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-NH₂)

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 2nd, 3rd and 6th position was get substituted by –NHCH₂CH₂NH₂ which was further reduced to –NH₂ using NaBH₄ 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.

\[
DS = \frac{330 \times (\% \text{ amino group} / 16)}{100 - \frac{15}{16} (\% \text{ amino group})}
\]  

(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\textsubscript{2}. The OH groups at 2\textsuperscript{nd}, 3\textsuperscript{rd} and 6\textsuperscript{th} position in the glucose back bone and galactose side chain can get substituted by NH\textsubscript{2} group (but the most probable position is 6\textsuperscript{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\textsubscript{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\textsubscript{2} (Figure 5.3). This is due to the binding of NH\textsubscript{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\textsubscript{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
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 5.4](image-url)

**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.
**5.3.4. 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 5.5.** Effect of temperature on the amination reaction of 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$_2$SO$_4$, 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. Crystallinity and Thermal properties

Native XG and XG-NH$_2$ showed crystallinity and exhibited a peak at 2θ angle of 23° in XRD analysis (Figure 5.7).
Figure 5.7. Crystalline nature of a) XG and b) XG-NH\textsubscript{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\textsubscript{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 ($\Delta$G$_{moduli}$). In 7 % (w/v) hydrogel, the $\Delta$G$_{moduli}$ was $1.2 \times 10^3$ Pa and 8% (w/v) hydrogel showed a less gel strength value of $0.7 \times 10^3$ Pa whereas
the gel strength of 9% gel was reduced to below $0.1 \times 10^3$ 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$_2$ did not show any sign of a good gel meanwhile, 8% aminated xyloglucan showed very high gel strength of $3.1 \times 10^3$ 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 highlights 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 ±°C.
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-NH₂ freeze dried powder.](image)

Whereas the native XG and XG-NH₂ at dry condition showed plate like structures. In aqueous medium XG-NH₂ forms a gel and has intercalated fiber structure. This observation clearly differentiates the existence of a different morphology of XG-NH₂.

![Figure 5.13. SEM image of a) & b) XG-NH₂ gel in aqueous medium.](image)
due to hydrogel 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

![Antimicrobial studies](image)

**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± °C.

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,
comparing to the chitosan in nutrient agar petri plates exposed to atmospheric contamination at room temperature (28± °C).

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.