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Biophysical Chemistry 121 (2006) 14–20

Biophysical Chemistry

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Morphology and gelation of thermosensitive xyloglucan hydrogels

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Received 26 October 2005; received in revised form 8 December 2005; accepted 9 December 2005
Available online 9 January 2006

Abstract

Galactose modified xyloglucan is a thermally reversible hydrogel that is increasingly used in the biomedical field due to the ease of altering the gelation time and temperature by modifying the galactose removal ratio. However there is little information concerning the morphology and rheological properties of the hydrogel under physiological conditions. Differential scanning microcalorimetry (DSμC) showed the thermal gelation process to occur over a broad temperature range (5–50 °C). The rheological properties of the hydrogels were investigated as a function of concentration, temperature and ionic strength. The final elastic moduli of the hydrogels increased with increases in concentration. Isothermal rheology suggests that the gelation occurred in two distinct stages, which was influenced by the solution media. Scanning electron microscopy (SEM) was used to characterize the morphology of the xyloglucan which were thermally gelled at 37 °C. The resultant morphology was strongly dependent on the concentration of the hydrogel. Strong hydrogels were only obtained at 3 wt.% at 37 °C, and the morphology characterized by an open 3-dimensional network, comprised of thin membranes. It is proposed that the first stage of the isothermal gelation is the formation and growth of the thin membranes, followed by the formation of a three dimensional network.

Keywords: Xyloglucan; Hydrogel; Rheology; Scanning electron microscopy; Morphology

1. Introduction

There has been growing interest in the use of thermally sensitive in situ forming, physical hydrogels for biomedical applications [1,2] as minimally invasive scaffolds for tissue engineering. Because of their low interfacial tension and high molecular and oxygen permeability, hydrogels are ideal tissue engineering constructs [3]. Consequently, hydrogels have been investigated for cell gene therapy [4], enzyme and cell encapsulation [5–7], drug delivery [8–11], joint cushioning and lubrication [8,12] and as environmental shape memory materials [4]. The utility of hydrogels as tissue engineering scaffolds appears to be related to microscopic [13] and macroscopic [14] porosity within the structure of hydrogels. Microscopic pore interconnectivity and the volume of water phase present within the hydrogel ensures cellular viability, permits cell migration, increases transportation of nutrients, oxygen and metabolites [15], and influences drug release profiles and enzymatic degradation [8]. The shape and size distribution of the pores also influence cellular function [8]. By exploiting these features it may be possible to mimic features of the natural extracellular matrix, and control tissue structure and cellular functions [16].

Xyloglucan is a neutral, non-toxic polysaccharide [17], whose degradation products consist of naturally occurring saccharides and are assumed non-toxic, although the experimental evidence for this conclusion is limited. Xyloglucan is extracted from the tamarind seed and is a major component of higher plant cell walls [18]. It is composed of a β-1,4 linked D-glucan backbone where the O-6 positions of the

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doi:10.1016/j.bpc.2005.12.005

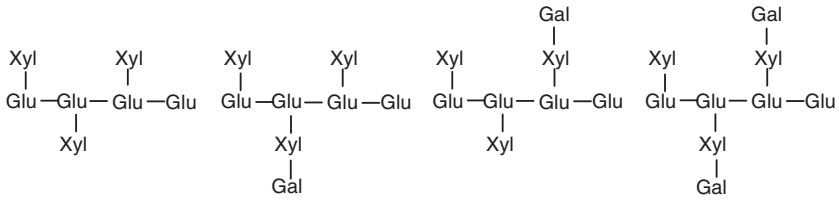

Fig. 1. Backbone structure of xyloglucan [17].

glucopyranosyl residue are partially substituted with α-D-xylopyranose residue [11,19]. Fig. 1 shows the backbone structure of xyloglucan and Fig. 2 presents the structure of the galactopyranose, glucopyranose and xylopyranose which make up the polysaccharide.

Thermally responsive xyloglucan is formed using fungal β-galactosidase to remove more than 35% of the galactose residue [17]. Shirakawa et al. [17] found that the optimum removal ratio was between 35% and 50%. Using DSC and rheology they related gelation temperature to the concentration of xyloglucan in the pre-gel solution. Miyazaki et al. [11] found that increasing the concentration of the pre-gel solution from 1% to 2% decreased gel temperature from 27 to 22 °C as well as the gel time. A lower and upper transition from sol–gel and gel–sol, respectively, were found, and the gel was shown to be thermo-reversible upon cooling. It was also determined that with a higher galactose removal ratio there was an increase in temperature range of the gelation peaks, hence a broader gelation range [17].

While tissue engineering applications for xyloglucan are not extensive, it has been used as skin patches [20], oral and rectal delivery of drugs [11], and for intraperitoneal injections [21]. It was drug loaded in the latter two applications and was found to provide stronger bioavailability of the relevant drug and longer residence times than previous commercial suppositories [22]. Importantly there was no apparent tissue damage [11] implying that xyloglucan hydrogel is a biocompatible material that can be implanted non-invasively via injection in tissue engineering applications.

X-ray scattering studies of the gel nano-structure of galactose-modified xyloglucan, showed that flat structures were formed from lateral stacking of rod-like chains [23] whereas the addition of ethanol to the solution created a random structure consisting of condensed phase aggregates amongst dilute, single chain areas [24].

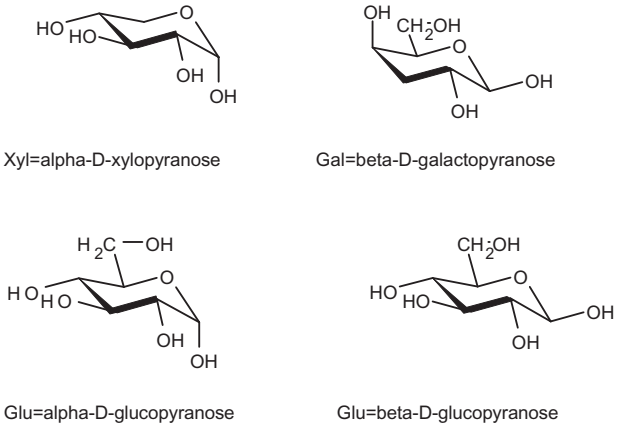

Fig. 2. Glucose units that exist in the three oligomer structures in xyloglucan.

This study aims to investigate the thermal, rheological properties of thermally gelling xyloglucan with a galactose removal ratio of 48% with consideration of its use as an injectable tissue engineering scaffold. The effect of changing the ionic strength of solution media on the properties of the hydrogel were determined by comparing deionised water and phosphate buffered saline (PBS) as solution media. This study also presents for the first time, the morphology of the gel networks as determined using electron microscopy.

2. Methodology

2.1. Materials

Xyloglucan with a 48% galactose removal ratio was prepared by enzymatic modification from tamarind seed xyloglucan, according to previous method [17]. It was purified by dissolving 1 wt.% xyloglucan in deionized water with a magnetic stirrer at a temperature between 0 and 5 °C. The solution was then precipitated out in 60% ethanol at room temperature and washed with 60% ethanol through a sintered glass filter and flask with attached vacuum pump. An additional wash was conducted using acetone. The precipitate was then dried at room temperature for two days in a vacuum oven.

2.2. Sample preparation

Sample solutions were prepared by dissolving the purified xyloglucan in either deionised water or PBS at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 wt.% and stirred in an ice bath until no particles were visible, and stored at 0–5 °C.

2.3. Rheology

Rheological measurements of the elastic (G') and viscous (G'') shear moduli and gelation temperature were taken for solutions of 2.0, 2.5 and 3.0 wt.% in both deionised water and PBS using a Bohlin CS-50 rheometer in parallel plate configuration, and within the linear viscoelastic region of the gels. Measurements were conducted using frequencies of 0.1, 0.316, 1.0, 3.19 and 10 Hz with the strain rate held at 1.25% with an initial stress of 0.245 MPa. The isothermal studies were conducted at 37 °C to determine the induction time of gelation, or the time where the phase shift was independent of frequency. For this experiment the sample was placed into the

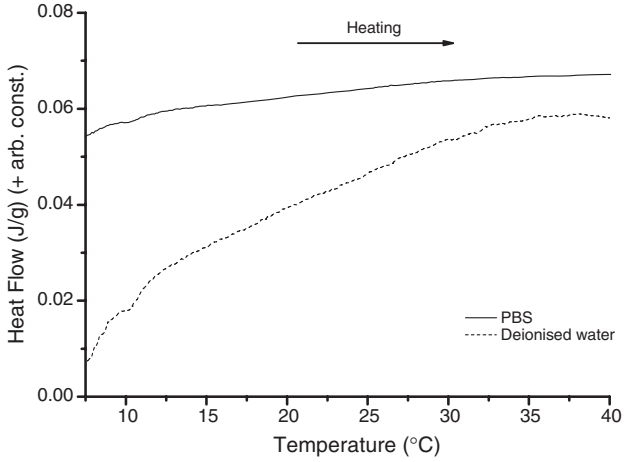

Fig. 3. DSμC heating curve for 0.5 wt.% xyloglucan in deionised water and PBS (arbitrary constant on *Y* scale).

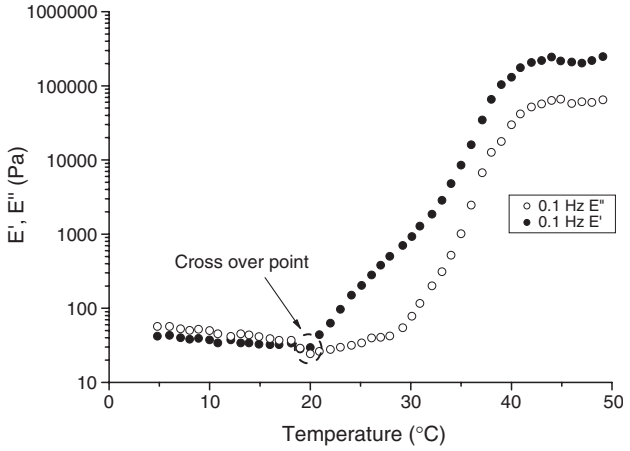

Fig. 5. Shear modulus versus temperature for a 3 wt.% xyloglucan in PBS solution (0.1 Hz).

rheometer at 5 °C and allowed to rest for 90 s before raising to 37 °C. Rheology was also conducted using temperature ramping of 1 °C intervals over the range 5–50 °C.

2.4. Differential scanning microcalorimetry

Differential scanning microcalorimetry (DSμC) was used to determine the gelation temperature at the lower concentrations (0.5, 1.0 and 1.5 wt.%) in deionised water and PBS solutions. The samples equilibrated for 30 min before the temperature was ramped from 5 to 50 °C at 1 °C/min, using a N-DSC II differential scanning microcalorimetry (Model 6100, Calorimetry Sciences Corporation; baseline noise range, 30 nW). Aliquots of xyloglucan solution and an appropriate control (deionised water and PBS solution, respectively) were used. The scan was conducted under atmospheric pressure to avoid damaging the delicate hydrogel networks by pressure-influenced thermal events [8]. To avoid significant evaporation the maximum temperature was limited to 50 °C. The samples were scanned in triplicate and the baseline was subtracted from the heat flow, which was normalized against the xyloglucan weight.

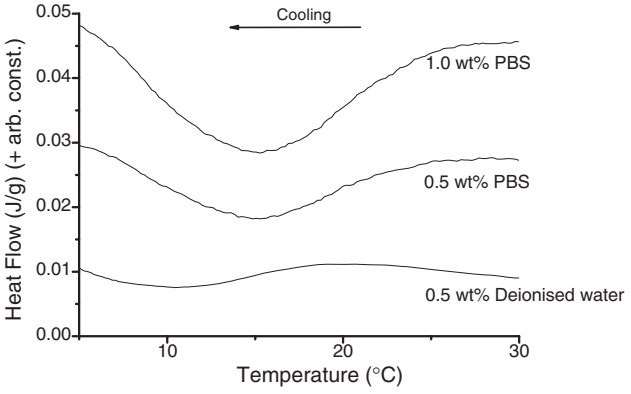

Fig. 4. DSμC cooling curve for 0.5 wt.% and 1.0 wt.% xyloglucan in deionised water and PBS (arbitrary constant on *Y* scale).

2.5. Scanning electron microscopy (SEM)

Samples were gelled at 37 °C on a microscope slide before being quenched with liquid nitrogen and freeze dried under vacuum for 48 h. The sections were then sputter-coated with gold using a Bal-Tec SCD 005, Balzers for 180 s before SEM was preformed using a Hitachi S-570 SEM with an accelerating voltage of 15 kV and a magnification ranging from 50 to 1000 times.

3. Results and discussion

3.1. Thermal and rheological characterisation

The gelation characteristics of the xyloglucan hydrogels at concentrations ranging from 0.5 to 3 wt.% were studied using DSμC and rheology in both water and PBS to determine if there was a change in the gelation behavior due to changes in ionic strength. At concentrations less than 2.0 wt.%, rheological characterisations showed that the physical gels were weak and their elastic modulus, (E'), did not increase as temperature increased. Consequently, DSμC was used to study the gelation behavior at concentrations below 2 wt.% (at higher concentrations this technique was inadequate because the gels became viscous, even at low temperature, making their insertion and removal from the apparatus difficult).

Table 1
Rheological properties of themosensitive xyloglucan at different compositions

Concentration (wt.%)	Cross over point at 0.1 Hz (°C)		Gel temperature (°C)		Maximum elastic modulus (MPa)	
	Water[a]	PBS[a]	Water[a]	PBS[a]	Water[b]	PBS[b]
2.0	18±2	23±2	37±2	36±2	0.15±0.03	0.17±0.04
2.5	17±2	21±2	35±2	36±2	0.20±0.06	0.25±0.04
3.0	18±2	20±2	34±2	35±2	0.48±0.05	0.32±0.07

[a] Errors estimated from the best possible resolution of the tan δ graphs.
[b] Errors were calculated using the standard deviation of the mean method (95% confidence interval).

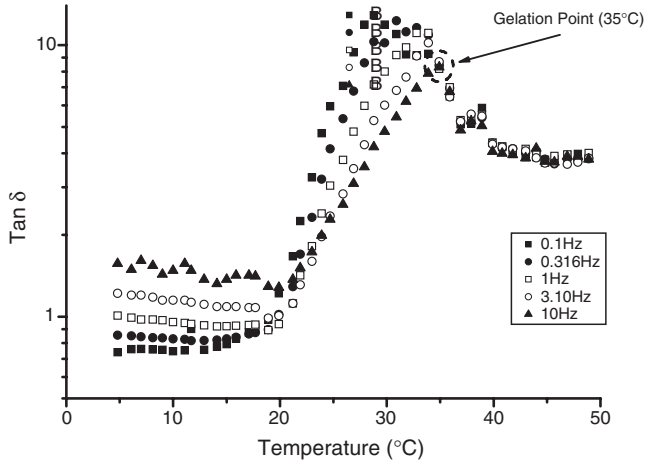

Fig. 6. Tan δ verses temperature curves for 3 wt.% xyloglucan in PBS solution.

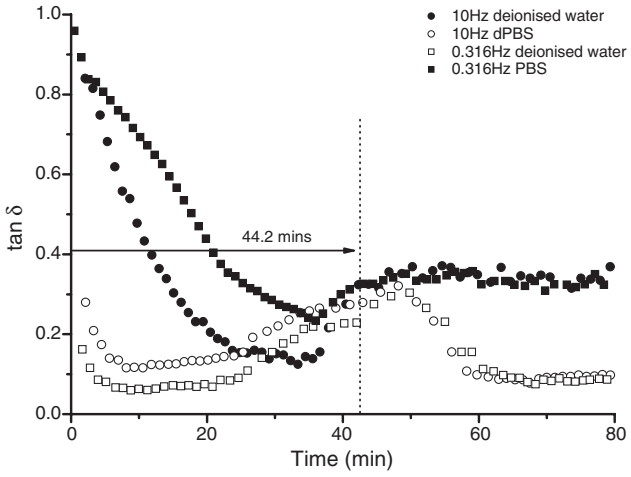

Fig. 8. Isothermal tan δ curves of 3.0 wt.% xyloglucan in deionised water and PBS at 37 °C. The induction time for gelation in both media is shown.

Figs. 3 and 4 show the DSμC heating and cooling curves, respectively, of the 0.5 wt.% solutions in both deionised water and PBS. The thermograms in both media exhibited a broad and non-resolved endotherm during heating between 9 and 50 °C indicating that the gelation process occurred slowly over this temperature range and began at low temperatures (Fig. 3). However, the endotherm of the PBS media was not as pronounced as that of the deionised water media. The cooling thermograms are shown in Fig. 4. Exotherms were observed in all the samples and the area of these exotherms increased with increasing concentration of xyloglucan (for example 1 wt.% PBS Fig. 4). The temperature onset of the exotherm was increased in PBS, indicating that the ionic strength was accelerating the gel to sol transition. Due to the low temperature limitations of the DSμC instrument, some of the exotherms could not be resolved.

Fig. 5 shows the change in the elastic modulus (E') and the viscous modulus (E'') as a function of temperature for the 3 wt.% xyloglucan in PBS. The cross over point of the E' and E'' gives an indication of the transition from the initial dominant viscous liquid-like behavior to elastic solid or gel-like behavior [25]. All compositions exhibited a cross-over point in the temperature range of 17–23 °C and was largely insensitive to concentration and solution media in the ranges investigated (Table 1). The samples exhibited frequency dependence prior to the cross-over point, i.e., increasing E' with increasing frequency, indicating the presence of a weak gel at these low temperatures. Following the cross-over point, there was a rapid increase in the E' attributed to the formation of the network gel structure. The gel point, as determined by the frequency independence in tan δ (Fig. 6, Table 1), was also insensitive to the composition of xyloglucan and ionic strength of the solution media in the concentration ranges investigated. At temperatures above 40 °C, the maximum elastic moduli were achieved for all samples (Table 1) and was found to increase with increasing concentration. Interestingly, the maximum elastic moduli were also insensitive to the ionic strength, except for the 3 wt.% solution which formed a significantly weaker gel (0.32 ± 0.07 in PBS compared to 0.48 ± 0.05 for water). In this instance, the higher ionic strength may be interfering with the hydrophobic associations of the xyloglucan.

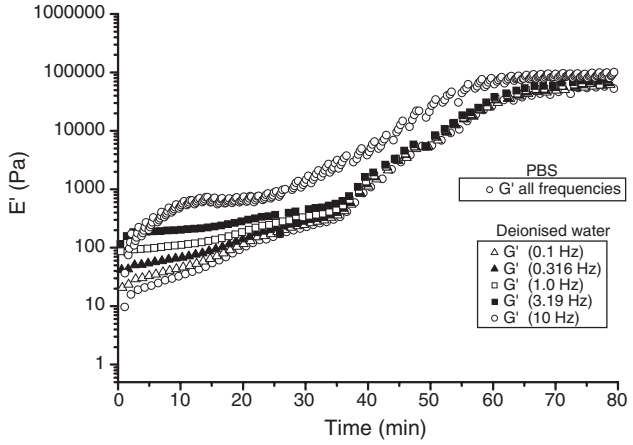

Fig. 7. Isothermal gelation (37 °C) of xyloglucan showing the elastic modulus E' as a function of time and solution media.

Table 2
Comparison of shear modulus with other hydrogels

Material	Modulus (Pa)	Reference
Xyloglucan	G' = 150,000–480,000	Experimental
2 w/v% chitosan/GP	G' = 6000	Chenite et al. [27]
Chitosan/xanthan (MW = 10^6 g/mol)	G' = 60,000	Magnin et al. [28]
1 w/v% agarose	G^* = 12.5	Balgude et al. [29]
Matrigel (basal)	G' = 34	Semler et al. [30]
Matrigel (crosslinked)	G' = 118	
Biomatrix I (ECM)	G' = 20–35	Snyder [31], Parsons and Coger [32]
Type I collagen	G' = 5–60	Knapp et al. [33], Parsons and Coger [32]
PHPMA	G^* = 250 G' = 2600	Woerly et al. [34]
1 w/v% Pluronic-PAA	G' = 100	Huibers et al. [35]
Polylysine-*b*-polyleucine copolymer	G = 12–4273	Breedveld et al. [36]

G^* = the complex modulus.

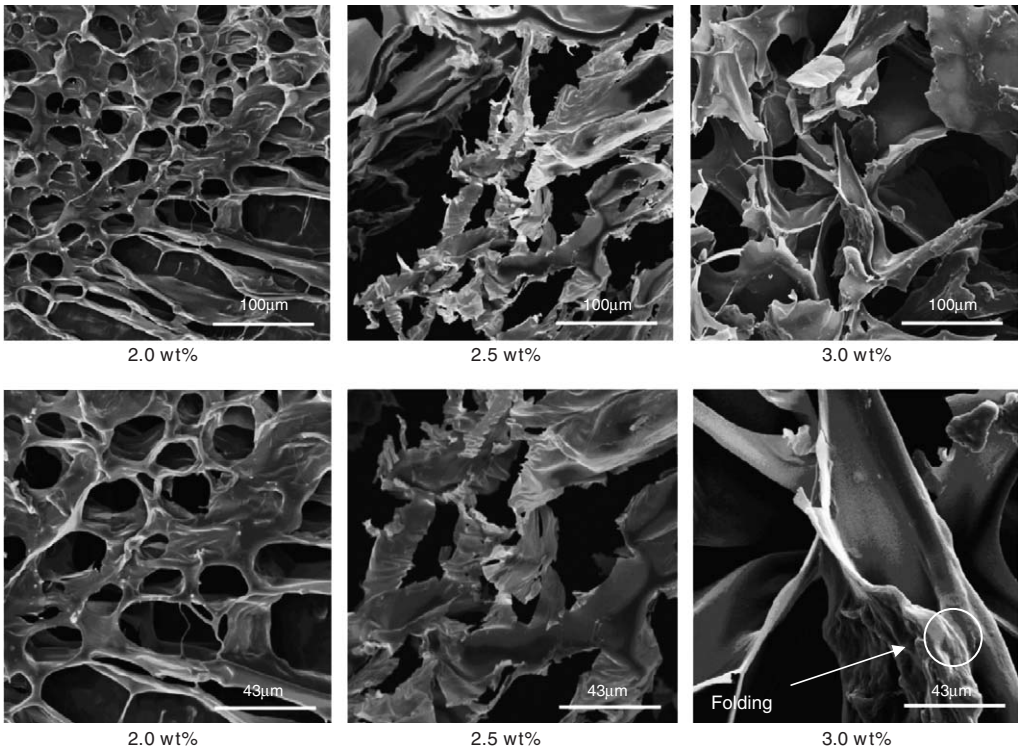

Fig. 9. SEM images of the surface morphology of xyloglucan at different concentration and magnifications.

Isothermal rheological experiments were also conducted at 37 °C with consideration of future in vivo studies where the sol will be injected into the body and allowed to gel in situ. Concentrations less than 3 wt.% failed to form strong physical gels at 37 °C regardless of the type of solution media and hence only the 3 wt.% compositions were investigated. Fig. 7 shows the elastic modulus as a function of time for the deionised water and PBS solutions. The deionised water sample exhibited a steady rise in E' which was also frequency dependent up to the gel point at (44.7 min, frequency independence of tan δ, Fig. 8) at which point there was a dramatic increase in E'. The introduction of PBS as the solution media had a significant effect on the isothermal gelation behavior. Fig. 7 shows that the PBS has an initially higher elastic modulus which is frequency independent at all times compared to the deionised water solution. The corresponding tan δ curves (Fig. 8) indicate that both systems exhibit similar gelation times (formation of a 3 dimensional network), however the elastic modulus is dominating both before and after gelation. The PBS also decreased the time to reach the maximum elastic modulus (60 min compared to 73 min for deionised water). The final elastic moduli for the 3 wt.% xyloglucan gels were insensitive to solution media and were approximately 100,000 Pa. This value is several orders of magnitude higher than other biological or synthetic physical hydrogels (Table 2).

3.2. Microstructure characterization and gelation mechanism

Macro-porous hydrogels can be examined using scanning electron microscopy (SEM) after freeze drying [22] when the material has an adequate modulus to avoid the structural collapse during dehydration [26]. The morphology of xyloglucan gels,

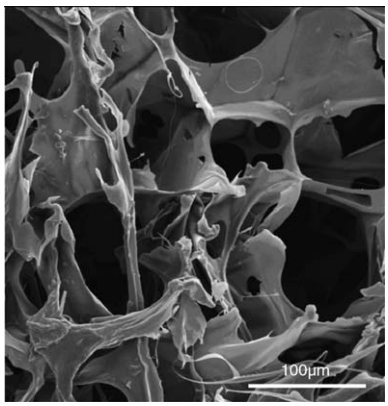

Fig. 10. Low magnification image of 3 wt.% xyloglucan in water.

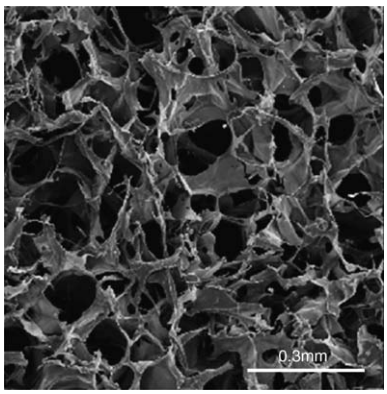

Fig. 11. Low magnification image of 3 wt.% xyloglucan in water.

whose temperature was held at 37 °C for approximately 5000 s prior to quenching in liquid nitrogen, was examined using SEM. Fig. 9 shows SEM of a range of concentrations (2.0, 2.5 and 3 wt. %) of xyloglucan gels. The SEM of 2.0 wt.% shows the gel structure collapsed, confirming that only weak gels formed at this concentration. However, some large flat sheets approximately 150 μm in length and 80 μm in width are present. A lath sheet-like structure was formed within the 2.5 wt.% sample and sheets were more numerous, larger (200 μm in length and 100 μm in width) with inclusions of macropores. There was almost no interconnection between laths and it is possible that the large sheets were torn. However, a significantly stronger gel was formed which did not collapse upon freeze drying when the concentration of xyloglucan was further increased to 3 wt.%. The SEM images showed a sheet-like or membrane structure with significant interconnection forming a 3-dimensional network (an example of this is highlighted in Figs. 10–11). The morphology of the final hydrogel was insensitive to the solution media. Fig. 10 also shows membranes fused together forming an interconnected cellular 3-dimensional network. In many instances, the membranes appear to be torn, probably as a result of the freeze drying process. The average pore ranged between 50 and 150 μm in size.

4. Conclusion

Xyloglucan is a thermally reversible hydrogel that has a significantly higher modulus than most other hydrogels. In this study the microstructural and rheological results indicated that the gelation of xyloglucan occurs as a 2 stage process. The first step, which is not concentration dependent, is the initial formation of large membrane structures in the pre gel. This accounts for the initially high modulus and, as the sheets refract light, also explains why the pre-gel solution is opaque. The formation of the membrane structures appears to be accelerated by the presence of ions in the PBS. The second stage involves the joining of membranes into a very strong 3-dimensional network. This process and the final morphology is independent of the solution media.

Acknowledgements

I would like to thank W.D. Cook for use of the rheometer, and I. Harper for use of the SEM. This project was funded by the CRC for Polymers and the ARC project DP0450618.

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