From the absorption of this coloured complex, the DS value of the aminated xyloglucan was calculated using the Equation 5.1.

amino group

$$DS = \frac{330 \times (\%\ \text{amino group}/16)}{100 - \frac{15}{16}(\%\ \text{amino group})} \qquad (5.1)$$

5.2.2.3. Solubility studies

Solubility of aminated xyloglucan was studied using solvents of different polarity. A 2% (w/v) concentration of both xyloglucan and aminated xyloglucan was used. The solvents such as acetic acid, sulphuric acid, hydrochloric acid and nitric acid, DMSO, THF, DCM, benzene, toluene, carbon tetra chloride, DMF were used for solubility studies.

5.2.2.4. Antimicrobial activity

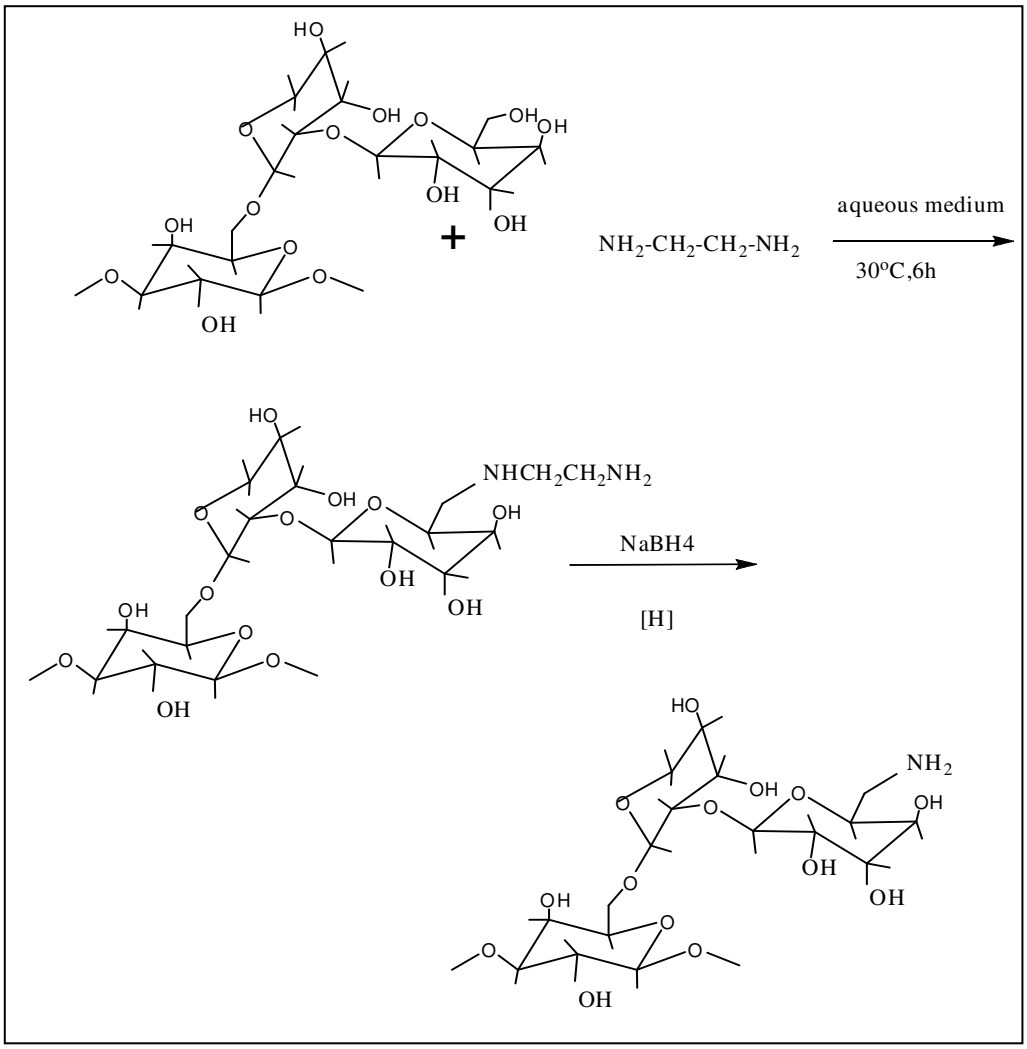
Antimicrobial activity was studied by using nutrient agar. Appropriate concentration of nutrient agar was made into a gel. The gel was mixed with aminated xyloglucan at neutral pH in a Petri dish and allowed to set, subsequently exposed to atmospheric

contamination at room temperature. The antimicrobial activity was determined by visual observation of the colonies formed and compared with chitosan as the control.

5.3. Results and discussions

5.3.1. Preparation of XG-NH_2

The aminated xyloglucan was synthesised by reacting the extracted xyloglucan with ethylene diamine and followed by reduction using $NaBH_4$ (Scheme 5.1).



Scheme 5.1. Synthesis of amino xyloglucan (XG-NH_2).

The formation of XG-NH_2 was characterized by FTIR and NMR analyses. The synthesised product exhibited characteristic -NH_2 band signals in FTIR spectra (Figure 5.1).

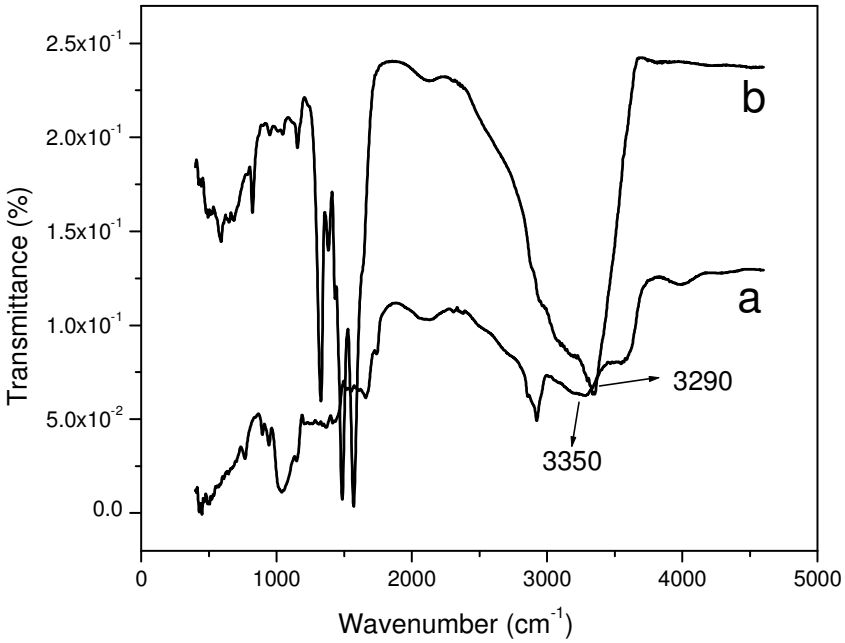


Figure 5.1. FTIR spectra which confirms the amination on xyloglucan a) XG, b) XG-NH_2.

The wide band observed at 3290 cm^{-1} is ascribed to the hydroxyl groups of xyloglucan. In amino xyloglucan, this band gets appears as a sharp band with a shift to 3350 cm^{-1} due to the binding of –NH_2 functional group to the xyloglucan structure. Generally, the bands of –NH_2 (primary amines) are identified as two identical sharp peaks in the hydroxyl band region, but here, due to the high volume of the hydroxyl groups, the bands of amino groups are merged with the hydroxyl bands and hence is not clearly visible, however, the sharpness of the band obviously indicates that primary

hydroxyl group is substituted by an amino group. As expected, the band is shifted to higher frequency region in the amino xyloglucan, because of lower percentage of hydrogen bonding than that of unmodified xyloglucan. The tendency for inter/intra molecular hydrogen bonding is comparatively less with nitrogen than oxygen which is reflected in the spectra of XG-NH_2. The OH groups at 2^{nd}, 3^{rd} and 6^{th} position in the glucose back bone and galactose side chain can get substituted by NH_2 group (but the most probable position is 6^{th}). This was confirmed by the IR spectra of galactose deficient (alpha galactosidase hydrolysed product) aminated xyloglucan. The cleaved galactose was separated from the glucan chain by dialysis through an ultra filtration membrane (Millipore) of MW cut off 10000. Both the galactose part and the glucose part gave characteristic peak for NH_2 group.

The proton-NMR of XG data showed a sharp singlet at 3.7 ppm which was found to be diminishing in the spectra of XG-NH_2 (Figure 5.3). This is due to the binding of NH_2 group in the position of primary hydroxyl groups. And hence the signal was shifted to the lower field (3.3 ppm). This is further attributed to the low electro negativity of nitrogen compared to oxygen. The singlet at 5.1 ppm indicates the presence of NH_2 group.

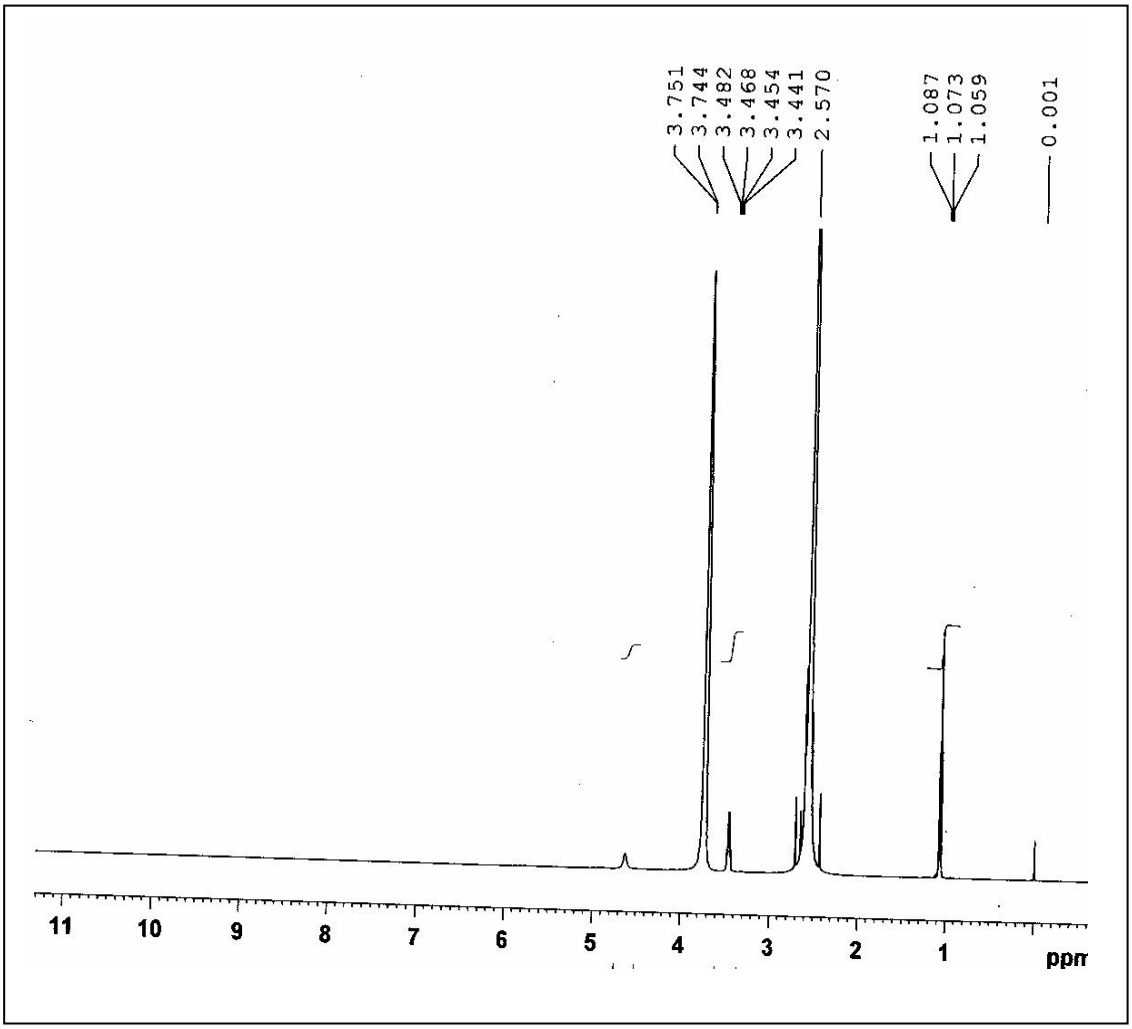


Figure 5.2. NMR spectra of xyloglucan.

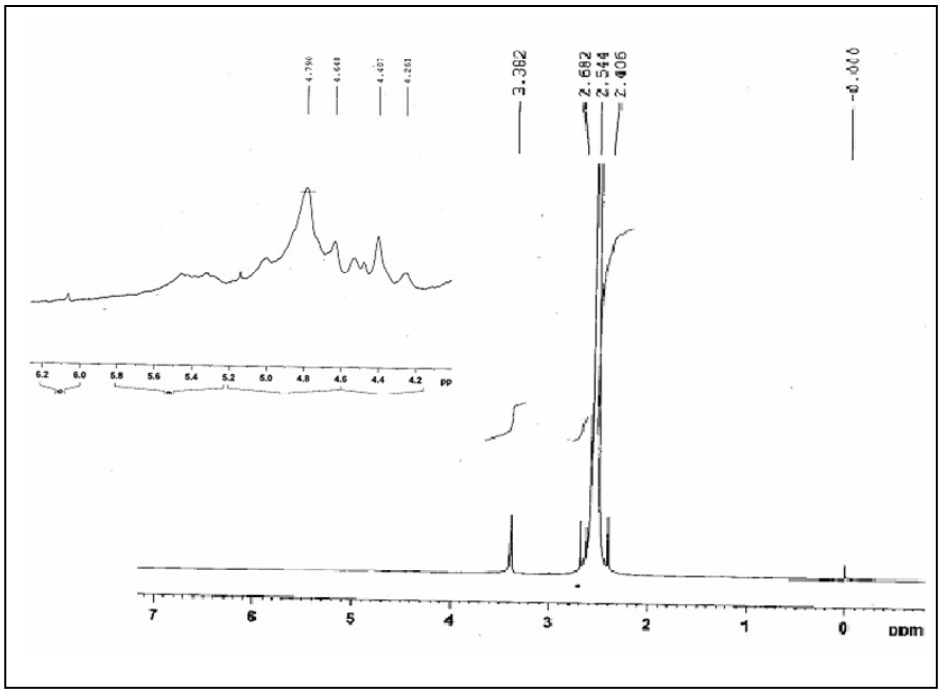


Figure 5.3. NMR spectra which confirms the amination of xyloglucan.

This peak was absent in the spectra of native xyloglucan (Figure 5.2). The peak was minute to be detected well (expanded form) because of the very low degree of substitution. From the FTIR and NMR analyses, the formation of XG-NH_2 was confirmed.

5.3.2. Effect of time duration on the amination of xyloglucan

Figure 5.4 shows the effect of reaction time on DS at 28 ±°C. The DS increases with the increase in reaction time. Significant decrease was observed on increasing the time up to a day. The enhancement of DS by prolonging the duration of reaction from 1h to 6 h is a direct consequence of the favorable effect of time on swelling of xyloglucan as

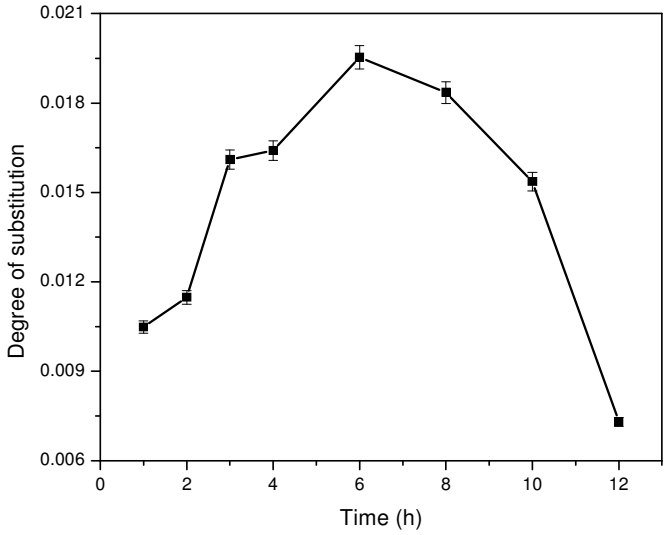


Figure 5.4. Effect of time duration on amination reaction of xyloglucan.

well as the diffusion and adsorption of the reactants with in turn ensures better contacts between the aminating agent and the xyloglucan.

5.3.3. Effect of temperature on the amination of xyloglucan

Amination of xyloglucan was performed at different temperatures (4°C, 10°C, 20°C 30°C, 40°C, 50°C, 60°C, and 80°C). The dependence of DS on reaction temperature was shown in Figure 5.5. It was observed that DS increases from 0.21% to 0.416% prominently as the reaction temperature increases from cold condition (4°C) to room temperature (28 ±°C) and thereafter decreases drastically from 40 °C onwards. It is due to the favorable effect of temperature on the optimum swellability of xyloglucan for the amination to occur.

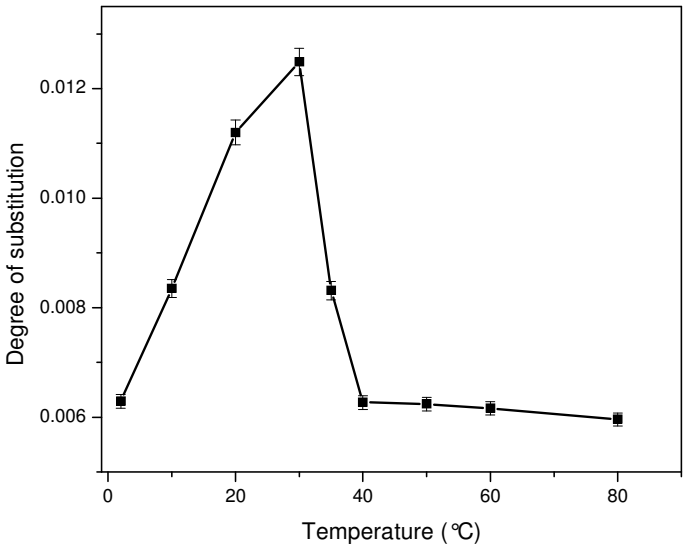


Figure 5.5. Effect of temperature on the amination reaction of Xyloglucan.

5.3.4. Effect of concentration on amination

The ethylene diamine concentration was varied from 10% to 50 % (w/v) at 30 °C and the results were shown in Figure 5.6. There is a distinct pattern of the increase in DS on increasing the concentration of ethylene diamine. As the concentration of aminating reagent increases above 40% the DS reaches an optimum, and thereafter excess aminating agent hinders further amination and there is only a marginal increase of DS. Hence it was concluded that 40% of ethylene diamine in water was optimum for the amination reaction. Aminating efficiency or the percentage DS depends on the temperature, duration of the reaction and the concentration of the aminating agent. By altering these variables the percentage DS can be improved. The conditions were optimized at a temperature of 28 ± 2 °C with 40 % of aminating agent for 6 h.

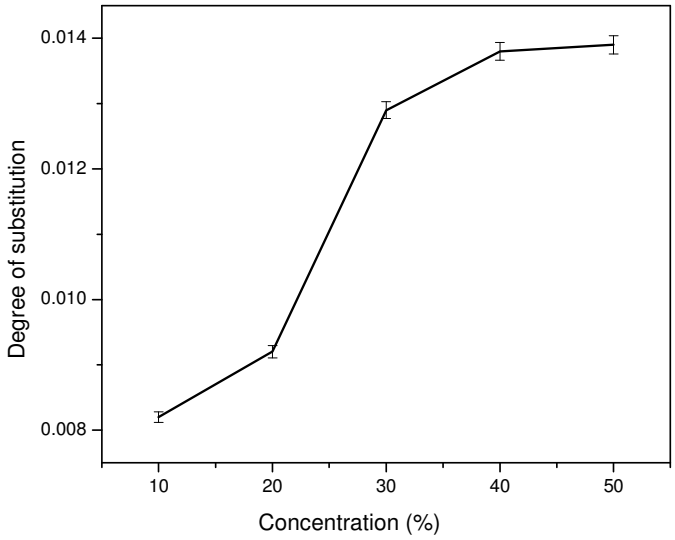


Figure 5.6. Effect of concentration of aminating agent on xyloglucan.

MALDI TOF MS results showed that xyloglucan has a molecular weight of 298 KDa and aminated xyloglucan has a lower value of 240 KDa. The XG-NH_2 is soluble in water, mineral acids like HCl, H_2SO_4, HNO_3 and the organic solvents like DMSO but partially soluble in acetic acid. The solubility properties are similar to the native XG and no significant change is observed. The solubility of XG-NH_2 was very attractive that it retains the solubility characteristics of native XG. It is interesting that XG-NH_2 retains good solubility in water too.

5.3.5. Crysatllinity and Thermal properties

Native XG and XG-NH_2 showed crystallinity and exhibited a peak at 2θ angle of 23º in XRD analysis (Figure 5.7).

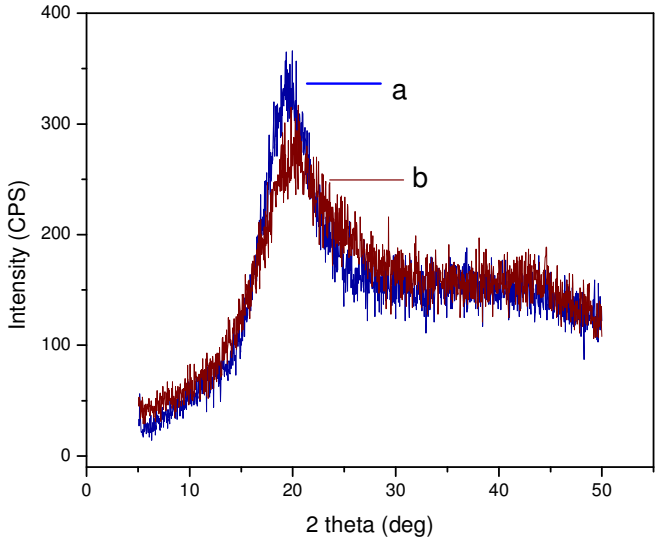


Figure 5.7. Crystalline nature of a) XG and b) XG-NH_2 by X Ray Diffraction pattern.

The degree of crystallinity of XG was found to be 21.33% and for aminated XG it was 13.74%. In other words, the XG was more crystalline than cationic xyloglucan as expected.

5.3.6. Thermal analysis

The TGA of XG shows weight loss events in two stages. The first stage regimes between 35 and 100 °C and shows about 9% loss in weight. This may correspond to the loss of adsorbed and bound water. The second stage of weight loss starts at 270 °C and continues up to 350 °C with a 62% weight loss due to the degradation of xyloglucan. There is a marked difference between the thermal properties of XG and XG-NH_2 sample. The latter has three stage of weight loss between 35 and 550 °C. The first stage of weight loss starts at 60 °C and continues up to 220 °C, during which there was 20% weight loss due to the degradation of xyloglucan.

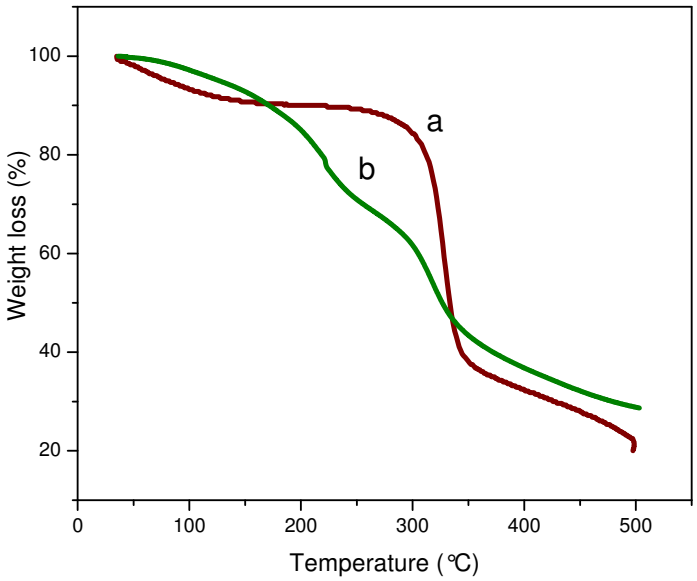


Figure 5.8. Thermal properties of a) XG, b) XG-NH_2 by thermogravimetric analysis.

The second stage from 220 to 300 °C and the third stage from 300 °C to 500 °C may contribute to the decomposition of different structure of the modified xyloglucan. Below 170 °C, the aminated xyloglucan had lower weight loss than xyloglucan. Subsequently, weight loss increases steeply with temperature. But above 330 °C the aminated xyloglucan had a lower weight loss than xyloglucan. Results are shown in Figure 5.8.

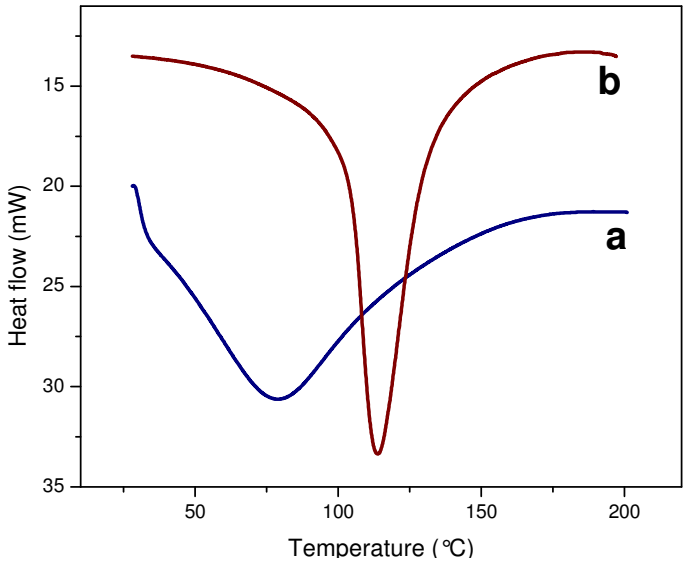


Figure 5.9. Thermal properties of a) XG, b) XG-NH_2 by differential scanning Calorimetry.

The DSC curves of XG and XG-NH_2 show an important qualitative difference (Figure 5.9). The XG showed a broad melting point around 78 °C meanwhile the melting point of XG-NH_2 was observed at 115 °C.

5.3.7. XG-NH_2- Formation of hydrogels

Aminated xyloglucan was tested for its gel formation competency. They formed very strong gels in water and in NaOH (Figure 5.10). The *insitu* gel formation of XG-NH_2 in water was attributed to the complexation reaction between NH_2 groups and water molecules to form NH_3^+ --- OH^- which holds the water molecules inside the matrix of aminated xyloglucan. It is known that, only at particular concentration range, polymers forms gel. It is imperative to study the strength of gels formed at different concentrations in water and NaOH. The dynamic viscoelastic measurements have

been used as an excellent tool for understanding the gel formation and gel strength. It uses oscillatory shear stress or strain and measure the response from the developing gel. Figure 5.11 shows the frequency dependence of the storage shear and the loss shear moduli of the XG-NH_2 gels.

Figure 5.10. Aqueous XG-NH_2 gel.

The polymer if it is a gel, the storage modulus value (G') will be higher than loss modulus (G'') and if it is a sol, the G″ will be higher values than G'. The XG-NH_2 does form hydrogels above 7 % (w/v). The G' was larger than G'' in the experimental conditions at 30 °C, indicating that aminated xyloglucan formed a gel structure. However, below 7 wt % of XG-NH_2 it could not form hydrogels because the number of helices and aggregates is not enough to form a percolated network in water. In addition, the gel strength is also determined by calculating the difference between their storage and loss moduli (ΔG_{moduli}). In 7 % (w/v) hydrogel, the ΔG_{moduli} was 1.2 x 10^{3} Pa and 8% (w/v) hydrogel showed a less gel strength value of 0.7 x 10^{3} Pa whereas

the gel strength of 9% gel was reduced to below 0.1 10^3 Pa. Hence, the strong hydrogel was formed at 7% (w/v) XG-NH_2. The frequency dependent rheological analysis of 7, 8, and 9 % (w/v) XG- NH_2 in aqueous NaOH showed gel formation. In NaOH, 7 % (w/v) XG-NH_2 was did not show any sign of a good gel meanwhile, 8 % aminated xyloglucan showed very high gel strength of 3.1 x 10^3 Pa and its gel strength reduced, since higher concentration of a polymer causes phase separation, resulting in a weak gel, similar trend is also seen in 9 % (w/v) hydrogel .

The phase angle value is less than 40 degree for all the cases, which shows that they are true gels. When compared with the animal derived polysaccharide amines such as chitosan, an 8 wt % aqueous solution could not form a strong gel, where as aminated xyloglucan can form a gel. This higlights the specific gel forming ability of aminated xyloglucan in both water and NaOH.

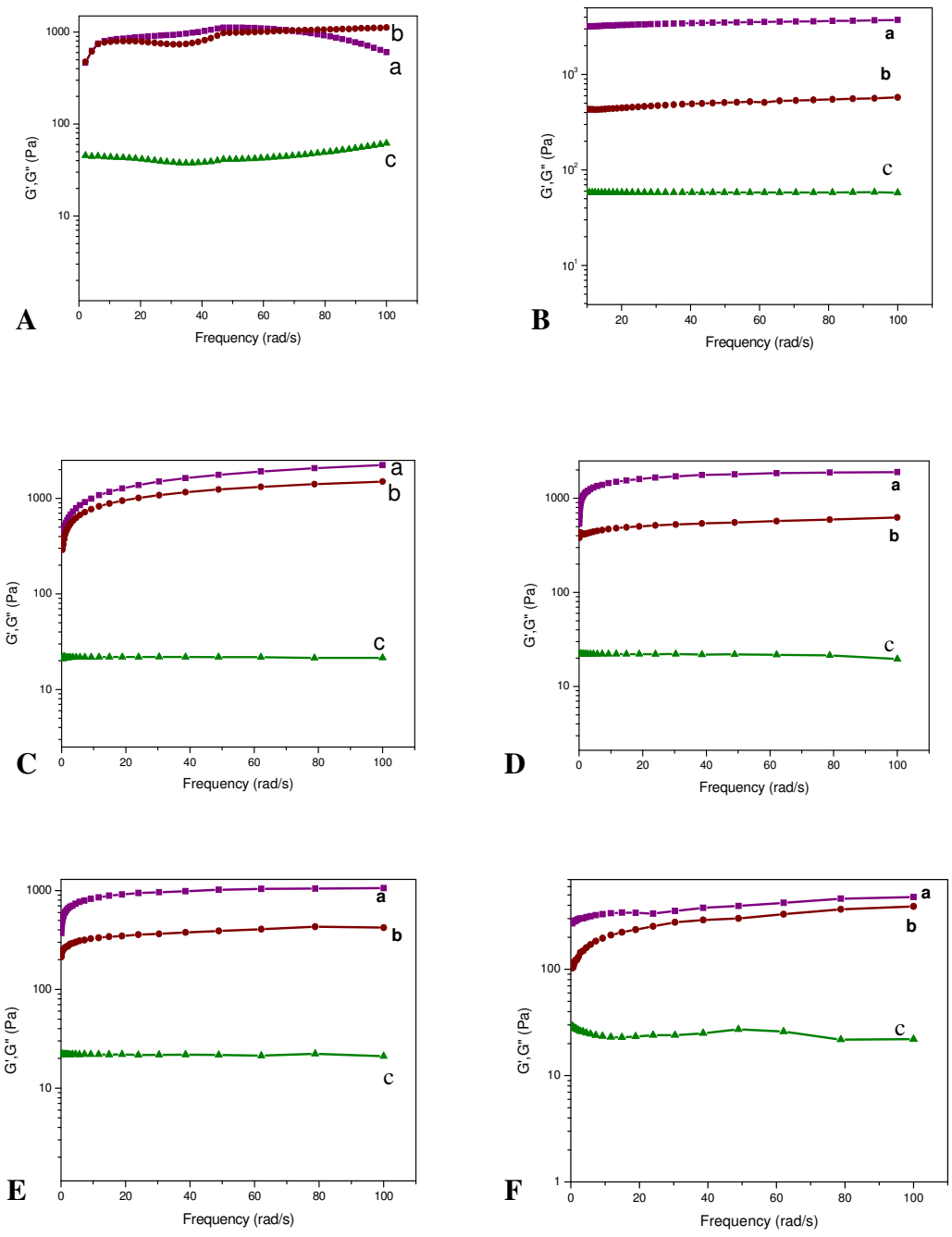


Figure 5.11. Rheology of aminated xyloglucan give the gel strength and confirm the formation of gel. Gel formation at different conditions A) 7% (w/v) gel in NaOH B) 8% (w/v) gel in NaOH, C) 9% (w/v) gel in NaOH, D) 7% (w/v) gel in water E) 8% (w/v) gel in water, F) 9% (w/v) gel in water at 28 ±ºC.

5.3.8. Morphology

A flat ribbon like chain structure was observed for the XG hydrogels in SEM analysis. This structure formation was due to the intercalation of water molecules (Figure 5.12).

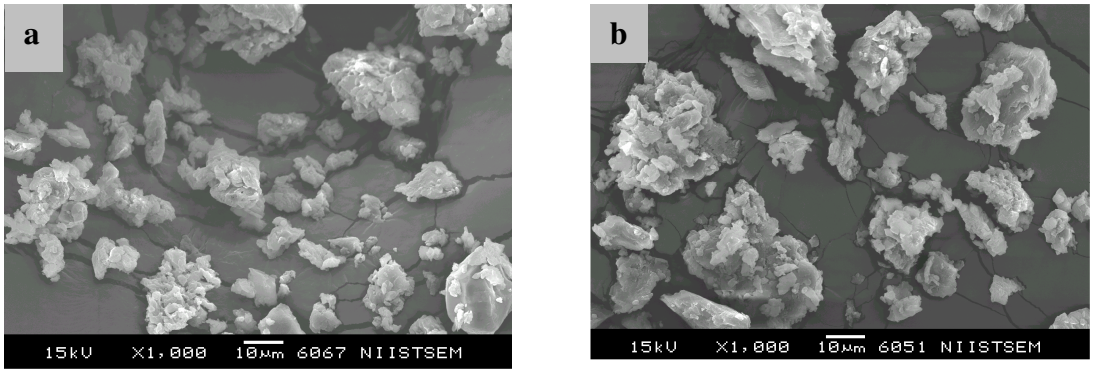


Figure 5.12. SEM image of a) XG, b) XG-NH_2 freeze dried powder.

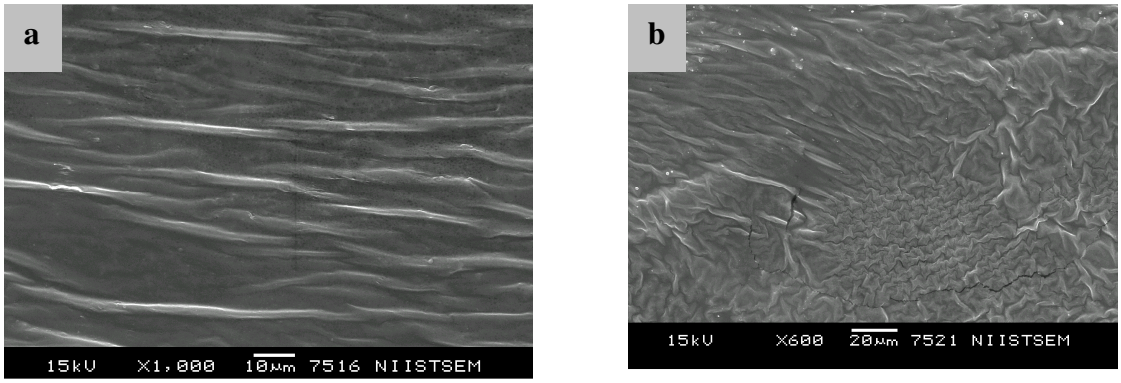


Figure 5.13. SEM image of a) & b) XG-NH_2 gel in aqueous medium.

Whereas the native XG and XG-NH_2 at dry condition showed plate like structures. In aqueous medium XG-NH_2 forms a gel and has intercalated fiber structure. This observation clearly differentiates the existence of a different morphology of XG-NH_2

due to hydogel formation (Figure 5.13). The similar morphology of XG and XG-NH_2 at dry condition may due to the lower DS values.

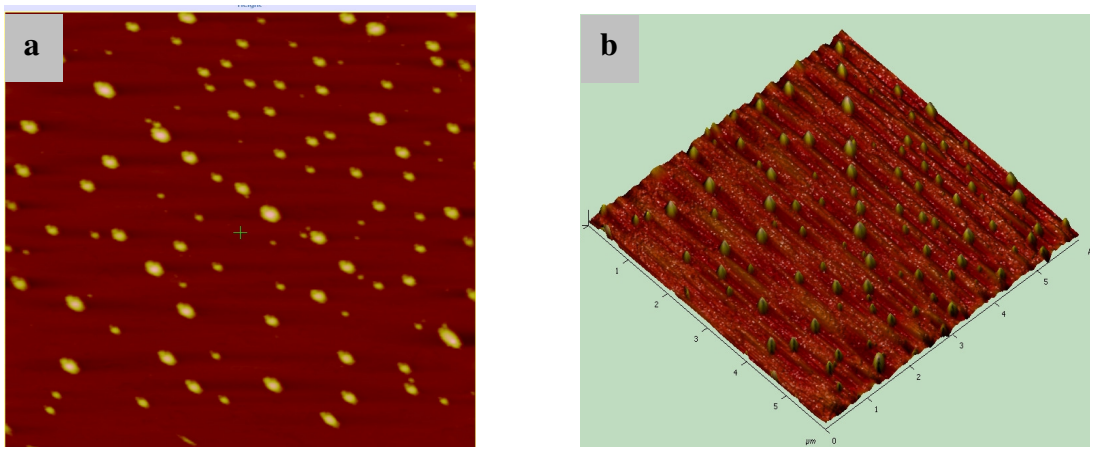


Figure 5.14. AFM image of XG-NH_2 a) Two dimensional image, b) Three dimensional image at low concentration. At low concentration it forms a self assembled spherical structure.

A very interesting observation of aminated xyloglucan is the self assembled spherical nano-particle formation at very low concentration (0.2 % w/v) in aqueous medium. The self assembled XG-NH_2 showed particle size of 60 nm was confirmed by AFM analysis (Figure 5.14).

5.3.9. Fluorescence of XG- NH_2 and their hydrogel

Biocompatible and water soluble hydrogels with fluorescence property especially blue are very attractive for various applications in medical filed like fluorescence tagging.

In solid state XG-NH_2 shows a green fluorescence. At 350 nm, amino xyloglucan showed green fluorescence with an emission at 479 nm but xyloglucan showed no blue florescence since it has an emission at 412 nm. At 425 nm wavelength, the emission

was at 489 and 494 nm respectively. Both XG and XG-NH_2 showed fluorescence behavior at all wavelengths investigated (Figure 5.15).

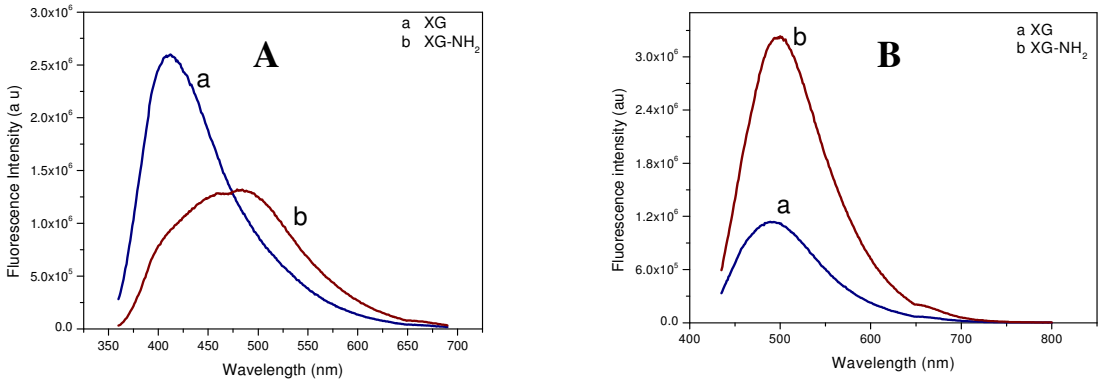


Figure 5.15. Fluorescent Analysis of XG and XG-NH_2 excited at A) 350nm, B) 475nm.

In all the cases XG-NH_2 exhibited bathochromic shift (red shift). Shift of a spectral band to longer wavelengths is mainly due to the influence of substitution or a change in environment. Changes in the environment include both chemical and physical changes and secondary interaction such as H- bonding, chain entanglements and the crystallite nature.

To explore the effect of solvent (water) specifically the existence of fluorescence in hydrogel of XG-NH_2, the fluorescence study was conducted in aqueous condition at 275 nm wavelength. For obtaining excitation wavelength first studied the absorption characteristics of aqueous solution of XG and XG-NH_2 using UV Visible spectroscopy. Both XG and XG-NH_2 gives absorption maxima at 275nm (Figure 5.16 A).

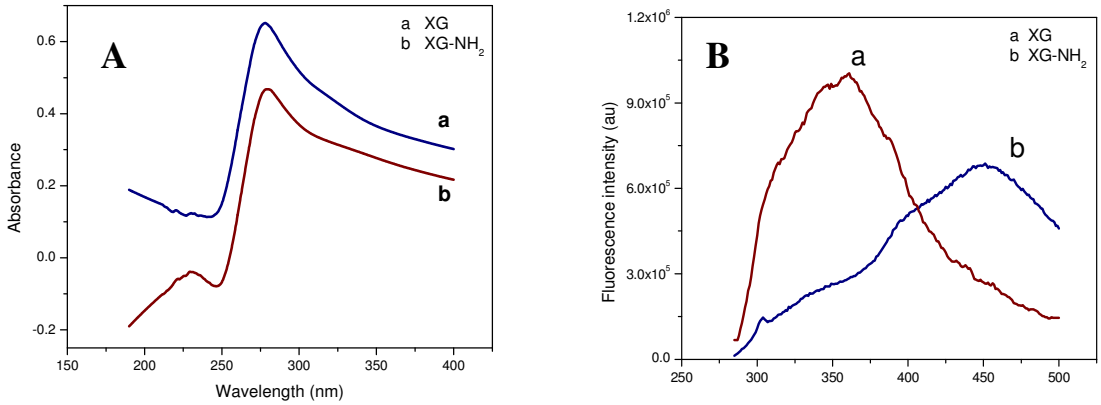


Figure 5.16. Fluorescent analysis in aqueous medium A) UV-Visible spectrum B) fluorescent analysis of XG and XG-NH_2 excited at 275nm.

In the presence of water molecules, XG-NH_2 showed blue fluorescence at 450 nm whereas the XG emission peak was observed at 359 nm (Figure 5.16 B). The valuable observation of this study is that the XG-NH_2 also has blue fluorescence emission properties in aqueous medium.

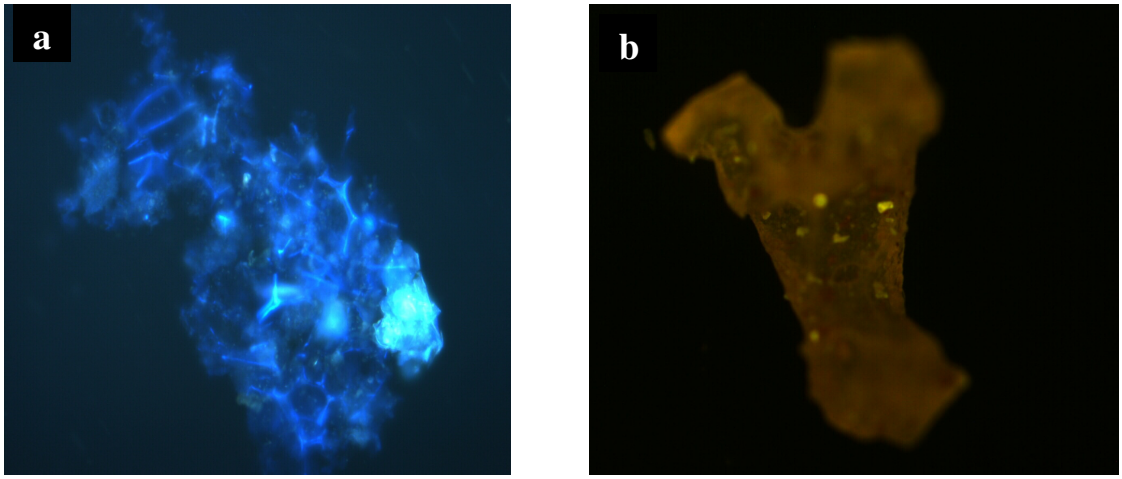


Figure 5.17. Fluorescent micrograph of a) aqueous XG-NH_2 gel, b) solid XG-NH_2.

A blue fluorescent micrograph of XG-NH_2 has been shown in Figure 5.17. This modified xyloglucan has potential applications in the medical and biotronics filed because it possess biocompatibility, strong hydrogel behavior with very useful blue fluorescence.

5.3.10. Antimicrobial activity and Texture profile

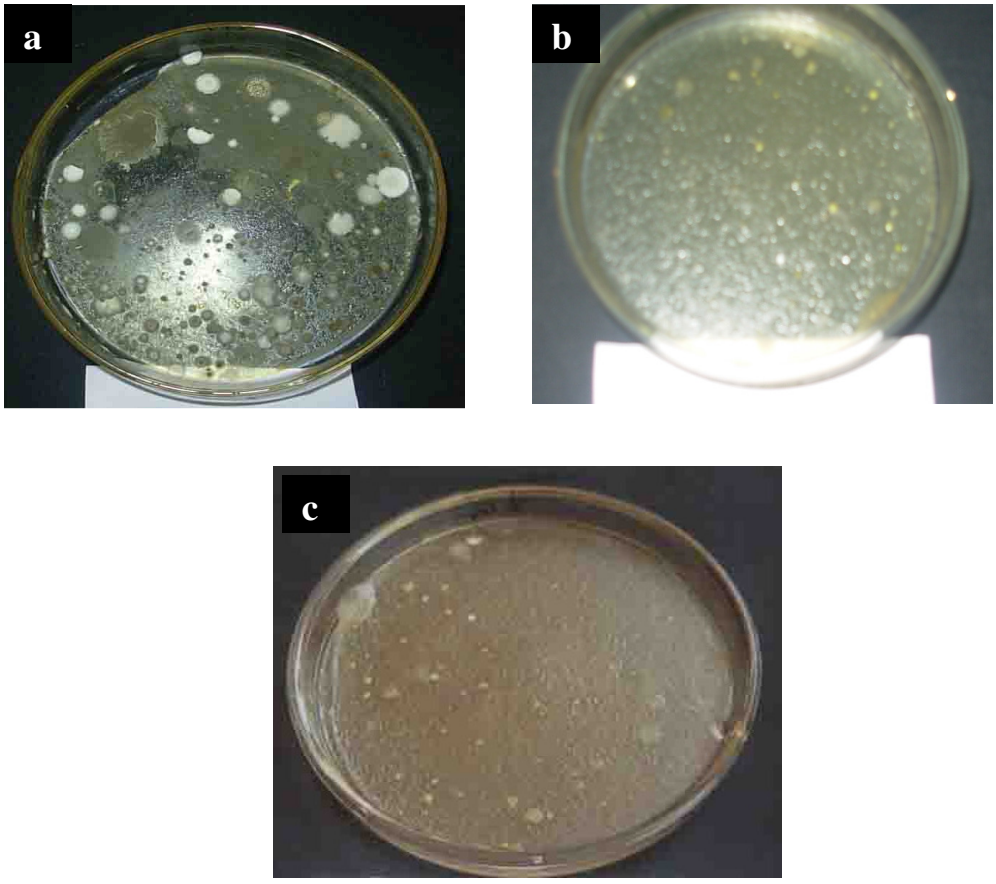


Figure 5.18. Antimicrobial studies of a) Nutrient agar plate as such, b) with chitosan gel, c) with 1 % (w/v) XG-NH_2 gel. Incubated for h at 28± ºC.

The XG-NH_2 shows good antimicrobial activity in comparison to chitosan since it allow less growth of the organisms, as seen from the growth of bacterial colonies,

compared to the chitosan in nutrient agar petri plates exposed to atmospheric contamination at room temperature (28± ºC).

5.3.11. Texture analysis

The hardness (HA), adhesiveness (AD) springiness (SP), cohesiveness, and chewiness of 7% XG-NH_2 hydrogel were studied. Hardness of gel was 2.89 N which was higher than the other xyloglucan gels. Adhesiveness is a surface characteristic and depends on a combined effect of adhesive and cohesive forces, and also viscosity and viscoelasticity. The XG-NH_2 hydrogel showed the negative value for adhesiveness (-0.62). Springiness or elasticity is a sensitivity of gel rubberiness in the mouth, and is a measure of how much the gel structure is broken down by the initial compression. High springiness appeared when the gel structure was broken into few large pieces during the first texture profile analyzer compression, whereas low springiness resulted from the gel breaking into many small pieces. Less springy gels will break down more easily during mastication than a firm and springy gel. Aminated xyloglucan gel has high springiness value of 1.0714. Chewiness is the quantity to simulate the energy required for masticating a semi-solid sample to a steady state of swallowing process. It is the product of gumminess and springiness. Chewiness of XG-NH_2 gel was 2.3389. Cohesiveness is an index that how well the product withstands a second deformation relative to its behaviour under the first deformation. It has a cohesiveness value of 0.7554, and shows a good gumminess property and the value is 2.1831. Texture profile analysis showed that aminated xyloglucan gel had good springiness, hardness, and

gumminess nature. These properties are very useful in food, feed, paper, pharmaceutical and adhesive industries.

5.4. Conclusions

We have accomplished the synthesis of a blue fluorescent cationic xyloglucan hydrogel by a facile synthetic strategy viz. amination of xyloglucan. The amination of xyloglucan polymer was confirmed by FTIR and NMR analyses. It evinces a good fluorescence property and emits blue fluorescence in aqueous medium and yellowish green fluorescence in solid state. The strength of hydrogels is good enough to be a strong hydrogel. It forms a self assembled nano particles of size 60 nm in aqueous medium at a very low concentration. The aminated xyloglucan also possesses good thermal properties compared to native xyloglucan. In addition, its antimicrobial and texture properties are very promising to find extensive use in food related applications. This cationic xyloglucan hydrogels can also be used as a substitute for the animal derived chitosan in various other applications such as for fluorescence tagging, joint cushioning and lubrication, pharmaceutical and as a food additive because of its excellent colligative properties.

CHAPTER 6

Transparent xyloglucan-chitosan complex hydrogels for different Applications

This chapter reports the oxidation of xyloglucan and its transparent hydrogel formation with chitosan. The xyloglucan was oxidized by using periodate and the oxidized xyloglucan was blended with chitosan which formed versatile irreversible transparent hydrogels.

6.1. Introduction

There has been growing interest in the use of sensitive hydrogels for biomedical, cosmetic and food applications [Rosiak *et al.*, 1999; Hoffman, 2002]. Hydrogels are widely used especially in medicinal field as ideal tissue engineering constructs for cell gene therapy [Petrini *et al.*, 2003], enzyme and cell encapsulation [Aebischer *et al.*, 1991; Emerich *et al.*, 1992; Zielinski *et al.*, 1994], drug delivery, joint cushioning and lubrication [Miyazaki *et al.*, 1998; Barbucci *et al.*, 2002; Cohn *et al.*, 2003; Drury *et al.*, 2003; Crompton *et al.*, 2005], because of their low interfacial tension and high molecular and oxygen permeability. The cosmetic and personal care product manufacturers claim that there is burgeoning trend for transparent products, for instance, those which use clear formulation techniques in their gels and emulsions. Xyloglucans are the main glycans that interlace cellulose microfibrils in most flowering plants. Besides, being a structural component of primary cell walls [Hayashi, 1989; Hayashi *et al.*, 1994], xyloglucans play other important roles, namely the control of cell expansion, effect on growth, and as a reserve of carbon in seeds of many dicotyledons. Xyloglucan extracted from tamarind seed is a polysaccharide which has (1→4)-linked β-D-glucan main chain, substituted at O-6 by single-unit α-D-xylopyranosyl side-chains. Some of them are further substituted at O-2 by β-D-galactopyranose [Hayashi *et al.*, 1994; Yuguchi *et al.*, 2004]. Tamarind seed xyloglucan (TSX) is soluble in water and used in Japanese food industries as a thickener because its solution has high viscosity and stability against heat, pH, and

shear [Shirakawa *et al.*, 1998]. The x-ray diffraction data proved that xyloglucan takes a 2-fold helix conformation. It shows synergism with helix-forming polysaccharides such as agarose, carrageenan, and xanthan. Xyloglucan along with xanthan or gellan or curdlan is reported to have synergistic interaction at low temperatures and results in an increase in the elastic moduli [Nitta *et al.*, 2003]. Pure xyloglucan extracted from tamarind seed as such does not form a gel; however, it forms a thermoreversible gel in the presence of a large amount of alcohol or sugar or by the addition of a polyphenol such as epigallocatechin gallate [Yamanaka *et al.*, 2000; Nitta *et al.*, 2004; Yuguchi *et al.*, 2004]. It was also reported that xyloglucan alone can form a gel if a part of the galactose is removed. The gel strength became greater with increasing removal ratio of galactose from Xyloglucan [Miyazaki *et al.*, 1998; Suisha *et al.*, 1998; Kawasaki *et al.*, 1999]. The gelation scheme is different for an aqueous solution of enzymatically degraded xyloglucan gel and the xyloglucan gelation in presence of alcohol and polyphenols. In the case of enzymatically degraded xyloglucan, the cross-linking domains are composed of aligned xyloglucan chains in the shape of flat plates, whereas no ordered structure was found for the cross-linking domains in the xyloglucan /ethanol system at lower temperatures.

The present invention relates to a crystal clear, colourless, nontoxic, biodegradable, biocompatible novel thermostable gel from xyloglucan and chitosan co-polymer and characterisations aim to demonstrate its wide range of potentials in different areas such as cosmetic, food and biomedical. The gel can be used for a variety of applications, such as make-up cosmetic or basic cosmetic such as face wash, milky lotion, cream or

foundation, with excellent elasticity and aging stability, giving refreshing feeling, free from stickiness and having excellent usability, as a ultraviolet protective agent or as a tissue adhesive which can be used, including haemostasis, wound sealing, tissue engineering or localized drug delivery as capsules & tablets and also can be used as a food ingredient, and supplement for metabolic disorders since it is not digested by the human digestive enzymes and yields zero calorie to the diet.

6.2. Experimental

6.2.1. Materials

Xyloglucan extracted from tamarind seed which was purchased from the local market in Trivandrum, Kerala, India and extracted as described in chapter 2. All other chemicals used were reagent grade.

6.2.2. Methods

Oxidized xyloglucan and xyloglucan chitosan composite gel was characterized by FTIR spectroscopy, UV-Visible spectroscopy, scanning electron microscopy, High resolution transmission spectroscopy, Thermogravimetric analysis, Differential Scanning Calorimetry, MALDI-TOF mass spectrometer, X ray Diffraction pattern, rheometer and food texture analyser.

6.2.2.1. Oxidation of xyloglucan

The oxidation reaction of xyloglucan was carried out in aqueous solution by using periodate as oxidizing agent. The volume ratio of sodium periodate (1%) to xyloglucan

(10%) was taken as 1:1. Continued the reaction under stirring for 6 h in the dark at 25 ± 2 °C. Then the reaction was quenched by the addition of ethylene glycol. The oxidized xyloglucan was purified by dialysis against distilled water for 12 h and the product formed was precipitated with ethanol and vacuum dried at 30±2 °C [Gomez *et al.*, 2007]. Reaction was controlled by adjusting the periodate concentration and duration of the reaction.

6.2.2.2. Preparation of xyloglucan chitosan composite gels (Chitam gel)

Appropriate amount of oxidized xyloglucan was mixed with 1% chitosan in 1% aqueous acetic acid under stirring to prepare the gels. The effect of pH (3 to 6) and concentration of chitosan (0.01g-0.04g) on the gel strength was investigated. The influence of addition of flavours (like vanillin), sweeteners [like sucrose (20% (w/v) and aspartame (1% (w/v)], salts [like NaCl (1.5% (w/v)] and citric acid (0.6% (w/v) was examined by rheology and texture analysis. These ingredients are incorporated into the xyloglucan chitosan gel by dissolving appropriate amount during the preparation of the gel.

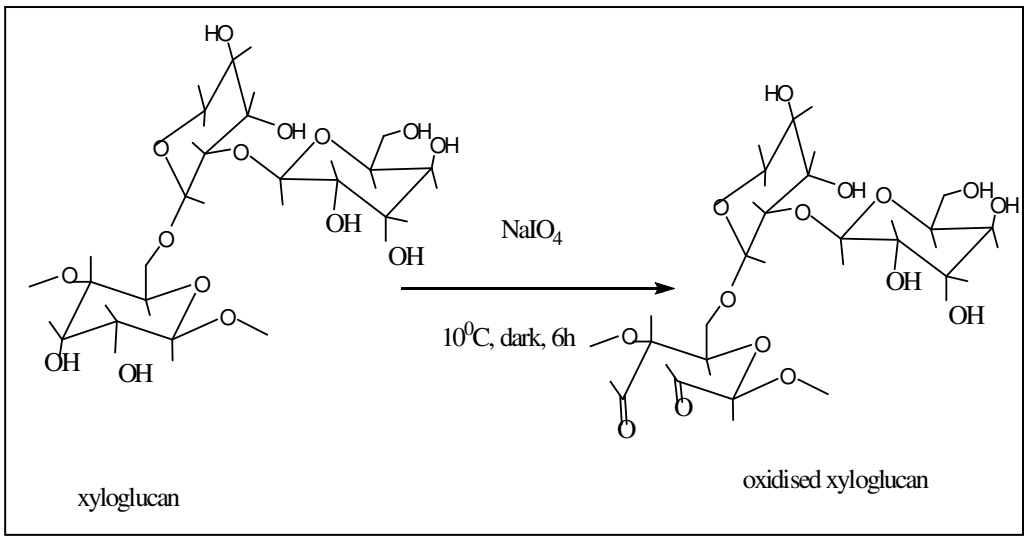
6.2.2.3. Antimicrobial tests

Antimicrobial activity was studied by using nutrient agar plates. Appropriate amount of nutrient agar gel was taken in a petri dish along with xyloglucan, xyloglucan chitosan gel or chitosan. This plate was exposed to atmosphere at room temperature (28 ±3 ºC) to contaminate. The bacterial colonies was counted after 48 h of growth.

6.3. Results and discussions

6.3.1. Characterization of Oxidised XG and their composite transparent gels with chitosan

Oxidation causes the rupture of carbon– carbon bond and to form two aldehyde groups in each oxidized monomeric unit (Scheme 6.1).



Scheme 6.1. Mechanism of oxidation of xyloglucan by periodate.

Hydroxyl groups on the carbons 2 and 3 of the repetitive unit were oxidized by sodium periodate. Therefore, new reactive groups having larger rotational freedom along the polymer backbone were obtained which can be used for further chemical modifications. The xyloglucan has a molecular weight of 298.51 KDa and the oxidized xyloglucan observed with a lower value of 169.115 KDa (from MALDI-TOF). The decrease in molecular weight may be due to the oxidative degradation of xyloglucan in the presence of sodium periodate. The oxidation of XG using $NaIO_4$ and the formation

of gel were analyzed by FTIR (Figure 6.1). The oxidation of XG (i.e. conversion of secondary hydroxyl groups into aldehydic functionality) is confirmed by the appearance of characteristic –CO band at 1742 cm^{-1}. The formation of gel between oxidized XG and chitosan is restructured via the reaction between -CHO (of oxidized XG) and $–NH_2$ (of chitosan).

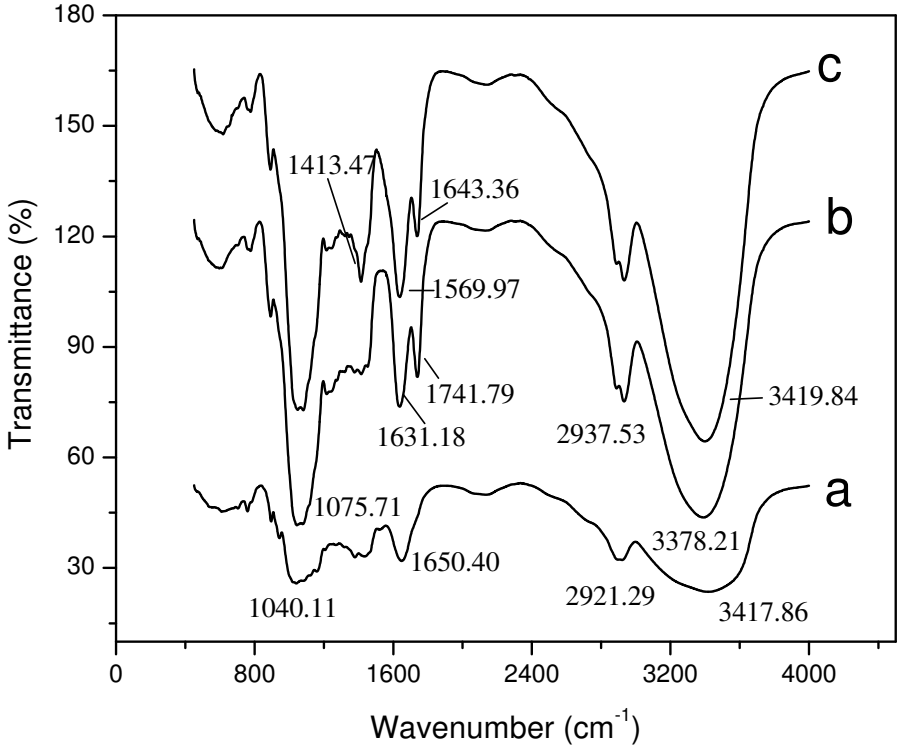
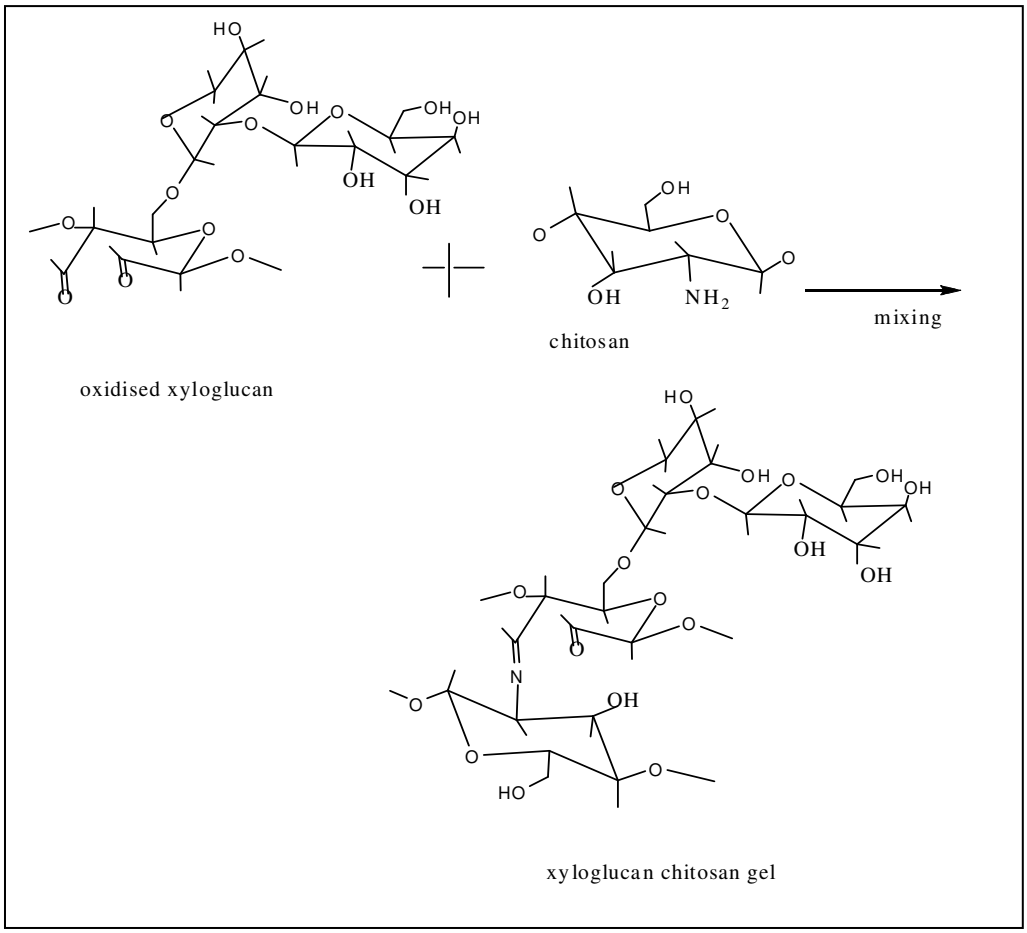


Figure 6.1. FTIR spectra of a) XG, b) oxidised XG, c) xyloglucan chitosan composite.

This reaction leads to the formation of imine groups (-C=N-) by the elimination of water molecule (Scheme 6.2). In the gel, the –CO groups disappeared and a new band was observed at 1644 cm^{-1} which corresponds to – C=N- groups. In addition, the hydroxyl absorption in oxidized XG was lowered to 3378 cm^{-1} due to the enhanced H-

bonding facilitated by the –CO groups *vis-à-vis* the absorption observed in native XG at 3420 $cm.^{-1}$



Scheme 6.2. Mechanism of formation of xyloglucan - chitosan composite gel.

6.3.2. Xyloglucan chitosan complex gel (Chitam gel) and their microstructure

Hydrocolloid gels are very close to either a liquid or to a solid. The liquid-like properties result from the fact that the major constituent (> 80%) is water. The solid-like behaviour is due to the network formed, and charactersied by a finite elastic modulus. The term 'physical hydrogel' has been coined to describe non-covalently crosslinked network to distinguish it from chemical hydrogels formed by covalent

bonds. Chemical hydrogels are commonly water-swollen networks of hydrophilic homopolymers or copolymers. For polysaccharide hydrogels, the term 'junction zone' has been frequently used to describe the crosslink, since each crosslink involves aggregates of ordered molecular chains like helices. The bonds involved in the junction zones are generally non-covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds, etc [Nishinari *et al*., 2000]. These transparent gels are basically falls under the category of chemically crosslinked hydrogels. Here, the oxidized xyloglucan formed a strong transparent gel with chitosan without any crosslinking agent (Figure 6.2).

Figure 6.2. Transparent xyloglucan -chitosan composite gel (Chitam gel).

The physical intermolecular interactions occur, either involving cooperative association of the chain segment belonging to different polymers, in this case chitosan and xyloglucan, in the formation of junction zones, or via chain chain association by virtue of charge-charge attraction. The hydrogel pattern is attributed to the formation

of complex between oxidized xyloglucan and chitosan which is attributed to the intermolecular interaction by covalent linkage between aldehyde group of xyloglucan and amino group of chitosan, which resulted in the removal of water molecule. The presence of more reactive carbonyl group in xyloglucan to some extent helps to form a gel by the inter and intra molecular hydrogen bonding and synergetic gel formation. Gel strength depends on the concentration of xyloglucan and chitosan. Physically, no difference has been noticed between these gels, but rheological analysis showed a remarkable difference in their strength.

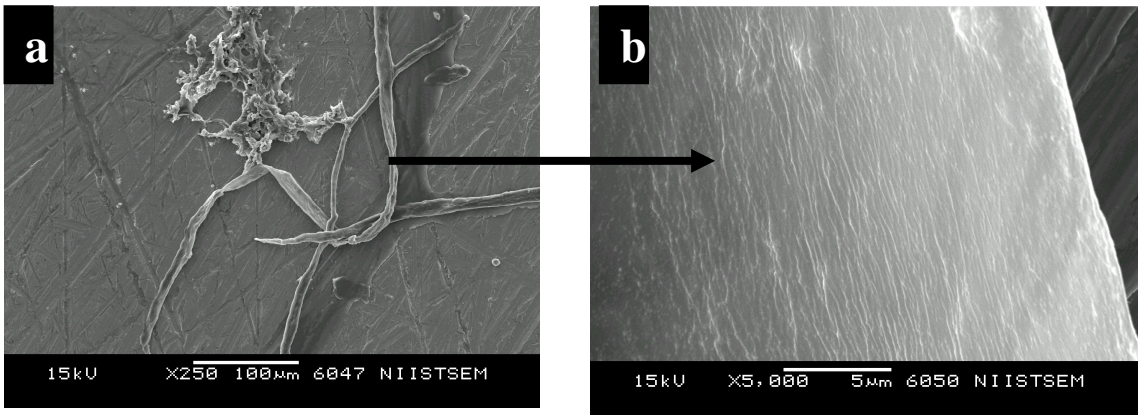


Figure 6.3. SEM image of a & b) xyloglucan at different magnifications which indicates its fiber nature in water.

From scanning electron micrograph it was clear that xyloglucan forms a uniform fibrous structure in water (Figure 6.3). But the gel formed by oxidized xyloglucan with chitosan has a intercalated network structure, having self assembled particles of 2-4 micron size (Figure 6.4).

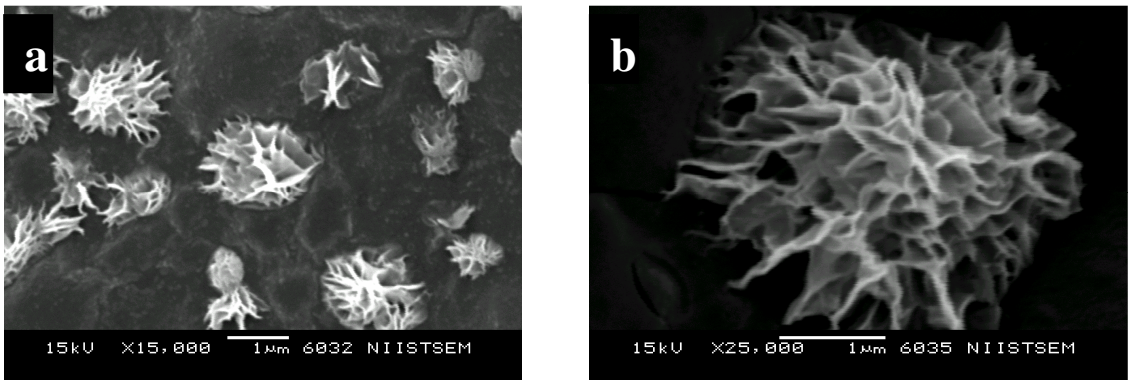


Figure 6.4. SEM image of a & b) xyloglucan chitosan gel at different magnifications.

This was confirmed by HRTEM (Figure 6.5). The xyloglucan-chitosan has a definite intercalated self assembled gel structure having elongated particles of chitosan of 0.2-0.4 micron length and 50-70 nm breadth, with smaller (~100-150 nm) spherical particles of xyloglucan embedded in the gel. At a higher magnification (inset of Figure 6.4 b), the spherical structures of xyloglucan is seen clearly.

Scanning electron micrograph gives the surface morphology of xyloglucan as uniform fibers where as HRTEM reveals the internal net work structure of the gel. Hence in High resolution transmission electron micrograph, we can actually identify the inner structure of each fiber and particles in the gel network.

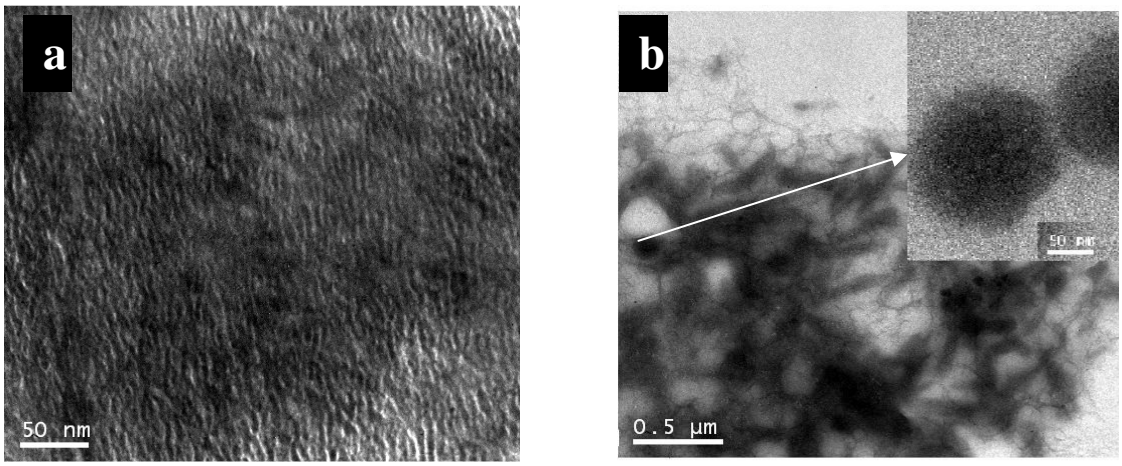


Figure 6.5. HRTEM image of a) xyloglucan and b) xyloglucan chitosan gel. Network formation in chitam gel was confirmed by this micrograph.

6.3.3. Thermal properties of Chitam gels

The DSC curves of xyloglucan and xyloglucan chitosan complex gels showed a marked difference (Figure 6.6). The xyloglucan and chitosan had a broad melting point of around 78 °C and 66 °C respectively and the melting peak of the chitosan was broader than the xyloglucan. Sharp peak indicates its crystalline nature and stability against heat. The melting point of xyloglucan chitosan complex in the ratio 1:1 and 1:4 is similar and was found to be 62 °C which was lower than the melting point of its components. This may be due to its lower interaction and/ or, the low inter and intra molecular hydrogen bonding. This transparent gel was highly influenced by the ratio of mixing of xyloglucan and chitosan (chitosan weight ratio changes from 0.1g to 0.4g). The melting temperature of the complex formed in the ratio 1:2 and 1: 3 was 85 °C.

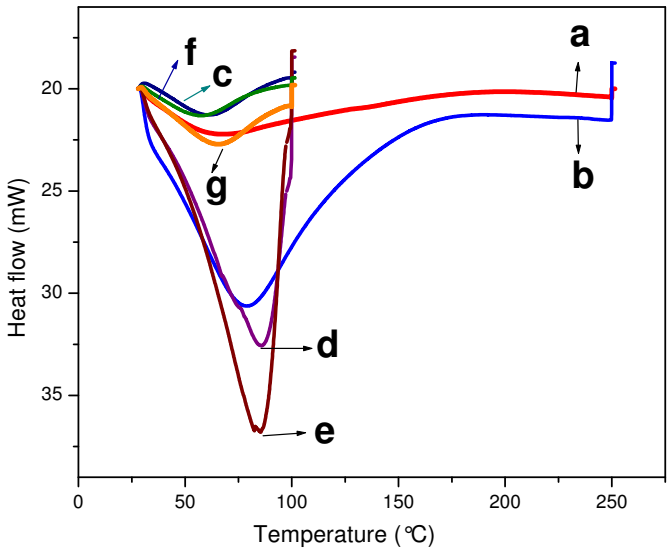


Figure 6.6. Heat flow properties of a) xyloglucan, b) chitosan, c) xyloglucan chitosan composite ratio 1:1, d) 1:2, e)1:3, f)1:4, g) 1:0.5 studied by differential scanning calorimetry.

Melting point at higher temperature and the presence of a sharp peak indicates that this complex at this composition needs higher energy for melting. In addition, the inference that, the sharpness of the peak is related to the strength of the complex was later established by the rheological measurements. Complex with lower percentage of chitosan (1:0.5) showed an endothermic broader melting peak at 65 °C similar to the chitosan alone.

Thermal properties of xyloglucan, chitosan, xyloglucan chitosan complex at different concentrations were studied by thermogravimetric analysis. TGA of xyloglucan showed a weight loss in two stages. The first stage ranges between 40 and 152 °C and shows about 10% loss in weight. This may correspond to the loss of adsorbed and bound water. The second stage of weight loss started at 289 °C and continued up to

351 °C during this time there was 49% weight loss due to the degradation of xyloglucan.

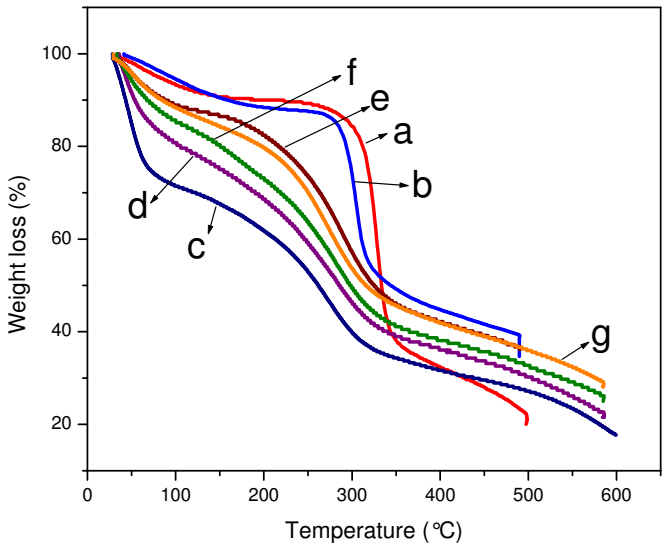


Figure 6.7. Thermal properties of a) xyloglucan, b) chitosan, c) xyloglucan chitosan composite ratio 1:1, d)1:2, e)1:3, f)1:4, g) 1:0.5 data obtained by thermogravimetric analysis.

The pattern of degradation was same for all the cases. The results are shown in Figure 6.7. Compared to chitosan, the xyloglucan has a slightly higher thermal stability and the weight loss increased steeply with the temperature. In chitosan, the first stage ranges between 43 °C and 180 °C. The second stage of decomposition was observed from 277 to 321 °C. Weight loss corresponds to this stage is 31%. Xyloglucan chitosan complex formed in the ratio of 1: 1 has a lower thermal stability, as it lost 24 % of its weight at 67 °C. In the composite form, the intermolecular attraction such as hydrogen bonding or Van der walls force may weaken and this in turn results in its

degradation at a lower temperature. At 300 °C, the weight loss was 62 %. Complex formed in the ratio 1: 3 are more stable than the other gels.

6.3.4. Rheological properties of the Chitam gel

The visco elastic nature of the gels can be determined by the dynamic measurements based on small oscillatory shear deformation experiment. Blends of two or more biopolysaccharides exhibit complex and often spectacular properties, which depends not only on the total polymer concentration, but also on the relative proportion of the polysaccharide components. This is also a function of the synergism between the primary and secondary structure of the component chains. Effect of xyloglucan chitosan ratio on gel strength was studied by dynamic rheology, which applies an oscillatory shear stress or strain and measure the response from the gel. The polysaccharide gels are classified into strong and weak gels by the visco elastic nature. In a strong gel G′ is always greater than G″ and is relatively independent of a wide frequency range. The variation of the storage modulus (G') and loss modulus (G'') with the frequency of oscillation (ω) for the gel are shown in Figure 6.8. In all the cases investigated, G′ was larger than G'' indicating a true gel nature. In the experimental conditions, both moduli showed a marginal increase with frequency (ω) indicating that oxidised xyloglucan -chitosan composite forms a strong gel, in the ratio of 1:3.

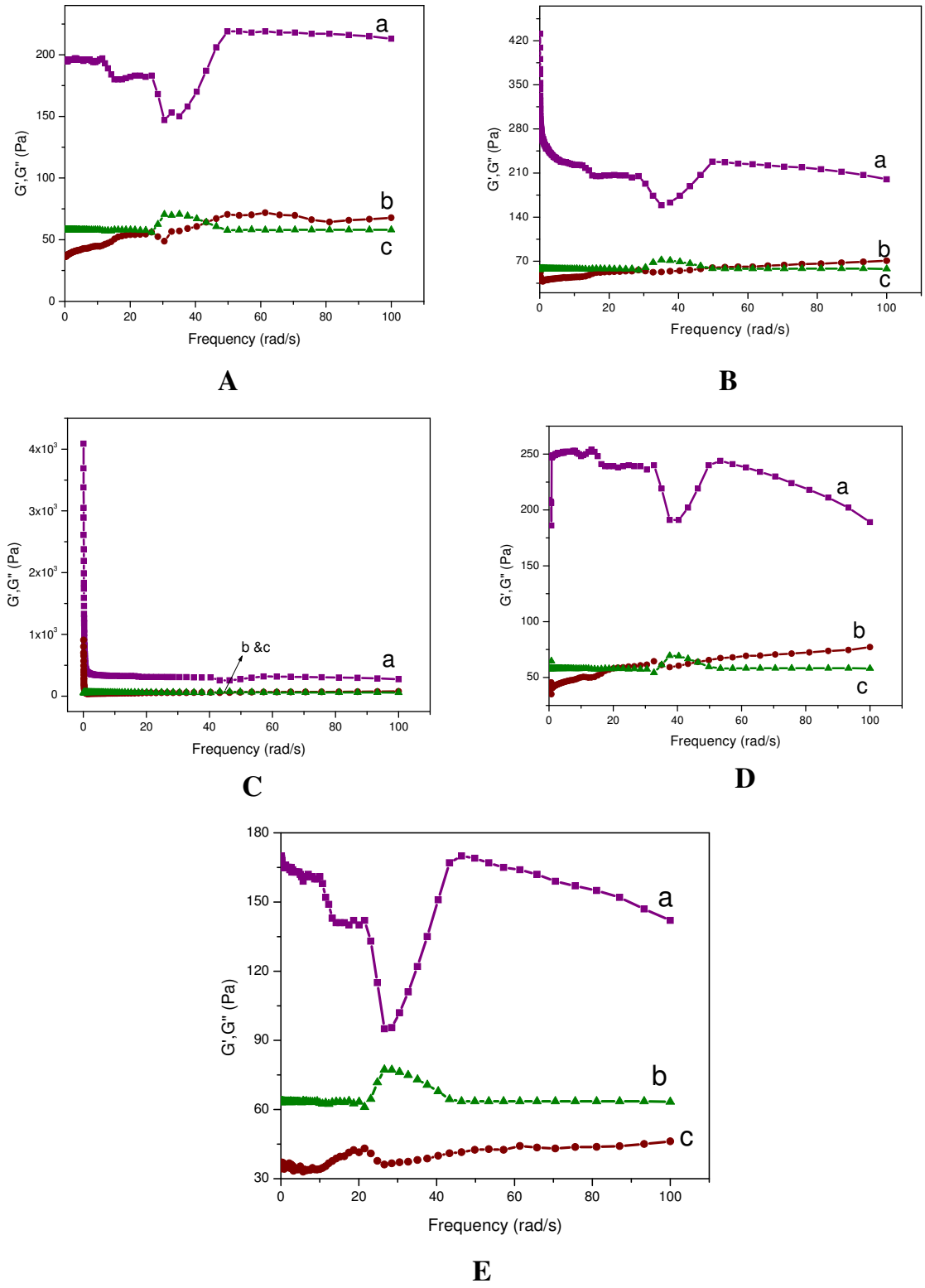


Figure 6.8. Effect of chitosan concentration on the xyloglucan-chitosan gel strength**:** [(a) storage modulus, (b)loss modulus,(c)phase angle)]. A) oxidized xyloglucan chitosan gel ratio (w/w) 1:1, B) 1:2, C)1:3, D)1:4, E) 1:0.5.

Above this concentration, due to mutual exclusion effect, one of the components in the system becomes biphasic. The phase angle values against frequency curve also agree with the above observation. In all the cases, phase angle value is in between 50 and 60 degree, which indicates the formation of a soft and ideal gel. This type of non toxic, soft transparent gel has wide use in pharmaceutical, cosmetic and food industry.

The effect of pH on the gel strength was also studied (Figure 6.9) by varying the pH (3, 4, 5, and 6). Rheological measurements indicate that gel strength decreased at below pH 4.0 and increases with increase in pH. Gel near pH 6.0 gives more strength and ideal rheological properties. Above this pH there is a precipitation tendency for the gel, as the chitosan becomes relatively insoluble.

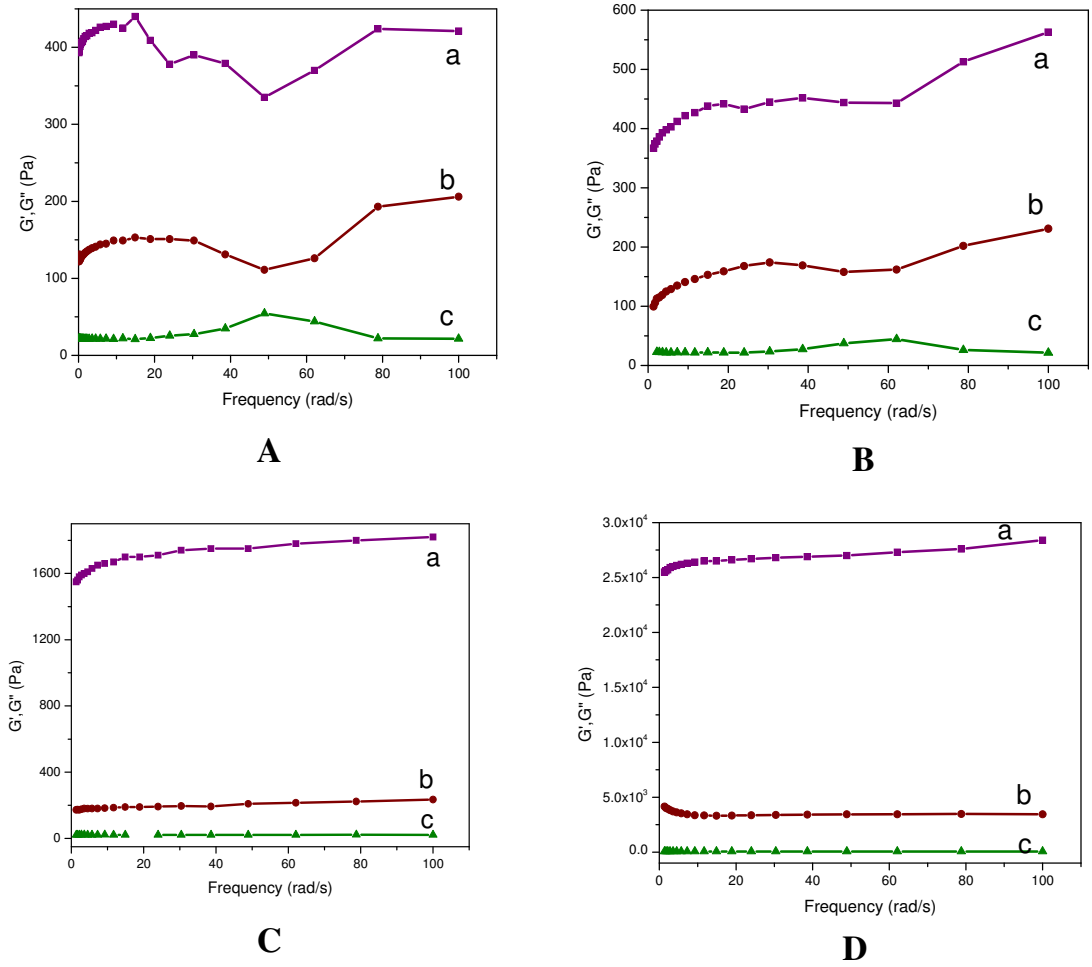


Figure 6.9. Effect of pH on the gel strength [(a) storage modulus, (b)loss modulus,(c) phase angle)] of xyloglucan-chtosan gel studied by a dynamic rheometer in the frequency sweep mode, at different pH values A) pH 3, B) pH 4, C) pH 5, D) pH 6.

Effect of incorporation of flavors, sweetener and salt on the rheology of the gel was studied. The results were shown in Figure 6.10. From figure it was clear that, in all the cases storage modulus is higher than the loss modulus, showing the preservation of a true gel structure. Even though, the storage modulus was higher than loss modulus, the difference between the moduli was decreased. In the entire cases phase angle was in between 40 and 50º.

The mechanical spectrum characteristics of the components in the network , in which different junction zones coexist and contribute significantly to the visco elastic properties at different frequency scales. The slight decrease in the difference between the modulus due to the addition of the food ingredients, did not significantly affect the gel strength, which is a prerequisite for its application in the food industry. This is more important since due to the beta linkage of the glucan, the gel will not get digested by the enzyme in the human digestive system, and hence is a zero calorie ingredient, however the beneficial bifidobacteria in the small intestine may assimilate a part of it, bringing down the cholesterol and glucose in diabetics.

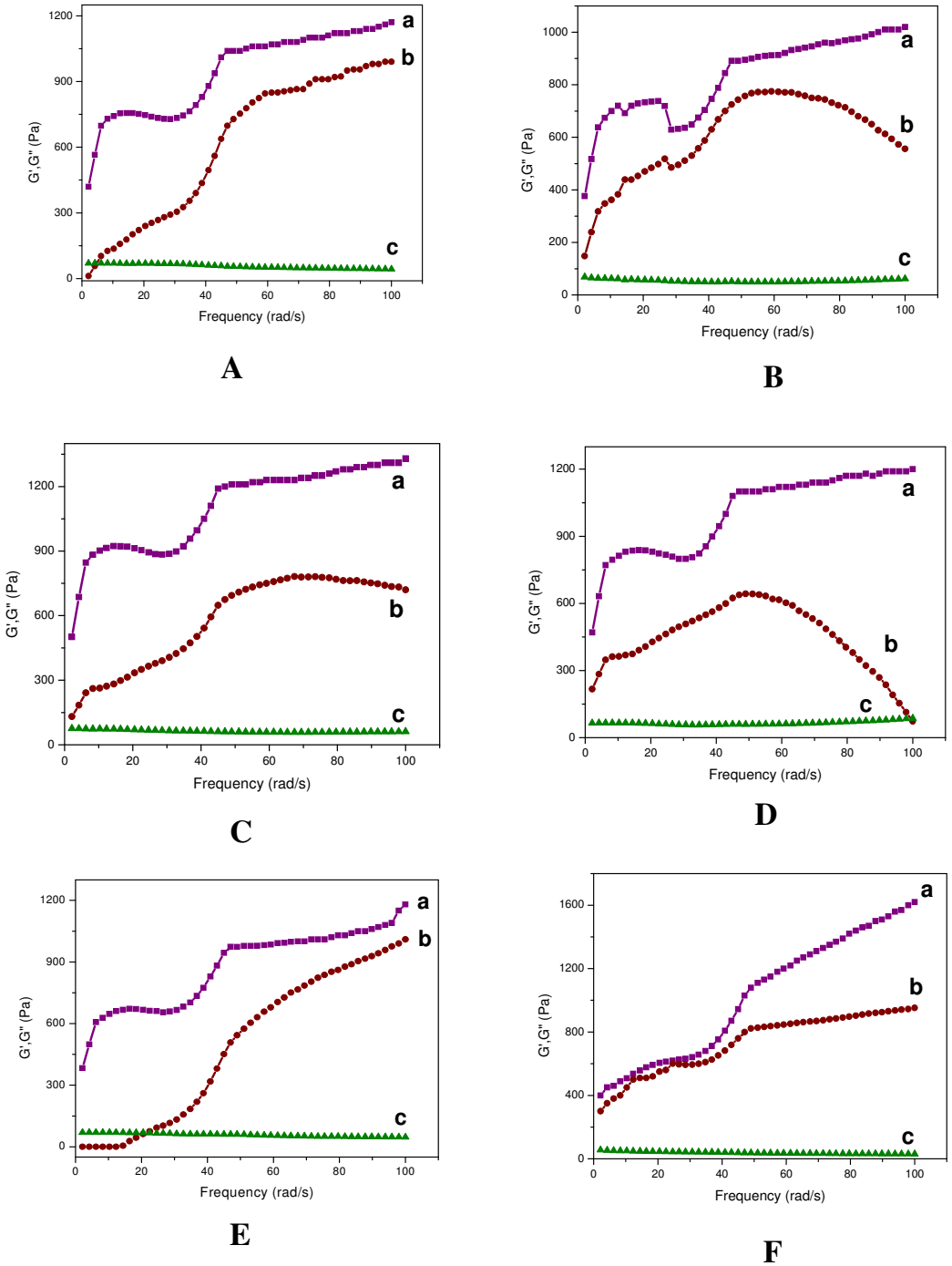


Figure 6.10. Effect of incorporation of food ingredients on gel strength by a dynamic rheometer in the frequency sweep mode: A) aspartame, B) sucrose, C) citric acid, D) flavour, E) Nacl, F) NaCl +sucrose+ flavour+ citric acid [(a) storage modulus, (b)loss modulus,(c)phase angle)] of xyloglucan-chtosan gel studied.

6.3.5. Texture studies of the composite gels

The hardness (HA), adhesiveness (AD) springiness (SP), cohesiveness and chewiness of the chitam gel as well as gels incorporated with salt, sweeteners, citric acid and flavours were examined. Results are shown in Table 6.1. Hardness of the 1:3 Chitam gel was 1.92 N which was higher than the other xyloglucan gels.

Table 6.1. Texture studies of transparent gel alone and in presence of additives

	Hardness	**Springiness**	**Cohesiveness**	**Chewiness**
Chitam gel	1.92	1	0.4717	0.9057
Sucrose	2.12	1.0909	0.4683	0.9928
Aspartame	1.52	1.0294	0.4195	0.6376
Citric acid	2.06	0.9211	0.4684	0.9649
NaCl	2.55	1	0.4416	1.1261
Flavour	1.94	1	0.4358	0.8454
Gel+NaCl+ Sucrose+Citric acid +flavour	0.19	1.0403	0.5197	0.0987

Springiness or elasticity is a sensitivity of gel rubberiness in the mouth, and is a measure of how much the gel structure is broken down by the initial compression. High springiness appeared when the gel structure was broken into few large pieces during the first texture profile analyzer compression, whereas low springiness resulted from the gel breaking into many small pieces. Less springy gels will break down more

easily during mastication than a firm and springy gel. Gel has springiness value of 1 and it has no effect on the addition of sweetners like sucrose and aspartame and also NaCl, citric acid, and flavours. Chewiness is the quantity to simulate the energy required for masticating a semi-solid sample to a steady state of swallowing process. It is the product of gumminess and springiness. Chewiness of gel was 2.34. Cohesiveness is an index that how well the product withstands a second deformation relative to its behaviour under the first deformation. It has a cohesiveness value of 0.47, and shows a gumminess property and the value is 0.906. Texture profile analysis showed that xyloglucan chitosan composite gel had good springiness, hardness, and gumminess nature and the addition of flavour, sweetners, and salts did not alter its textural properties to a large extent. These properties are especially useful and make the ease of modulation of gel in specialty food, and cosmetic industries.

6.3.6. Antimicrobial activity

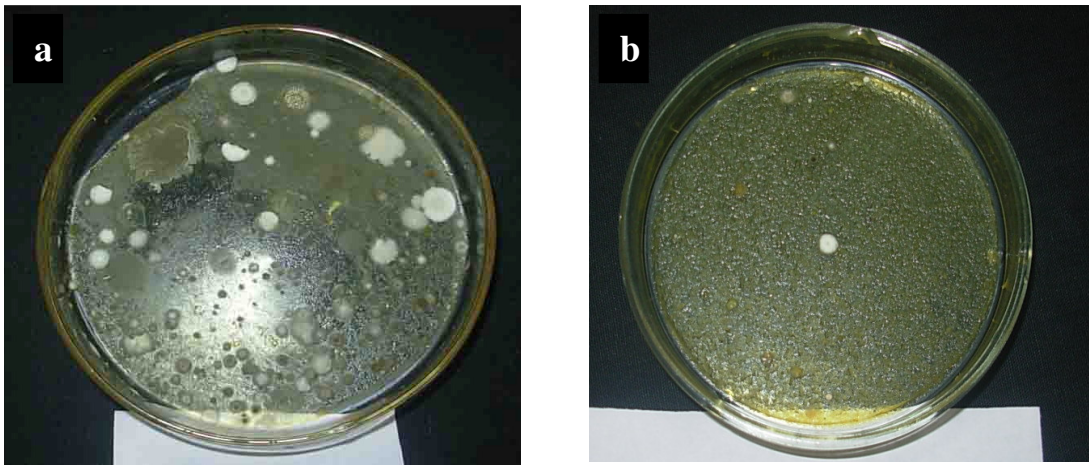


Figure 6.11. Antimicrobial studies at (28± ℃) for 48 h. a) Nutrient agar plate, b) Nutrient agar + Chitam gel

The petri plates containng nutrient agar with chitam gel was exposed to atmospheric contamination at room temperature (28± ºC) for 48 h. The composite gel showed good antimicrobial activity in comparison to xyloglucan since it did not allow the growth of the microbial colonies in the nutrient agar.

6.4. Conclusions

We have accomplished the synthesis and characterization of a transparent, colourless, nontoxic, biodegradable and biocompatible gel from oxidized xyloglucan and chitosan (Chitam). The oxidation of xyloglucan and its gel formation with chitosan are characterized by FT-IR. The gel has fibrous network structure in which oxidized xyloglucan has been observed in spherical and nanosize form. The hydrogel formation is attributed to the formation of complex between oxidized xyloglucan and chitosan which is attributed to the covalent linkage between aldehyde and amino group. The Chitam gels showed high gel strength at 1:3 composition. The Chitam hydrogel possesses good thermal properties compared to the native xyloglucan. In addition, its antimicrobial is very promising and in turn will find use in specialty food related applications. The texture studies show that it can also be used as an excellent food ingredient. The rheology of the chitam gel with the incorporation of common food ingredients such as sugar, sodium chloride, citric acid and flavor revealed that, the ingredients in the given concentration did not alter the gel nature. The gel which is transparent, colourless, thermostable, and biocompatible with ordered structure and from renewable resources and can be made cost effective is in great demand globally.

CHAPTER 7

Biodegradable biocompatible xyloglucan films for various applications

This chapter highlights the preparation, thermal and mechanical characterisation of xyloglucan films and xyloglucan chitosan and starch composite films. The swelling properties of the xyloglucan chitosan blend film, studied as a function of pH showed that the sorption ability of the blend film was high at pH 7.4. This indicates its controlled release property at that pH.

7.1. Introduction

In the past 50–60 years synthetic polymeric food packaging materials are used widely due to various advantages such as high strength, elongation, low cost, lightness and water resistance [Mali *et al.*, 2002]. These plastic materials are convenient, safe, strong and economical but not biodegradable. Polar biopolymers such as polysaccharides and proteins have been studied as potential alternatives in the film and plastic industries. Polysaccharides are known for their film-forming properties which have been intensively investigated for food and non-food applications. It has been shown that a wide range of film properties can be obtained due to the diversity of available polysaccharides [Lafargue *et al.*, 2007]. Edible and biodegradable films are thin, transparent and flexible materials obtained from natural biopolymers, such as starch, gelatin, etc [Fairley *et al.*, 1996; Paschoalick *et al.*, 2003; Vicentini *et al.*, 2005; Bergo *et al.*, 2007]. Biopolymer films are excellent vehicles for incorporating a wide variety of additives, such as antioxidants, antifungal agents, antimicrobials, drugs, colors, and other nutrients [Rhim *et al.*, 2006].

The interest in the study of biopolymer films has witnessed a steady increase as they are environmentally friendly alternatives to synthetic, non-biodegradable films and have been used to coat different products. Several investigations have been conducted to study the properties of protein, polysaccharide, and lipid-based films; and these raw materials were successfully formed into films or coatings [Artharn *et al.*, 2009; da Silva *et al.*, 2009; Ferreira *et al.*, 2009; Leon *et al.*, 2009; Limpisophon *et al.*, 2009;

Vasconez *et al.*, 2009]. The industrial application of these films for non-food uses is limited due to their brittleness, hydrophilic nature and their low mechanical strength. Plasticizers such as glycerol can be used to overcome these limitations by improving the processing and the flexibility [Lourdin *et al.*, 1997; Krogars *et al.*, 2003; Mehyar *et al.*, 2004]. Another way to reduce these drawbacks is the use of modified polysaccharide or the incorporation of another biopolymer, nanolayers of layered silicate or synthetic polymer such as poly (vinyl alcohol) (PVA), poly esters, etc [Tharanathan, 2003; Pandey *et al.*, 2005; Dicharry *et al.*, 2006]. Blending of two different polymers is common in work with synthetic polymers to achieve the desired properties for plastic applications and in biopolymers for food applications. When blending two polymers, it is important to know whether they are miscible or whether they will phase separate [Wang *et al.*, 2007].

After cellulose, hemicelluloses constitute the second most abundant class of polysaccharides found in nature. Xyloglucan coming under the category of hemicellulose is biodegradable neutral polysaccharide with structure similar to cellulose. The backbone consists of an α-1, 4-glucan residues about 80% of which are substituted by xylose. About 40% of xylose units are substituted with galactose. This substitution is the main reason for its solubility compared to cellulose [Rindlav-Westling *et al.*, 2003]. Chitosan provides unique functional, nutritional, and biomedical properties. It shows a good film forming property also. Starch is inexpensive, widely produced in the world, and possesses excellent film-forming

properties, and has therefore been widely used as a single biopolymer in studies on edible or biodegradable films [Nitta *et al.*, 2004; Zhang *et al.*, 2007].

Investigations on the preparation of biocompatible thermostable transparent xyloglucan films and composite films with chitosan and starch were carried out. The characterisation of its mechanical, chemical, thermal, and swelling properties and morphological features which have wide range of potentials in packaging, controlled release of drugs, cosmetics etc was carried out. The controlled release property was studied by using streptomycin as the model drug.

7.2. Experimental

7.2.1. Materials

Tamarind kernel powder and Njavara rice was purchased from the local market at Trivandrum, Kerala, India. All the other chemicals used are of analytical grade. The xyloglucan from tamarind kernel powder and starch from njavara rice was extracted as explained in chapter 2.

7.2.2. Methods

Xyloglucan films and composite films with chitosan and starch were characterised by Universal Testing Instrument, Contact angle measurement device, Screw gauge, Scanning electron microscopy, Thermogravimetric analysis, Differential scanning calorimetry, MALDI-TOF mass spectrometer, and X ray Diffraction pattern.

7.2.2.1. Film preparation

Xyloglucan solution (1%, 1.5%, 2% and 2.5%) was prepared in 1% aqueous acetic acid. After the complete dissolution, the sample was filtered through cheese cloth. Glycerol was added at 25% (w/w) of the total solid weight in solution. Mixed well and then cast on to flat acrylic plates. After drying the films at 50 ±2 ºC for 24 h, they were peeled from the plates. The dried films were conditioned in a desiccator at 25 ±2ºC for 48 h prior to testing.

Xyloglucan chitosan composite film (XG-CH) was prepared by mixing xyloglucan (1%, 2%, 3% and 4%) with 1% (w/v) chitosan solution prepared as above. A series of xyloglucan-chitosan composite films were prepared by mixing 50 ml of 1, 2, 3, and 4% xyloglucan solution with 50 ml of 1% chitosan solutions. 25% (w/w) (total solid weight in solution) glycerol was added as a plasticizer.

Similarly, xyloglucan starch composite films (XG-ST) were prepared. Aqueous starch solutions of concentrations 1 % (w/v) were prepared by heating, beyond their gelatinization temperature (90 ± 2 °C) for 20 min under stirring. The solutions were then cooled to 25 °C. A series of xyloglucan-starch composite films were prepared by mixing 50 ml of 1 and 2 % (w/w) xyloglucan solution with 50 ml of 1 % (w/w) starch solutions, respectively, with magnetic stirring. 25 % glycerol was added as plasticizer.

7.2.2.2. Film Thickness

Film thickness was measured using a Screw gauge (Dollar, Ultrascience Aids, Mumbai, India). Five thickness measurements were taken along the gauge length of each specimen and the mean value was used in calculating the film tensile strength.

7.2.2.3. Film Moisture Content

The moisture content of the film was determined gravimetrically by oven drying at 105°C for 24 h.

7.2.2.4. Swelling property of films in water and as a function of pH

The dried films of known weight were allowed to swell in solutions of acidic, neutral, and alkaline pH - namely, 2, 7.4, and 10 at room temperature (28 ºC) and at 100 rpm by preparing 0.1M solutions of KCl-HCl buffer, phosphate buffer, and glycine-NaOH buffer, respectively. The swollen films were removed from the solution at regular intervals and dried superficially with filter paper, weighed, and replaced in the same bath. The degree of swelling, S_w in the film was calculated as

$$Sw = \frac{We - Wd}{Wd} \qquad (7.1)$$

where W_e is the weight of the film in the swollen state and W_d is the dry weight of film.

7.2.2.5. Drug loading and in vitro drug release studies

Drug loading into the xyloglucan chitosan blend film was studied by using streptomycin as the model drug. Appropriate amount of the xyloglucan (1%, 50ml) and chitosan (1%, 50 ml) solution, glycerol and the drug streptomycin were mixed well and casted on an acrylic plate. Dried at 50 ºC for 24 h and they were peeled from the plates. The dried film was conditioned in a desiccator at 25 ºC for 48 h.

For the study of in vitro drug release, small amount of drug loaded film was taken in phosphate buffer of pH 7.4. Kept the sample for 180 min with constant shaking at 100 rpm. At regular intervals, certain amount of medium was replaced by fresh phosphate buffer. The amount of drug released was determined by a UV- visible spectrophotometer at 300 nm. The drug release kinetics was studied by Higuchi and Korsmeyer Peppas method.

The experimental results are expressed as an average of five independent measurements. Differences were considered to be significant at $SD < 0.01$.

7.3. Results and discussions

Xyloglucan was successfully extracted from the previously defatted and deproteinated tamarind kernel powder (TKP) by hot water extraction at a pH of 3.0. The xyloglucan was precipitated from the extract with 95% ethanol (38% yields). The dried xyloglucan powder contains 9.7 % moisture, 0.33 % lipids, 0.285 % ash and negligible protein. MALDI TOF MS results showed that xyloglucan has a molecular weight of 298 KDa.

Njavara rice starch with amylose content of 20 ± 2 % was extracted successfully and used for the film preparation.

7.3.1. Film Preparation

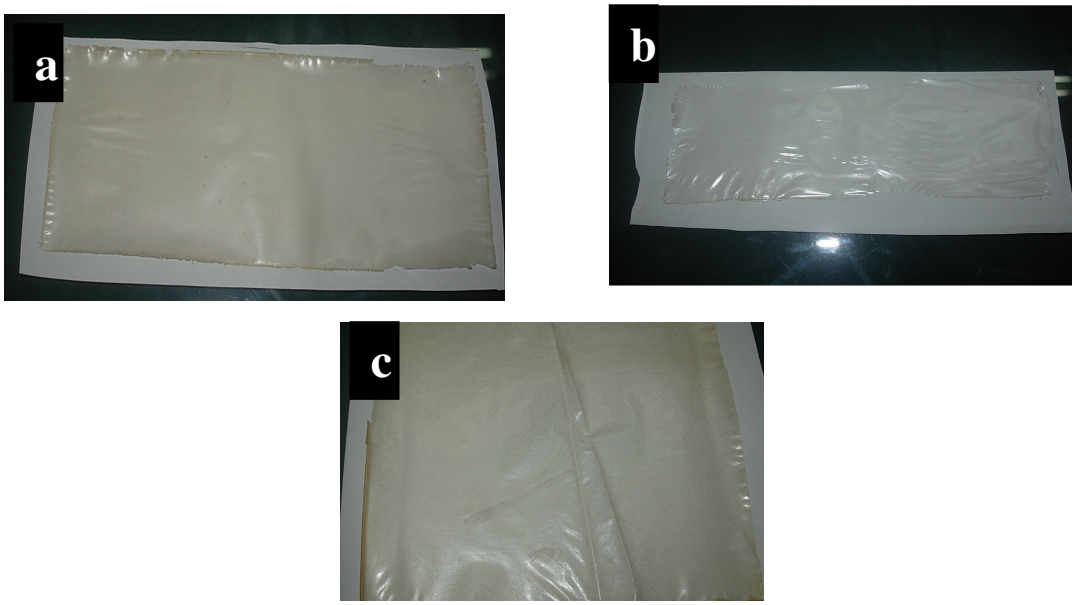


Figure 7.1. Transparent xyloglucan films a) XG film, b) XG-ST film, c) XG-CH film

The different types of transparent films from xyloglucan (xyloglucan film, xyloglucan chitosan film, and xyloglucan starch film) were prepared (Figure 7.1). The composition of the blend films were given in Table 7.1. The thickness of the films was found to be in the range of 0.07 to 0.1 mm and the moisture content of XG, XG-CH and XG-ST film was 1.89 %, 5.32 % and 3.09 % respectively.

Table 7.1. Composition of xyloglucan chitosan blend film

Xyloglucan Chitosan ratio	Xyloglucan mg/ml	Film thickness (mm)
1:1	10	0.07
2:1	20	0.09
3:1	30	0.08
4:1	40	0.08

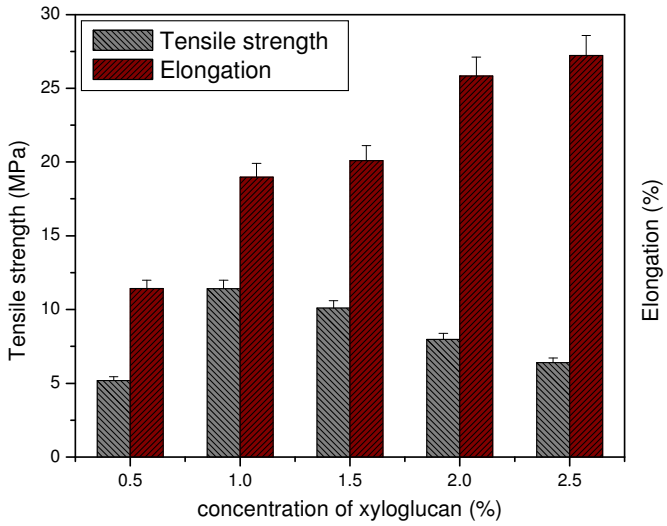


Figure 7.2. Effect of xyloglucan concentration on mechanical properties

A study of the mechanical properties of the films prepared with different concentration of xyloglucan revealed that the xyloglucan film prepared from 1% concentration was optimum and it gives high tensile strength than the other concentrations (0.5, 1.5, 2 and 2.5 %), but the flexibility or the % elongation increases with increase in xyloglucan concentration (Figure 7.2). For the improvement of the film strength,

xyloglucan was separately blended with chitosan and starch and the mechanical properties were studied. Results showed that XG-CH blend films have high tensile strength than XG-ST and XG films. Order of increase in film strength was XG - CH > XG- ST > XG. But the flexibility of the XG-CH film was lower than XG and XG-ST film (Figure 7.3).

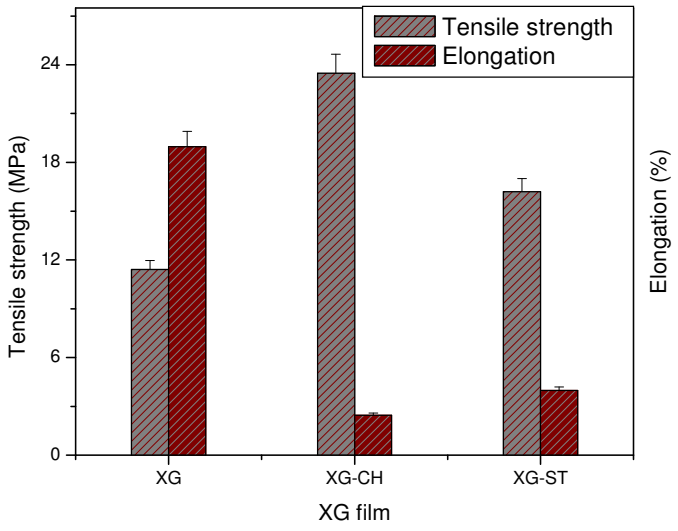


Figure 7.3. Effect of polysaccharide blending on mechanical properties of xyloglucan film

Considering the above results the concentration ratio of xyloglucan and chitosan in the blend film needs to be optimized to obtain strong as well as flexible films. From the mechanical test, it was clear that XG-CH blend film in the ratio 1:1 has high tensile strength. Flexibility of the film increases, whereas the tensile strength decreases with increase in xyloglucan concentration (Figure 7.4). In acidic solution, the glucosamine units of chitosan are ionized to the soluble form of $R\text{-}NH_3^+$ and the non-ionic polymer xyloglucan cross-links are formed between the hydroxyl and amino groups of chitosan

and the hydroxyl group in xyloglucan, giving good compatibility. The main reason for the increase in tensile strength in xyloglucan chitosan composite film is the strong interaction between them via the formation of hydrogen bonds [Mathew *et al.*, 2008].

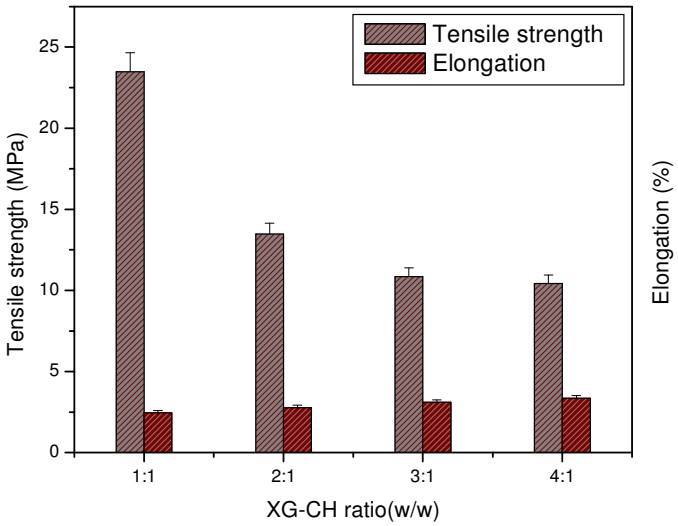


Figure 7.4. Effect of xyloglucan concentration in XG-CH film on mechanical properties

The tensile strength and the flexibility are important mechanical properties for the characterization of films. Films intended for dermal drug delivery must be flexible enough to follow the movements of the skin and provide a good feel, and at the same time resist the mechanical abrasion caused, for example, by clothes. A film for skin drug delivery should be hard (high tensile strength) and tough (high flexibility) [Silva *et al.*, 2008]. The strength of xyloglucan chitosan blend film, which shows an ideal film property, will find applications in medical, cosmetic and food industries.

7.3.2. Contact angle measurements

Hydrophobicity/non-wettability is a highly desirable property for materials that are used in a wide range of applications. The contact angle of water is one of the basic wetting properties of packaging materials and is an indicator of the hydrophilic/hydrophobic properties of the material. Usually, more hydrophilic material shows the lower contact angle.

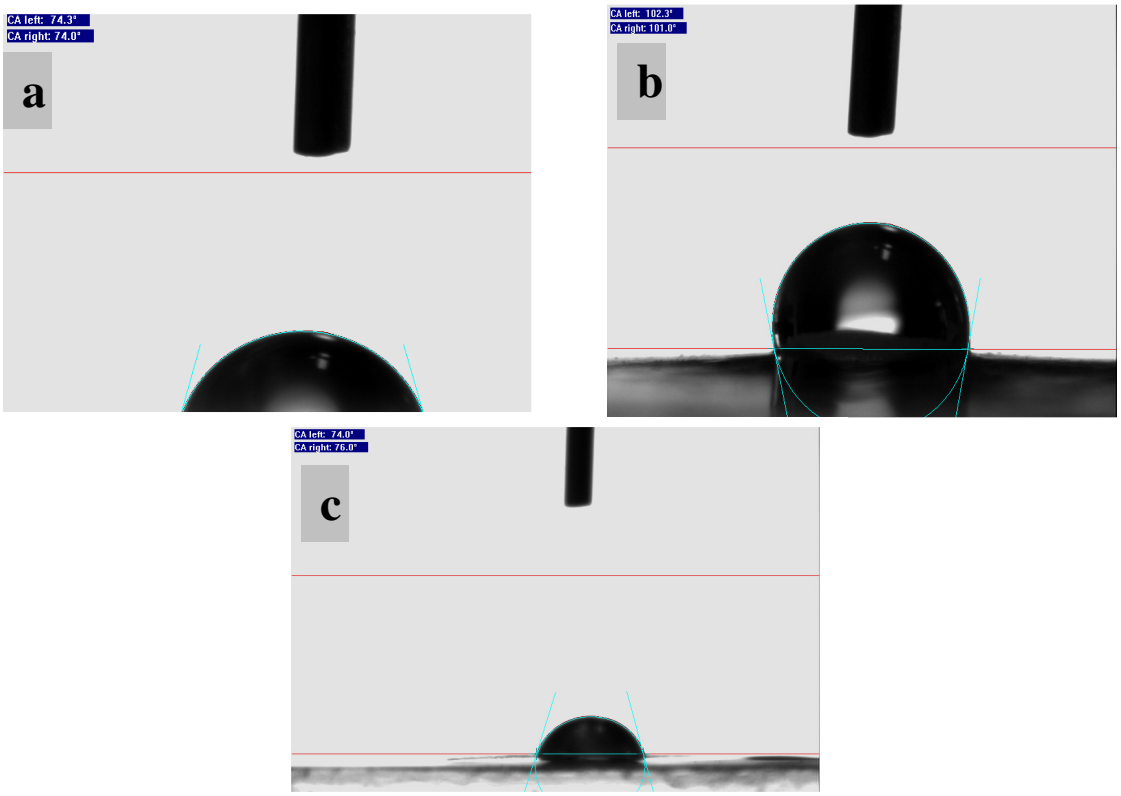


Figure 7.5. Contact angle measurements of a) XG, b) XG-CH, c) XG-ST films

Contact angle measurement of the films studied indicated that, XG-CH blend film showed high contact angle (102 º) value than xyloglucan film (76 º) (Figure 7.5) where as chitosan film showed lower hydrophobicity with a contact angle of 45 º [Silva *et al.*,

2008]. This indicates that blending of chitosan on xyloglucan increases its hydrophobicity. This may be due to the high crystalline nature of the chitosan since hydrophobicity is directly related to crystallinity. XG-ST blend film, shows low hydrophobicity than the xyloglucan film (74 °), because of the lower crystalline nature of the starch compared to xyloglucan.

7.3.3. Surface morphology

SEM micrographs of the surfaces of XG film and the blend films were shown in Figure 7.6.

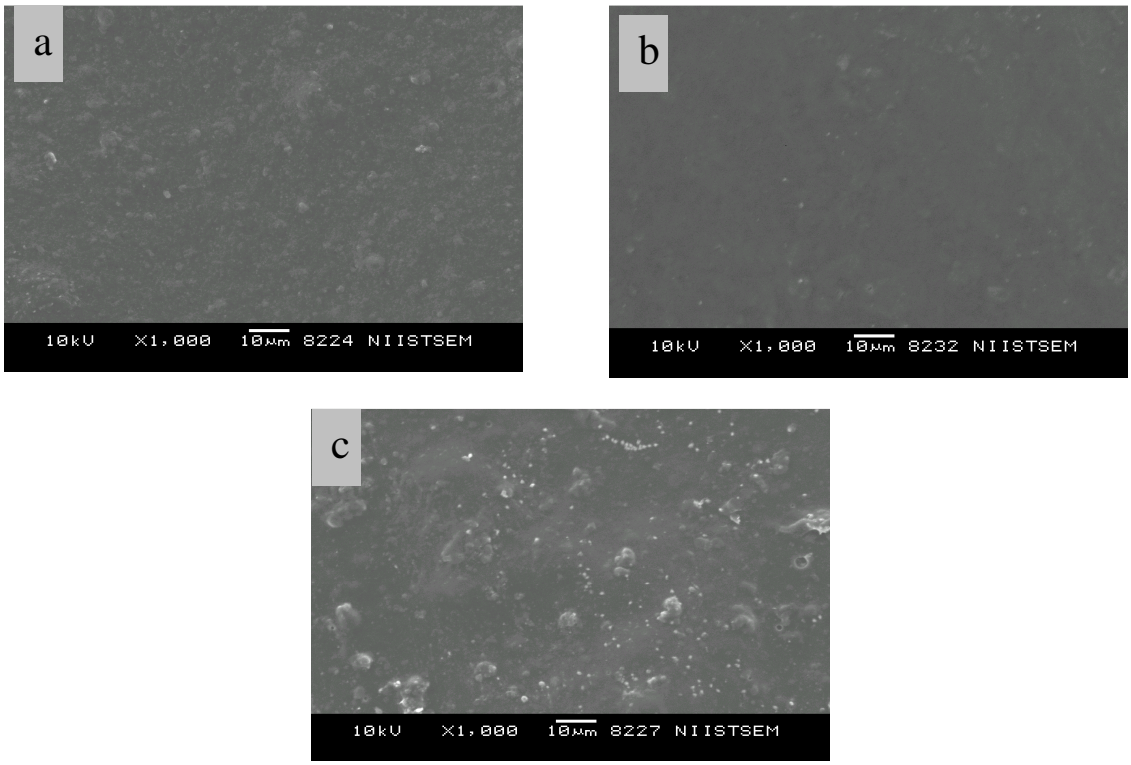


Figure 7.6. Morphological analysis of XG, b) XG-CH and c) XG-ST composite films by SEM

Surface morphology of the XG-CH blend film is quite smooth and uniform. This may be due to the maximum number of electrostatic interactions between xyloglucan and chitosan and has a tight structure and improved network stability. XG- ST blend film and XG film surface was rough compared to XG -CH film.

7.3.4. X-Ray Diffraction

X-ray diffraction pattern of XG, XG-CH and XG-ST composite films were shown in Figure 7.7. Xyloglucan powder shows a highly crystalline peak at 2θ value 20°.

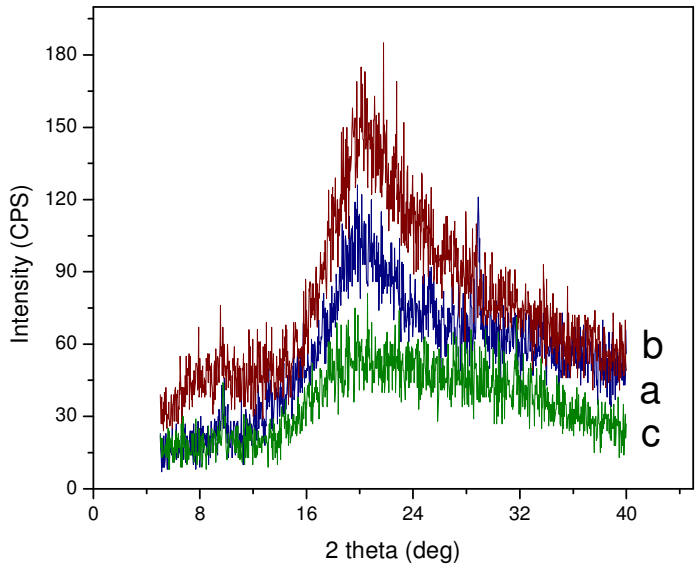


Figure 7.7. X-ray diffraction pattern of a) XG, b) XG-CH and c) XG-ST composite films

But in the xyloglucan film, the intramolecular interactions or the hydrogen bonding reduced the crystallisations, and the % of crystallinity is low compared to xyloglucan powder. XG-CH films prepared by casting from aqueous acetic acid solution were more crystalline form owing to the presence of the acetic acid solvent residue, which

might have hindered the formation of inter and intra molecular hydrogen bonds in chitosan and resulted in less dense packing [Mathew *et al.*, 2006]. Crystallinity of starch blended xyloglucan film was poor. This crystalline nature explains the high hydrophobic behaviour of the XG-CH blend film. However, the crystalline structure of xyloglucan was not significantly affected through the film preparation and blending as indicated by comparison of the XRD patterns. A relative crystallinity can be determined by comparing the area under the crystalline peaks with the area of the amorphous region under the peaks. The % crystallinity of the XG, XG-CH, and XG-ST films were found to be 9.09%, 36.38% and 7% respectively.

7.3.5. Thermal Analyses

Thermogravimetric analysis at a temperature ranging from 30 °C to 500 °C in nitrogen atmosphere at the rate of 10 °C/min was performed to examine the thermal stability of different types of films and their TGA curves were shown in Figure 7.8. In all the cases, weight loss takes place in three stages. XG and XG-CH film shows similar thermogravimetric patern and merge together. The initial or first stage between 55 to 130 °C corresponds to the loss of adsorbed and bound water. In XG and XG-CH second stage degradation starts at 264 ºC, and ends at 346 ºC, with 65% weight loss. But in XG-ST film second stage degradation starts at 264 ºC to 350 ºC, with a weight loss of 57%. In XG-ST third stage degradation occurs in between 480 to 560 ºC with 98% degradation. In XG and XG-NH_2 film third stage degradation starts at 450 ºC and final decomposition temperature was 530 ºC. 97% degradation was occurred at this

stage. From these results it was clear that XG and XG-CH film was thermally stable than XG-ST films.

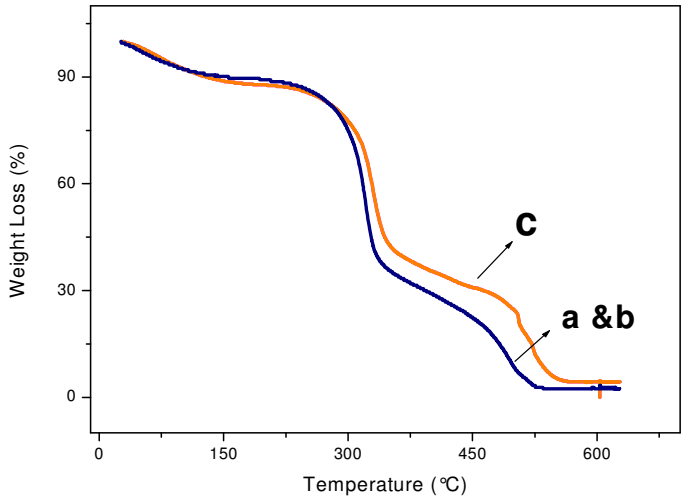


Figure 7.8. Thermogravimetric analysis of a) XG, b) XG-CH and c) XG-ST composite films

The DSC thermograms of films were shown in Figure 7.9. The samples were heated from 30 to 200 ºC at a rate of 10 °C/min under nitrogen. The endothermic peak around 76 °C corresponds to the melting temperature of xyloglucan film. Xyloglucan chitosan blend film has a broad peak at 120 °C which is higher than that of xyloglucan and xyloglucan starch blend film. This DSC data agrees with the XRD pattern. The high melting temperature of XG-CH films shows its high compatibility and a strong bonding between the xyloglucan and chitosan.

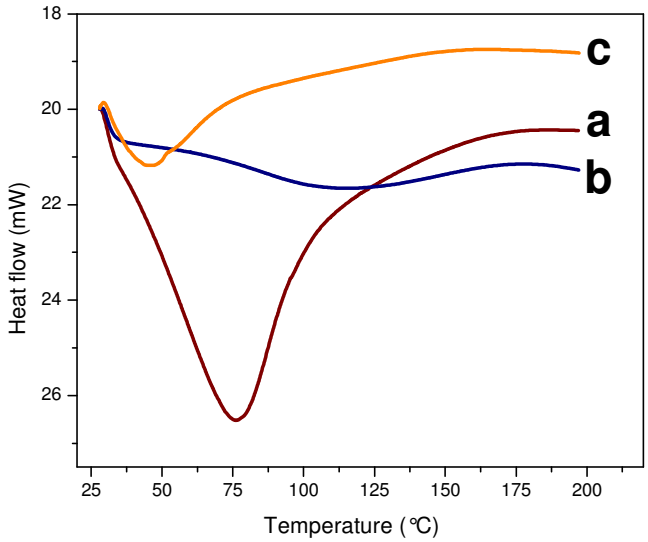


Figure 7.9. DSC analysis of a) XG, b) XG-CH and c) XG-ST composite films

The xyloglucan chitosan blend film exhibited high thermal stability than the parent xyloglucan film.

7.3.6. Swelling power or water absorption capacity

Swelling property is very important parameter in the case of drug delivery. Swelling characteristics of samples in three different pH (2, 7.4, and 10) were shown in Figure 7.10. Sample has a very rapid swelling at pH 7.4 which is the pH encountered in the duodenum area of the gastro intestinal system in humans where the drug has to be released. Sample at pH 10 shows slow swelling at the initial stage. Swelling power increases with time. Swelling power in acid pH (pH 2) was higher than the alkaline pH.

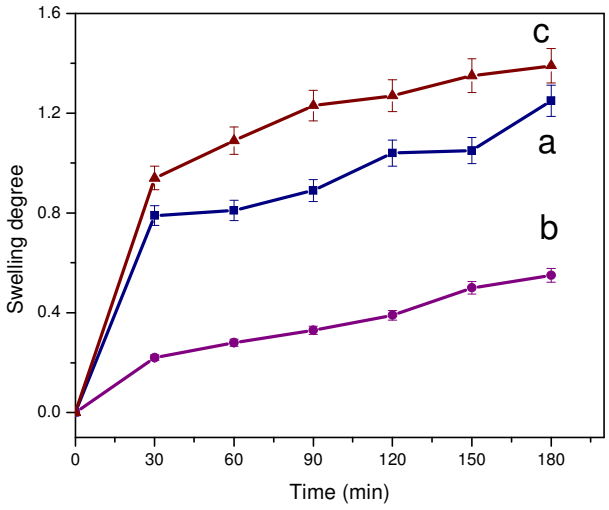


Figure 7.10. Swelling studies of XG-CH film as a function of pH. a) XG-CH in pH 2, b) XG-CH in pH 10, c) XG-CH in pH 7.4.

From these swelling studies it can be concluded that the XG-CH films are good enough for the controlled release applications.

7.3.7. Drug release studies

Figure 7.11 showed the controlled drug release profile of streptomycin loaded xyloglucan chitosan blend films. Streptomycin was encapsulated in a composite xyloglucan chitosan (1:1) film and then studied its controlled release efficiency which will be useful parameter for its usage as a capsule, patches and other aroma and cosmetic release systems in therapeutic and cosmetic field. Results showed that controlled release of drug occurs at a pH 7.4 and exhibit a small burst release in the initial stage and then a slow constant release. This initial burst effect could be attributed to the diffusion of the drug caused by rapid membrane swelling and also the faster release of the drug adsorbed toward the surface of the films matrix. Slow release

of drug in buffer of pH 7.4 has proved that the xyloglucan chitosan blend film can be used as a good carrier of drugs.

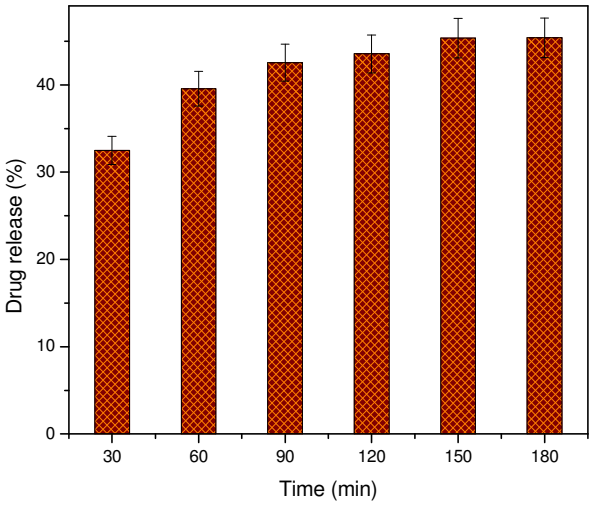


Figure 7.11. Controlled drug release profile of XG-CH film

It is generally accepted that increased hydrophobicity, electrostatic attractive forces and swelling behaviour of the materials could improve the controlled-drug release properties. The drug release kinetics was studied by Higuchi and Korsmeyer- Peppas model. Higuchi developed theoretical models to study the release of high and low water soluble drugs incorporated in solid and semisolid matrices. According to this model, drug release was described as a square root of time dependent diffusion process based on Fick's law. Plot of square root of time versus cumulative amount of drug released yields a straight line and slope gives the kinetics of drug release. Under some experimental situation, the release mechanism deviates from Fick's equation. In this case Korsmeyer Peppas developed a simple, semi empirical model relating exponentially the drug release to the elapsed time. The release data were analysed on

the basis of Korsmeyer Peppas and Higuchi kinetics. The release rates n and k of each model were calculated by linear regression analysis using Microsoft origin 6 software. Coefficients of correlation (r^2) were used to evaluate the accuracy of the fit. The r, n and k values were shown in Table 7.2.

Table.7.2. Drug release kinetics

	r^2	**constants**
Higuchi	0.8229	k =1.6334
Korsmeyer Peppas	0.8503	n =0. 8933

If n value is in between 0.5 and 1 the drug release follows a non-Fickian model (anomalous transport). Here the value is 0.89. So it follows the Korsmeyer- Peppas kinetics with non-Fickian diffusion. According to Higucchi kinetics, the slope of the straight line or the kinetic constant 'k' is one or more than one, then the particular system is considered to follow the Higuchi kinetics of drug release. Here the constants k has the value 1.6 which indicates that the mass transfer follows Higuchi kinetics, which also indicates diffusion controlled drug release. In both the case the r value is almost same. Here we conclude that xyloglucan chitosan films are quite suitable to be applied in drug delivery systems.

7.4. Conclusion

Xyloglucan shows a good film forming properties and a transparent thermostable film was successfully prepared having high tensile strength and flexibility. Xyloglucan film properties were improved by blending with chitosan than with starch. XG-CH film has high crystallanity, hydrophobicity, and thermal stability and swelling power in different pH. Because of inter and intramolecular hydrogen bonding, the XG-CH film in the optimum ratio, forms a strong, stable, and flexible films. Morphological studies by SEM confirm the smooth surface and compatibility with chitosan. Streptomycin drug was encapsulated in XG-CH film and is used successfully for the controlled release of drug by changing the pH suitably. This drug release obeys both Higuchi and Korsmeyer peppas kinetics. These non toxic transparent films were found to be ideal for the controlled release of drugs and this film has extensive application in packaging wound dressing, capsules, cosmetics etc.

CHAPTER 8

Summary and Conclusions

The interest in the application of natural and renewable polymers especially polysaccharides is increasing due to its non toxicity, water solublility, broad range of functional properties, in industrial applications of foods, textiles, paints, cosmetics and pharmaceuticals. The two major categories of natural storage polysaccharides, abundantly available in our region, namely α- glucans (starch) and β- glucans (xyloglucan) were studied to enhance the commercial utility of the same. A brief over view of the recent developments in the field of polysaccharide nanoparticles, gels and films, scope of the present work and a detailed description of materials, methods and techniques used for the extraction, purification and characterisation of the glucans are given. The important conclusions are

❖ Starch as an alpha glucan, has many properties which can be utilized in the medicinal field. The medicinal utility of starch from rice and cassava was investigated. A study was undertaken on the unique physico chemical properties of starch from the Njavara rice. So the rice starch was compared with, Chamba rice's starch which is our staple food. Njavara, unique rice, short duration cultivar grown only in certain pockets in Kerala state, south India and belongs to the family Oryza. This is the only cultivar traditionally used effectively in the Ayurvedic system of medicine in certain specific treatments like *Panchakarma*. It was found that the njavara starch has high gelatinisation temperature, water absorption capacity, solubility, swelling power, pasting properties, high gel strength and better rheological properties. The starch degrades at higher temperature and has a higher enthalpy of gelatinization. The better thermal properties make it useful in products which need to be processed at a higher

temperature. It is because of its high heat holding capacity, the njavara rice is specifically used in *Ayurveda* treatments which needs sustained heat application such as *kizhyi*. Texture profile analysis showed that the properties of both starches are similar, however njavara starch has better springiness and chewiness. Both the starches are of A type X ray diffraction pattern, which is typical of cereal starches and hence are of similar crystalline nature.

❖ The chemical modification of cassava starch by grafting with long chain fatty acids and utilizing this partially hydrophobic starch to make nanoparticles was accomplished. Nanoparticles loaded with drugs show drug release at right rate and dose at specific sites in the body for certain duration to realize the accurate delivery, which enhances the therapeutic effect and reduces the toxicity and side effects of the drug. Starch grafted with oleic acid was obtained in good yield compared to that grafted with stearic acid (DS 0.1 to 1.0). These starches become slightly hydrophobic and have good thermal properties. Grafted starch has a high swelling power at neutral pH compared to the native starch. This behaviour is useful in drug delivery applications. Starch nanoparticles were prepared by crosslinking the starch molecules with sodium tripoly phosphate and controlled precipitation with a solvent of appropriate dielectric constant and then stabilizing with a surfactant. Controlled release of the drug previously loaded in nano particle was studied by using indomethacin as the model drug. This modified cassava starch nanoparticles was found to be an excellent vehicle for the controlled release of drug by slow dissolution.

❖ Xyloglucan is a beta glucan and have applications in food, serving as a thickener and stabilizer, gelling agent, ice crystal stabilizer, and starch modifier, also in cosmetic and pharmaceutical industries. It is a neutral, non-toxic storage polysaccharide (backbone composed of 1, 4- linked β-D-glucopyranose residues) present in the seeds of the tamarind tree (*Tamarindus indica*) which is abundantly available in South India. Pure xyloglucan extracted from tamarind seed as such does not form a gel. Chemical derivatisation methods are employed to improve the properties of these intractable, inexpensive polysaccharides material. Chemical oxidation of xyloglucan was carried out to get dialdehyde xyloglucan. A transparent, colourless gel combining oxidised xyloglucan with chitosan in right proportion, having good compatibility for food and cosmetic applications was developed and named 'Chitam gel'. The ultra micro structure revealed that, the gel has a fibrous network structure of chitosan intercalated with oxidized xyloglucan as nano sized elongated particles. The transparent gel has superior thermal properties compared to native xyloglucan. The chitam gel is obtained in high yield since 10 g raw material produces 1Kg gel, with 100 fold yield and hence cost effective. The chitam gel had a viscosity of 4100 cP at 28 ±2 ºC, is thermostable from -20 to 90 ºC. Does not decompose on prolonged exposure to sun light or on exposure to ultra violet rays. It is as a zero calorie gel, not digested by digestive enzymes in humans, and useful as a diet replacement, especially for diabetic patients. It can be incorporated into beverages, Jam, jellies, marmalades, to give the desired viscosity, gel characteristics and mouth feel and also found compatible with edible colours, flavors and artificial sweeteners

(aspartame, alitame, saccharine). Very stable at acidic to neutral pH of 3 to 7 and hence can also be used as a vehicle for drugs in oral, topical, or wound healing patches. The chitam gel is easy to handle and hygienic and nontoxic. No need for heat or long periods of stirring for the gel preparation since the gel is formed at room temperature and at atmospheric pressure, costly processing equipments are not necessary. The desired final product viscosity can be tailor made from the chitam gel either modifying the percentage of xyloglucan, chitosan ratio or by diluting with suitable media. Apart from supplement functional foods (nutritional care) the chitam gel has applications in the area of cosmetic and personal care products, as a ultraviolet protective agent or as a tissue adhesive which can be used, including haemostasis, wound sealing, tissue engineering or localised drug delivery as capsules & tablets . The food and beverage market is also will benefit by the chitam gel as it has zero calorific value and can be used in beverages, jam, jellies, marmalades and ice creams useful for diabetic patients.

❖ Considering the recent trends in the increasing applications of chitosan, an animal derived cationic polysaccharide, which vegetarians does not prefer, in drug and gene delivery, we envisaged to synthesise a plant derived cationic polysaccharide from tamarind kernel xyloglucan. This was accomplished by a facile synthetic strategy viz. amination of xyloglucan. The resultant product has gelling property and emits blue fluorescence in aqueous medium and green fluorescence in solid state. At very low concentration in aqueous medium, it forms a self assembled nano particles of size of ~60 nm and this unique property have great applications in delivery of drugs, nucleic

acids etc. The product showed good thermal properties and higher melting point compared to the non aminated xyloglucan. The crystalline nature decreases after amination as seen from the x-ray diffraction pattern. This property can be used for a variety of applications such as in drug targeting and diagnostics, since the material is non toxic, plant derived, low cost and abundantly available. This cationic xyloglucan hydrogels can also be used as a substitute for the animal derived chitosan for gene therapy, tissue engineering scaffolds and in various other novel applications.

❖ The film forming property of the xyloglucan and its composites were studied and good quality films were produced. Xyloglucan as such showed a good film forming property. The xyloglucan- chitosan composites forms films with good tensile strength, however the tensile strength decreases with increase in the concentration of xyloglucan. The contact angle measurements showed that xyloglucan- chitosan blend film was more hydrophobic in nature. High melting point of this blend film indicates its strength. Controlled release of the drug previously loaded in the films was studied by using streptomycine as the model drug. The films can be used for the preparation of therapeutic patches, drug release and in food industry.

Future prospects:

The studies on the preparation of composite polysaccharide micro/nano particles and films for novel applications were accomplished to a large extent and the new findings will pave way for renewed interest and more research in this area. The unique properties of njavara rice starch such as high thermal, pasting and rheological

properties over the commonly available chamba rice starch, was found to be the main reason for its specific use in ayurveda treatments. There may be other important properties of this njavara rice, such as antioxidant property which brings about many health benefits, may have to be investigated in future.

Starch as such is very hydrophilic and is not suitable for controlled release applications. Fatty acid grafted starch nano particle was found to be a good vehicle for the controlled oral drug delivery. This grafted starch become slightly hydrophobic and has good thermal properties and high swelling power at neutral pH compared to the native starch, which may useful not only in drug delivery but also for a variety of applications in personal care products, and cosmetics, food and beverage. Starch nanoparticles were stabilized by crosslinking with sodium tripoly phosphate, but there may be other options to cross link and stabilize the starch nano particles. Many new surfactants and solvents may be used for this purpose. The storage stability of nano particles in both emulsion as well as powder forms are to be carried out in detail. Novel methods of making nano particles in large scale have to be developed, so that the availability of nano particles are enhanced, which can be used in many more fields.

Today's consumers in the cosmetic, food and beverage market are increasingly interested in healthy life styles, a trend which has produced a rising demand for health oriented products. Cosmetic and personal care products manufacturers claim that there is promising trend for transparent products for instance those which use clear formulation techniques in their gels and emulsions. The synthesis of a transparent,

colourless, nontoxic, biodegradable, biocompatible gel from oxidized xyloglucan and chitosan co-polymer having good thermal, antimicrobial and texture properties was accomplished. Hence the gels which is crystal clear, colourless, not thermoreversible, non toxic, biodegradable, biocompatible and having an ordered structure and from renewable resources and hence cost effective are in great demand globally. In addition, its antimicrobial and texture properties are very promising and hence the colourless, odourless, non toxic transparent gel will find use in specialty foods and in cosmetic applications

Development of many more conjugates of a variety of non-starch polysaccharide molecules like the present xyloglucan –chitosan composite, to give colourless transparent gels, having good compatiblity for food, cosmetic and other applications may be happening in the near future.

Cationic polysaccharides have wide applications in drug and gene delivery. Attempts have been made to make a plant derived cationic polysaccharide from xyloglucan. The resultant blue fluorescent gel will find use in new areas like biotronics and fluorescent labeling applications in biological area. Polycations and negatively charged nucleic acids can spontaneously form nanocomplexes by electrostatic interaction. Polycationic vector reduces the electrostatic repulsion between DNA and cell surface by neutralizing the negative charge and also protects it from enzymatic digestion by nucleases in serum and extra cellular fluids.

Xyloglucan as such showed a good film forming property. Xyloglucan chitosan composite forms a film with good tensile strength and is hydrophobic in nature and released streptomycin. Chemically modified xyloglucan gels and films with different functional properties will have significant applications in cosmetic, food and medical field.

Thus the xyloglucan from the tamarind seed kernel was found to be a versatile polysaccharide for further modifications to enhance its utility. Japan is using our tamarind kernel powder for a variety of functional properties and in India it is far under utilized. It is urgently needed to develop value added products from tamarind kernel. Further work can be done to develop tailor made particles, gels and films from this type of natural non toxic polysaccharides, which are cheap and abundant in nature, amenable to derivatisation, to impart new properties, and for better utilization of this natural resource.

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List of Publications

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1. Tholath Emilia Abraham, **Chandroth Kalyad Simi**, A transparent Chitam gel and a process for the preparation thereof. Patent filed through PCT on 15-12-2008. Number 0141NF2008 to 5 countries.

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Conference Presentations/Proceedings

1. **C.K. Simi** and T. Emilia Abraham. Investigation of thermal and rheological properties of njavara starch. International conference on Advances in Polymer Technology on 25 -27 September 2008 at Kochi.

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