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Global conformation analysis of irradiated xyloglucans

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ABSTRACT

Xyloglucan isolated and purified from tamarind seed was subjected to various degrees of γ-irradiation treatments - from 10 to 70 kGy, monitored for radiation damage and then studied using a new combined hydrodynamic approach with regards to conformation and flexibility. Radiation products were analysed with regard to molecular weight (weight average) M_w from size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLs), intrinsic viscosity [η] and sedimentation coefficient $s^o_{20,w}$. Sedimentation coefficient distributions and elution profiles from SEC-MALLs confirmed the unimodal nature of the molecular weight distribution for each sample in solution. The chain flexibility was then investigated in terms of the persistence length, L_p of the equivalent worm-like chain model. The traditional Bushin-Bohdanecky (intrinsic viscosity) and Yamakawa-Fuji (sedimentation coefficient) relations were used separately then combined together by minimisation of a target function according to a recently published procedure (Ortega A. and Garcia de la Torre, J. *Biomacromolecules*, 2007, 8, 2464-2475) and yielded an estimate for L_p in the range 4 – 9 nm using floating and fixed mass per unit length analysis protocols and "point" global analysis: irradiated xyloglucans behave as flexible structures in common with pressure/heat treated materials.

Keywords: Xyloglucan; γ-irradiation; chain flexibility; Bushin-Bohdanecky; Yamakawa-Fuji; Combined "global" analysis

INTRODUCTION

Xyloglucan (XG) from the seed kernels of *Tamarindus indica* L. is a plant cell wall polysaccharide consisting of a β (1→4)-D-glucan backbone, which is partially substituted at position 6 of the glucopyranosyl units mainly by single α-D-xylopyranosyl residues as well as by disaccharide side chains composed of β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl residues (Gidley, Lillford, Rowlands, Lang, Dentini, Crescenzi, Edwards, Fanutti & Grant Reid, 1991; Nishinari Yamatoya & Shirakawa, 2000). Its repeating unit belongs to the XXXG type, consisting of three consecutive xylosylated glucopyranose units (X) separated by at least one unsubstituted glucopyranose unit (G), whereby some of the xylose units bear a galactopyranose unit (L) (**Figure 1**). Recently, the structural motifs of the tamarind seed xyloglucan existing mainly as heptasaccharide (Glc_4Xyl_3, motif XXXG), octasaccharide (Glc_4Xyl_3Gal, motif XXLG), and nonasaccharide ($Glc_4Xyl_3Gal_2$, motif XLLG) have been reported (Hoffman, Jia, Peña, Cash Harper, Blackburn, Darvill & York,).

<Figure 1 near here>

Use of water soluble xyloglucan isolated from the endosperms of seeds of *Tamarindus indica,* is expanding from textile and food to pharmaceutical and cosmetic industries. This expansion of interest is due to its particular physicochemical properties and spectrum of biological activities (Burgalassi, Raimondi, Pirisino, Banchelli, Boldrini & Saettone, 2000; Shankaracharya, 1998; Sreelekha, Vijayakumar, Ankanthil, Vijayan & Nair, 1993). Many of the functional properties of xyloglucan, in common with other polysaccharides, depend on molecular weight. Production of polysaccharides of lower molecular weight by whatever means is of particular interest to the biomedical and healthcare industries, as lower molecular material offers the advantage of improved diffusion into biological tissues: the use of irradiative methods for producing lower molecular weight material is one such method and is explored here.

Several reports on the solution properties of xyloglucan from tamarind seed and *Detarium senegalense* have already been published (Lang & Burchard, 1993; Lang & Kajiwara, 1993; Picout, Ross-Murphy, Errington, & Harding, 2003; Wang, Ellis, Ross-Murphy & Burchard, 1997). The most recent work (Picout et al. 2003) has focussed on the need to provide adequate solubilisation particularly with regard the higher molecular weight material using appropriate assisted dispersal methods involving pressure and temperature. In this present study we are not able to follow this advised procedure primarily as it would obscure the effects of irradiation damage, which we are trying to assess. However since, with the exception of the un-irradiated sample, we are dealing with lower molecular weight xyloglucans the presence of incompletely dispersed material (which will be removed by ultracentrifugation anyway) will not be as serious a problem because of their higher solubility. We still though have to take particular care to ultracentrifuge all samples prior to analysis (and final concentration assignment).

Besides pressure and temperature treatment (Picout et al. 2003), other methods for depolymerisation/ degradation of xyloglucans have involved oxidative scission by hydroxyl radicals (Fry, 1999; Schweikert, Liszkay & Schopfer, 2000) and the generation of oligosaccharides by enzymatic treatment (Strickland, Darvill, Albersheim, Eberhard, Pauly & Pelley, 1999). However neither oxidative scission nor enzymatic treatments are aimed at preserving the structural features of the parent polymer. The application of irradiation treatment to xyloglucan in this study is designed to break the main chains without changing the structural features: there should be no substantial change in sugar composition of the heteropolysaccharides, the only major effect being a reduction in the size of the polysaccharide with increase in radiation treatment. In this regard a recently published study (Vodeničarová, Drimalova, Hromadkova, Malovikova & Ebringerova, 2006) probed the effect of various radiation treatments on the chemical and structural properties and molecular weight of tamarind seed xyloglucan. It was shown by those researchers that depolymerisation by γ-irradiation at doses up to 50 kGy resulted in no significant change in the degree of branching of the residual polymer chains, with a suggestion of a small increase >50kGy. The present study now focuses on the hydrodynamic properties of a series of tamarind seed xyloglucan fractions prepared by γ-

irradiation according to Vodeničarová et al. (2006) to assess the molecular flexibility of these molecules, and to take advantage of some recent advances in data analysis and to see if there is any significant change in flexibility at high irradiation doses.

MATERIALS

The xyloglucan sample XG-0, produced by CPN, spol. s r.o. (Dolní Dobrouč, Czech Republic), was irradiated in dry air at room temperature using a ^{60}Co source (Perun, Artim s.r.o., Prague, Czech Republic). The irradiation doses ranged from between 10 to 70 kGy yielding samples XG-10 (10 kGy), XG-20 (20 kGy), XG-30 (30 kGy), XG-40 (40 kGy), XG-50 (50 kGy) and XG-70 (70 kGy). Their monosaccharide compositions are shown in **Table 1**.

<Table 1 near here>

Samples were dissolved in phosphate buffered saline at pH 7.0 (I = 0.1 M) in screw capped tubes with constant stirring at low speed. During this period the temperature was raised to 80.0 ˚C for 10 minutes to obtain maximum solubility. Stirring continued at room temperature (20.0 ˚C) overnight at low speed. Samples were then subjected to preparative ultracentrifugation at 40,000 rpm for 15 min (Beckman L8-55 M Ultracentrifuge, Beckman Instruments, Palo Alto, USA) to remove any insoluble particles or aggregates. The concentration was then estimated using a differential refractometer (Atago DD5 – Jencons Scientific, Leighton Buzzard, UK) and refractive increment *dn/dc* of 0.152 mL g^{-1} (Wang et al. 1997).

METHODS

Sedimentation velocity

Sedimentation coefficients were evaluated using the Beckman Optima XL-I analytical ultracentrifuge (Beckman Instruments, Palo Alto, USA). Solvent (400 µL) - and sample (380 µL) were injected into the solvent and sample channels of the 12 mm, double

sectored carbon filled centrepiece. Cells were then loaded into an 8-hole titanium rotor and placed in the centrifuge. Samples were run at 40,000 rpm and 20.0 ºC throughout. Concentration profiles of the ultracentrifuge cells were registered using the Rayleigh interference optical system (Ralston, 1993).

Solute concentration distributions recorded over the time-course of the sedimentation experiment (scans every 4 minutes) were analysed using the finite-difference algorithm SEDFIT (Schuck & Rossmanith, 2000) in terms of an apparent distribution of sedimentation coefficients in the form of $g^*(s)$ versus $s_{T,b}$, where the * indicates that the distribution of sedimentation coefficients has not been corrected for diffusion effects. Scans were used from attainment of meniscus depletion up to the point at which the mid-point of the boundary had traversed a further ~20% of column length - which meant that ~40 scans were analysed (regularisation was set to 0.68). The sedimentation coefficient, s for each concentration (from the mode of the main peak position of the g*(s) distribution) was then corrected to standard solvent conditions (density and viscosity of water at 20°C) to yield $s_{20,w}$ (S) (Schachman, 1959). To eliminate the effect of non-ideality, $s_{20,w}$ was further extrapolated to zero concentration to obtain $s^o_{20,w}$. For asymmetric molecules in non-ideal systems (Harding, 1995, 1997) reciprocal ($1/s_{20,w}$) versus concentration plots generally give more reliable estimates for $s^o_{20,w}$ (Table 2).

Viscometry

The intrinsic viscosity of each xyloglucan sample was measured using a semi-automated viscosity measuring unit (AVS 310, Schott Geräte, Hofheim, Germany) at a temperature of (20.00 ± 0.01) °C in an Ostwald viscometer. Relative dynamic viscosities η_{rel} were calculated using (Huggins, 1942).

$$\eta_{rel} = \left(\frac{t}{t_0}\right)\left(\frac{\rho}{\rho_0}\right) \approx \frac{t}{t_0} \qquad (1)$$

where t, and ρ refer to the flow time and density for xyloglucans respectively, and t_0 and ρ_0 are solvent flow time and density respectively. Since concentrations were low (<2 x

10^{-3} g mL^{-1}) the density correction term was considered negligible. Reduced specific viscosities η_{red} (mL g^{-1}) were then determined across a range of concentration c (between 0.1 - 2 x 10^{-3} g mL^{-1}):

$$\eta_{red} = (\eta_{rel} - 1)/c \quad (2)$$

and intrinsic viscosities [η] were calculated from extrapolation of η_{red} to zero concentration (Huggins, 1942) to eliminate non-ideality.

Size Exclusion Chromatography coupled to Multi-Angle Laser Light Scattering (SEC-MALLs)

Molecular weights (weight averages, number averages and distributions) were estimated using SEC-MALLs (Wyatt, 1992). The SEC system used consisted of a Jasco HPLC pump (Model PU-1580, Jasco Corporation, Tokyo, Japan), a Phenomenex guard column (Phenomenex, Macclesfield, UK), and TSK (Tosoh Biosciences, Tokyo, Japan) Gel G4000 PW and Gel G3000 PW columns connected in series. An on-line de-gasser was used to remove gas from the eluent. A flow rate of 0.8 mL min^{-1} for the mobile phase was used at room temperature. A DAWN-EOS multi-angle laser light scattering detector and an Optilab rEX refractometer (Wyatt Technologies, Santa Barbara, USA) were used for light scattering intensity and concentration detection respectively. The mobile phase was the phosphate buffered saline at pH 7.0, I = 0.1 M and 100 μL samples of xyloglucan were injected into the size exclusion system after initially centrifuging at 40,000 rpm for 15 minutes (Beckman L8-55 M Ultracentrifuge, Beckman Instruments, Palo Alto, USA) and filtering through 0.45 μm filters (Whatman, Maidstone, England). It is also possible to estimate (z-average) radii of gyration R_g for particles large enough so there is a measurable dependence of the scattering function Kc/R_θ on scattering angle θ, where R_θ is the Rayleigh excess ratio (a measure of the difference in intensity between the scattered light and incident light), c is the concentration (g/mL) and K is a scattering 'constant' depending on the wavelength, refractive increment dn/dc (ml/g) and refractive index of the medium n_o. However in this study only for the unfractionated sample was there a significant angular dependence allowing R_g to be estimated (Patel, Picout, Ross-Murphy & Harding, 2006).

RESULTS AND DISCUSSION

Sample purity: sedimentation coefficient and molecular weight distributions

Sedimentation coefficient distributions were registered for the untreated (XG-0) and irradiated (XG-10 – XG-70) samples at all the concentrations studied. In every case the samples showed a strong degree of unimodality (**Figure 2**) but with some evidence of the existence of discrete (*i.e.* non-continuous) heterogeneity at higher molecular weights.

<Figure 2 near here>

The g*(*s*) distribution for each of these plots reflect the effect of γ-radiation on the macromolecular chain. The sedimentation coefficient, a very sensitive parameter to molecular weight, is seen to decrease steadily with increase in severity of radiation treatment (see **Table 2**) indicating a progressive scission of the polysaccharide chains. Additionally, it was also observed that there was an increase in the width of the main peak with increasing treatment. This is consistent with an increase in polydispersity, although quantitative estimates are difficult because contributions to the broadening could also arise from increased diffusion and decreased hyper-sharpening though decreased size and hence non-ideality (Dhami Harding, Jones, Hughes, Mitchell & To, 1995; Morris Li, Puaud, Liu, Mitchell & Harding, 2001). Molecular weight distribution analysis from SEC-MALLs confirms these observations (**Figure 3**), and quantitative estimates for the polydispersity (from the ratio of the weight average M_w to the number average M_n) are consistent with the treated samples being more polydisperse than the native material.

<Figure 3 near here> <Table 2 near here>

Intrinsic viscosity

The intrinsic viscosity was measured by extrapolation of the reduced viscosity η_{red} to zero concentration (Huggins, 1942) and was seen to decrease with increase in γ-radiation treatment (**Table 2**).

SEC-MALLs and weight average molecular weights

Elution profiles for the untreated and irradiated xyloglucans were largely unimodal, consistent with the sedimentation velocity data. Values for the weight average molecular weight (M_w) for all samples measured using SEC-MALLs are presented in **Table 2**, showing a steady decrease with increasing treatment. The polydispersity ratio M_w/M_n also increases after mild irradiation and remains essentially constant thereafter. A radius of gyration of (76 ± 2) nm was estimated for the untreated sample and "<50nm" for the other samples.

Conformational analysis

Hydrodynamic results obtained from SEC-MALLs, sedimentation velocity and viscosity measurement were further used to study the gross conformation of xyloglucans (Harding, Vårum, Stokke & Smidsrød, 1991), taking advantage of the fact that the radiation at various levels yielded different values for weight average molecular weight, M_w, facilitating the use of the "Mark-Houwink-Kuhn-Sakurada"- (MHKS)-type power law relations linking [η] and $s^o_{20,w}$ with M_w:

$$[\eta] \propto M_w^{\ a} \tag{3}$$

$$s^o \propto M_w^{\ b} \tag{4}$$

The MHKS exponent (*a*), and sedimentation exponent (*b*) are derived using double logarithmic plots of respectively, intrinsic viscosities and sedimentation coefficients versus molecular weights (**Figure 4**), and the values obtained are reported in **Table 3**. This procedure assumes a homologous series for the polymers (*i.e.* they all have approximately the same conformation type): any departure would reveal itself as non-linearity of the logarithmic plots.

<Figure 4 and Table 3 near here>

The values estimated for both parameters are suggestive of a flexible coil molecule. The exponents are within the typical range for flexible chains with dominant hydrodynamic interaction in good solvents. These values only refer however to the average properties across a polymer series: the lower limit of the power law coefficient suggests that there is no measurable excluded volume effect which is what is expected for macromolecules of such size (Tanford, 1961).

We can then also apply a Tsvetkov-Eskin-Frenkel (Tsvetkov, Eskin, Frenkel, 1970) relation for linear polymers to look for consistency between power law exponents *a* and *b*. If they are consistent then

$$a \approx (2 - 3b) \qquad (5)$$

Using this we estimate $a \sim 0.7$ from the measured b and this is in reasonable agreement with the value of ~ 0.6 estimated from equation (3).

Estimation of persistence length

The Bushin -Bohdanecky method (Bohdanecky, 1983; Bushin, Tsvetkov, Lysenko & Emel'yanov, 1981)

This is one of the most popular methods for estimating chain persistence lengths particularly for semi-flexible polymers, and has been applied to range of polysaccharides from charged polysaccharides including xanthan (Sato, Norisuye & Fujita, 1984), sodium hyaluronate (Mendichi, Soltes & Schierone, 2003) as well as galactomannans (Gomez, Navarro, Manzanares, Horta, Carbonell, 1997) and neutral polysaccharides. In its simplest form, the Bushin-Bohdanecky method involves plotting $\left(M_w^2/[\eta]\right)^{1/3}$ versus $M_w^{1/2}$ and from the slope L_p can be calculated using the following relation and tabulated values (Bohdanecky, 1983) of the coefficient B_0:

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_\eta + B_0\Phi^{-1/3}\left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2} \qquad (6)$$

From a plot of $\left(M_w^2/[\eta]\right)^{1/3}$ versus $M_w^{0.5}$ (**Figure 5**) we obtain a slope of (1.23 ± 0.02). Taking B_0 as ~ 1.025 (ref. 30), 2.86 x 10^{23} mol^{-1} for the Flory-Fox 'constant' Φ and a (molar) mass per unit length M_L of ~ 537 g mol^{-1} nm^{-1} calculated from the Glc/Xyl/Gal ratio of 1:0.6:0.3 (**Table 1**) the value obtained for L_p is ~ (4 ± 1) nm.

<Figure 5 here>

The Yamakawa-Fujii method

Hearst and Stockmayer (1962) first reported the sedimentation coefficient in relation to wormlike chain parameters, later refined by Yamakawa and Fujii (1973). The original relation given by Yamakawa and Fujii relating the sedimentation coefficient with persistence length was unfortunately misprinted; the correction was given by Freire and Garcia de la Torre (1992):

$$s^0 = \frac{M_L\left(1-\bar{v}\rho_0\right)}{3\pi\eta_0 N_A}\times\left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + ...\right] \qquad (7)$$

Yamakawa and Fujii (1973) showed that $A_2 = -\ln(d/2L_p)$ and $A_3 = 0.1382$ if the L_p is much higher than the chain diameter, d. Using the Yamakawa-Fujii procedure a plot of $s^o_{20,w}$ versus $M_w^{1/2}$ (**Figure 6**) yielded a slope of (7.6 ± 0.7) x 10^{-3}, and from equation. (7) and a fixed M_L of 537 g mol^{-1} nm^{-1} again the L_p = (8 ± 1) nm somewhat higher than from the Bushin-Bohdanecky analysis but still well within the flexible coil range .

<Figure 6 here>

Combined analysis

Different methods provide their own bias on results (Bohdanecky & Petrus, 1991; Picout Ross-Murphy, Jumel & Harding, 2002) and in response to this problem Ortega and García de la Torre have created a new software package, Multi-HYDFIT (Ortega & García de la Torre, 2007) which considers data sets of both intrinsic viscosities for different molecular weights and sedimentation coefficients for different molecular weights. It then performs a minimisation procedure finding the best values of M_L and L_p and chain diameter d satisfying the Bushin-Bohdanecky (Bohdanecky, 1983; Bushin et al. 1981) and Yamakawa-Fujii (Yamakawa & Fujii, 1973) equations. Extensive simulations have shown that values returned for M_L and L_p are insensitive to d so this is usually fixed (Ortega & García de la Torre, 2007).

For the xyloglucans we considered two possible cases:

1. chain diameter, d was fixed at 0.9 nm and the mass per unit length, M_L was fixed at 537 g mol^{-1} nm^{-1} (we considered each xyloglucan separately and as a pseudo-homologous series). A value of d ~ 0.9 nm can be calculated from the partial specific volume and mass per unit length using eqn. 23 of Bohdanecky (1983). The value for M_L is obtained from the ratio of the average molar mass of the structural unit (290) to its length (0.54 nm).
2. only the chain diameter, d was fixed at 0.9 nm.

The Multi-HYDFIT program then "floats" the variable parameters (L_p in case 1, L_p and M_L in case 2) in order to find a minimum of the multi-sample target (error) function (Ortega & García de la Torre, 2007), Δ.

In this procedure as defined in Ortega and García de la Torre (2007) , Δ is calculated using equivalent radii (or the ratio of equivalent radii), where an equivalent radius is defined as the radius of an equivalent sphere having the same value as the determined property. These 'determined properties' include the translational frictional coefficient, f (calculated from either the diffusion or sedimentation coefficients), intrinsic viscosity,

[η], radius of gyration, $R_{g,z}$ or the rotational relaxation time, τ . In the present study we are interested in the equivalent radii resulting from the sedimentation coefficient i.e. translational frictional coefficient (a_T) and from the intrinsic viscosity (a_I).

$$a_T = \frac{f}{6\pi\eta_0} \tag{8}$$

where η_0 is the viscosity of water at 20.0 ºC, and

$$a_I = \left(\frac{3[\eta]M_w}{10\pi N_A} \right)^{1/3} \tag{9}$$

where N_A is Avogadro's number.

The target function, Δ can be evaluated from this relation:

$$\Delta^2 = \frac{1}{N_s} \sum_{i=1}^{N_s} \left[\left(\sum_T W_T \right)^{-1} \sum_T W_T \left(\frac{a_{T(cal)} - a_{T(\exp)}}{a_{T(\exp)}} \right)^2 \right] \tag{10}$$

and this one

$$\Delta^2 = \frac{1}{N_s} \sum_{i=1}^{N_s} \left[\left(\sum_I W_I \right)^{-1} \sum_I W_I \left(\frac{a_{I(cal)} - a_{I(\exp)}}{a_{I(\exp)}} \right)^2 \right] \tag{11}$$

where N_s is the number of samples in multi-sample analysis, W_T and W_I are the statistical weights for equivalent radii a_T and a_I (from translation frictional coefficient and intrinsic viscosity data respectively) and the subscripts cal and exp represent values from calculated and experimental values respectively. Δ is thus a dimensionless estimate of

the agreement between the theoretical calculated values for the translational frictional coefficient (consequently the sedimentation coefficient) and the intrinsic viscosity for a particular persistence length, L_p (and mass per unit length, M_L in case 2) and the experimentally measured parameters (Ortega & García de la Torre, 2007).

In the first case (**Figure 7**) where both the diameter and the mass per unit length are fixed we estimate a persistence length of (7 ± 1) nm. In the second case (**Figure 8**) where the depression (blue end of the colour spectrum) in the contour plot (where Δ varies by less than the error in the experimental data) gives the same answer within experimental error, namely L_p = (6 ± 1) nm and M_L = (510 ± 60) g mol^{-1} nm^{-1}.

Furthermore using "point" global determinations, that is to say we use [η], $s_{20,w}$ and M values for each irradiation does to estimate L_p/M_L (and hence L_p for an M_L = (510 ± 60) g mol^{-1} nm^{-1}) for each dose) there is within the error margin no significant difference in persistence lengths between the samples. This is consistent with the sugar composition data (Table 1) which shows that the Glc: Xyl ratios of xyloglucan before and after γ-irradiation (< 50 kGy) showed no significant differences as they varied from 1: 0.68 to 1: 0.60, and the Xyl: Gal ratios varied from 2.1 - 1.9: 1. At 70 kGy, the Glc: Xyl ratio increased to 1: 0.78, but the Xyl: Gal ratios remained unchanged. The combined results indicate that the overall flexibility of the molecule is not markedly changed, i.e. the series of polymers is ~ homologous as far as hydrodynamic properties are concerned.

In this paper we have shown using three different approaches based on intrinsic viscosity [η], sedimentation coefficient ($s^{o}_{20,w}$) and weight average molar mass (M_w) that irradiated tamarind seed xyloglucan products adopt flexible conformations in solution (L_p ~ 6-7 nm) which is in good agreement with previous estimates (Freitas, Martin, Santos, Valenga, Buckeridge, Reicher & Sierakowski, 2005; Ren, Picout, Ellis & Ross-Murphy, 2004; Picout et al. 2003) using either intrinsic viscosity or radius of gyration (**Table 5**). We have also seen that different approaches in the estimation of the persistence length can lead to bias in the results and therefore it is more appropriate to characterize macromolecules using more than one hydrodynamic technique.

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Table 1: Monosaccharide composition of native and γ-irradiated xyloglucans

Sample	*Radiation kGy*	*Glc: Xyl :Gal (Mole ratios)*	*Xyl : Gal*
XG-0	0	1 : 0.68 : 0.32	2.1 : 1
XG-10	10	1 : 0.64 : 0.31	2.1 : 1
XG-20	20	1 : 0.63 : 0.31	2.0 : 1
XG-30	30	1 : 0.66 : 0.31	2.1 : 1
XG-40	40	1 : 0.64 : 0.32	2.0 : 1
XG-50	50	1 : 0.60 : 0.32	1.9 : 1
XG-70	70	1 : 0.78 : 0.36	2.2 : 1

Table 2: Hydrodynamic data for native and γ-irradiated xyloglucans

Sample	$s^{o}_{20,w}$ **(S)**	**[η] (mL/g)**	$10^{-4} \times M_w$ **(g/mol)**	M_w/M_n
XG-0	7.21 ± 0.03	405 ± 35	70.0 ± 5.0	1.1 ± 0.1
XG-10	4.66 ± 0.03	210 ± 10	27.0 ± 1.0	1.3 ± 0.1
XG-20	3.10 ± 0.04	170 ± 10	15.8 ± 0.3	1.4 ± 0.1
XG-30	3.30 ± 0.01	140 ± 10	12.7 ± 1.0	1.3 ± 0.1
XG-40	2.82 ± 0.04	135 ± 5	9.7 ± 1.0	1.3 ± 0.1
XG-50	2.80 ± 0.08	100 ± 5	6.0 ± 0.4	1.3 ± 0.1
XG-70	2.61 ± 0.02	75 ± 5	4.5 ± 0.3	1.1 ± 0.1

Table 3: Experimental power law values for xyloglucans and the theoretical values for random coils

Power law parameter	*Value*	*Theoretical (random coil)*
a	0.55 ± 0.03	0.5 – 0.8
2 - 3b	0.74 ± 0.03	
b	0.42 ± 0.01	0.4 – 0.5

Table 4: Individual estimates of L_p/M_L for each irradiated xyloglucan. Corresponding persistence lengths also given for M_L ~ 537 g mol^{-1} nm^{-1}

Sample	**L_p/M_L (nm^2 mol g^{-1})**	**L_p (nm)**
XG-0	0.011 ± 0.002	6 ± 1
XG-10	0.011 ± 0.002	6 ± 1
XG-20	0.017 ± 0.002	9 ± 1
XG-30	0.011 ± 0.002	6 ± 1
XG-40	0.015 ± 0.002	8 ± 1
XG-50	0.011 ± 0.002	6 ± 1
XG-70	0.011 ± 0.004	6 ± 2
Overall	0.013 ± 0.002	7 ± 1

Table 5: Persistence length estimates for xyloglucan using different approaches

***Persistence length* (nm)**	***Method (parameters used)***	***Reference***
4 ± 1	M_w, [η]	This study
8 ± 1	M_w, s	This study
7 ± 1	M_w, [η], s – fixed M_L (537 g mol^{-1} nm^{-1})	This study
6 ± 1	M_w, [η], s	This study
6 - 8	M_w, [η]	9
11	M_w, R_{gz}	39
5 - 8	M_w, [η]	40

LEGENDS OF FIGURES

Figure 1. Structural features of tamarind seed xyloglucan.

R: H (motif XXXG) or β-D-galactopyranose (motif XXLG).

Figure 2. g*(s) distributions for xyloglucans: XG-0 at a loading concentration c = 1.0 mg/mL (!). The main peak s value = 4.38 S, XG-20 at 1.0 mg/mL (7), s = 2.91 S, XG-50 at 1.2 mg/mL ( ), s = 2.71 S, XG-70 at 1.2 mg/mL (ξ), s = 2.31 S.

Figure 3. Molecular weight distributions for xyloglucans: XG-0 (!), XG-10 (,), XG-20 (7), XG-30 (B) and XG-40 (Λ).

Figure 4. Power law double logarithmic plots (a) viscosity**:** slope, $a = 0.55 \pm 0.03$, (b) sedimentation: slope, $b = 0.42 \pm 0.01$.

Figure 5. Bohdanecky plot for xyloglucan. L_p is calculated from the slope.

Figure 6. Yamakawa-Fujii plot for xyloglucan. L_p is calculated from the slope.

Figure 7. Plot of target function (Δ) vs. persistence length for xyloglucan treated as a pseudo-homologous series (at fixed $M_L = 537$ g mol^{-1} nm^{-1}).

Figure 8. Combined analysis plots for xyloglucan. The x-axis and y-axis represent L_p (nm) and M_L (g mol^{-1} nm^{-1}) respectively. The target function, Δ is calculated over a range of values for M_L and L_p. In these representations, the values of Δ function are represented by the full colour spectrum, from blue (Δ = 0) to red (Δ ≥ 1). The calculated minimum is indicated.

FIGURES

Figure 1

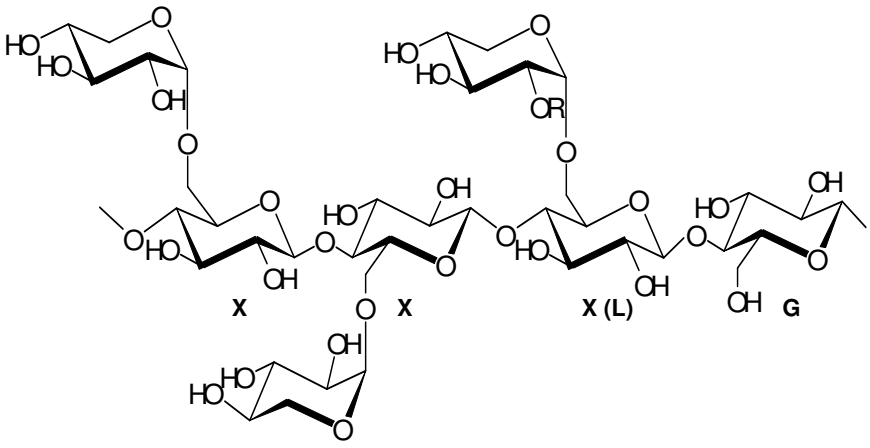


Figure 2

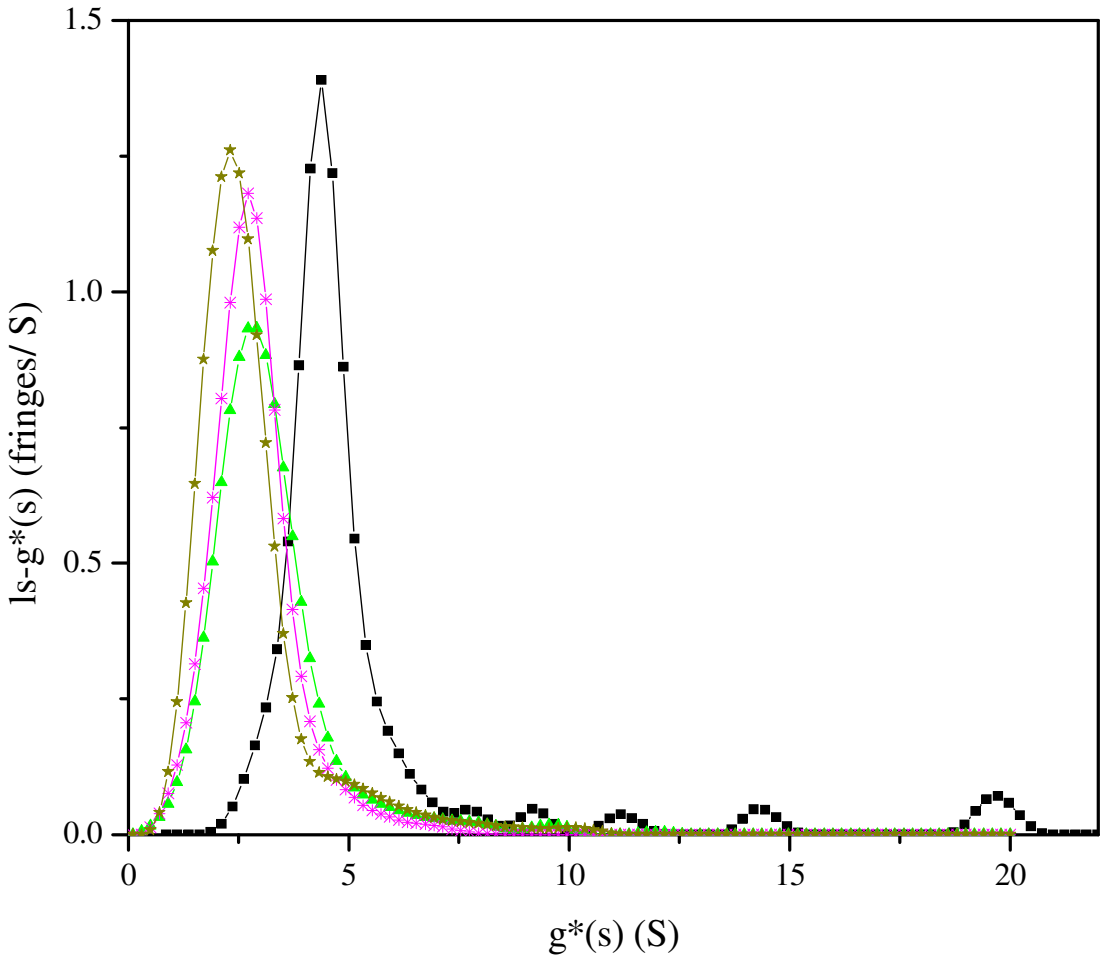


Figure 3

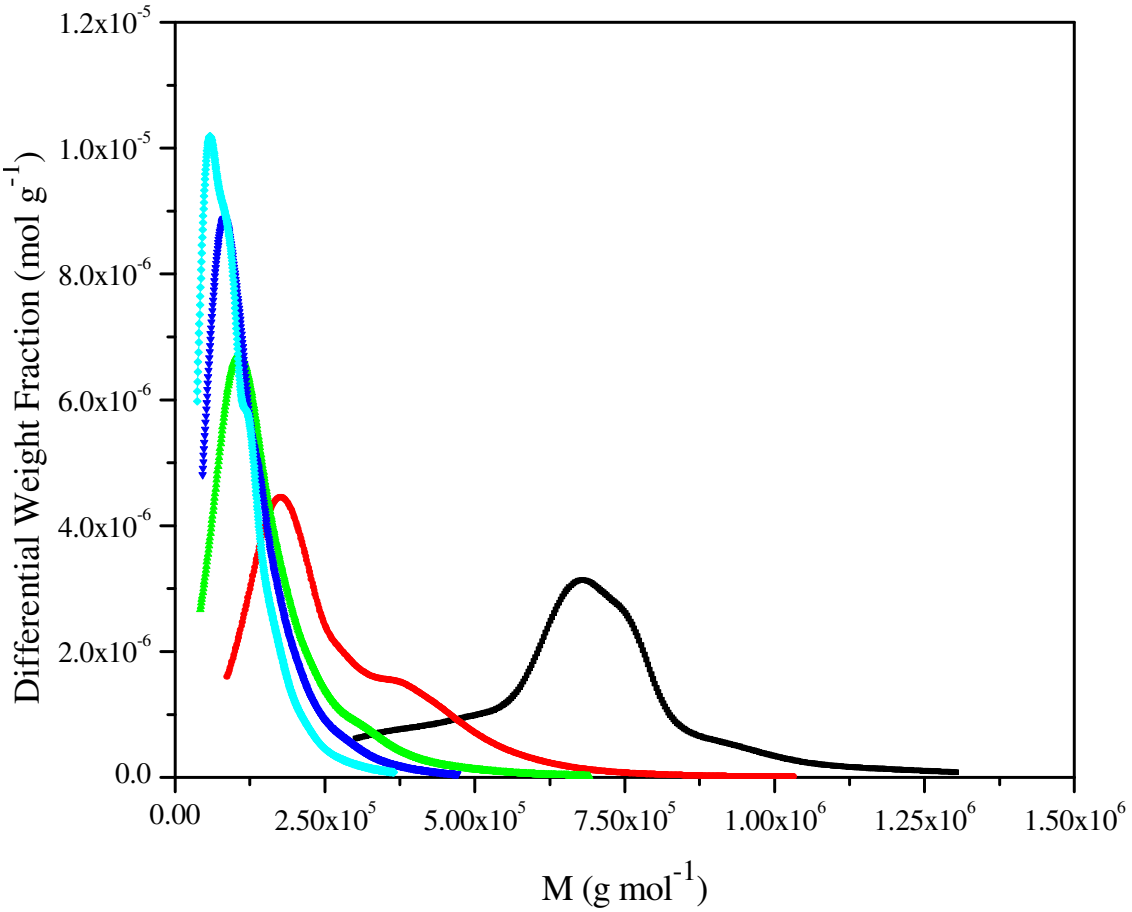
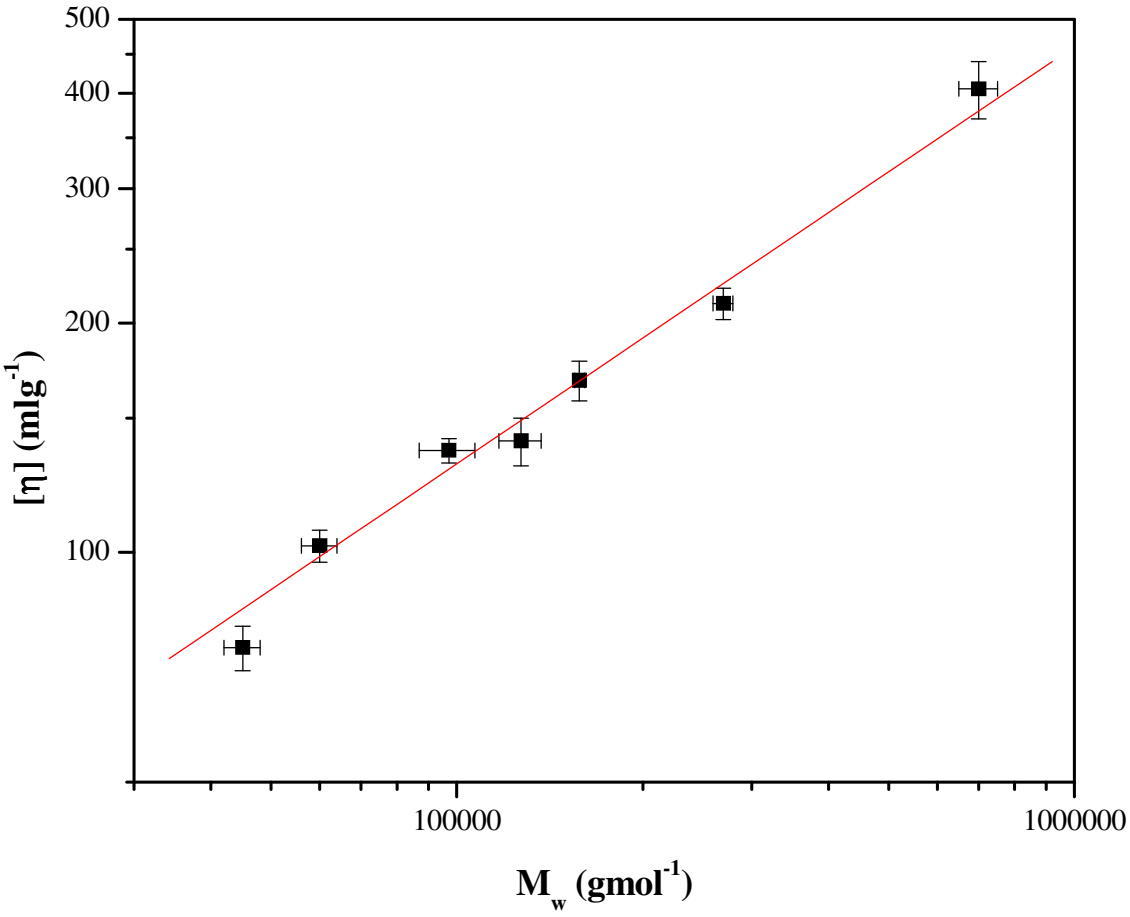

1.2x10-5
1.0x10-5
8.0x10-6
6.0x10-6
4.0x10-6
2.0x10-6
0.0
Differential Weight Fraction (mol g-1)
0.00
2.50x105
5.00x105
7.50x105
1.00x106
1.25x106
1.50x106
M (g mol-1)

Figure 4 (a)



(b)

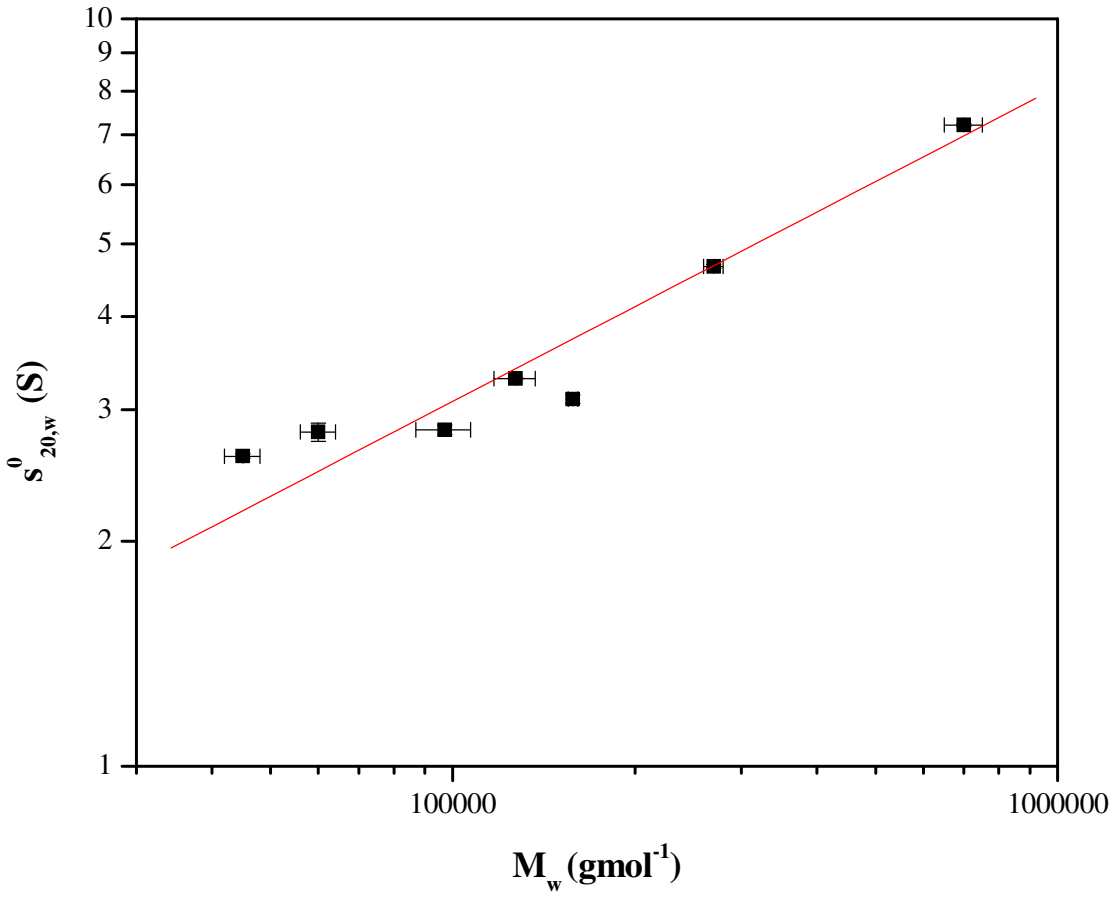
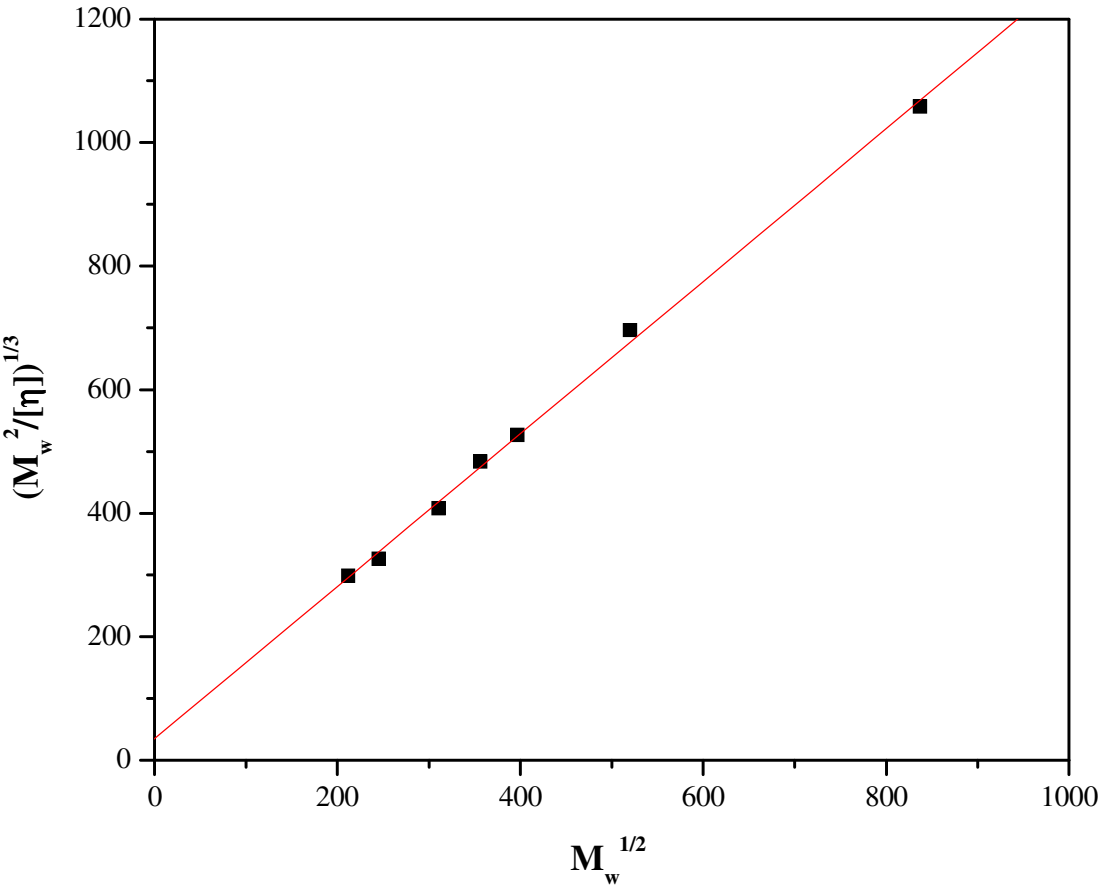


Figure 5


1200
1000
800
600
400
200
0
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200
400
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800
1000
$(M_w^2/[\eta])^{1/3}$
$M_w^{1/2}$

Figure 6

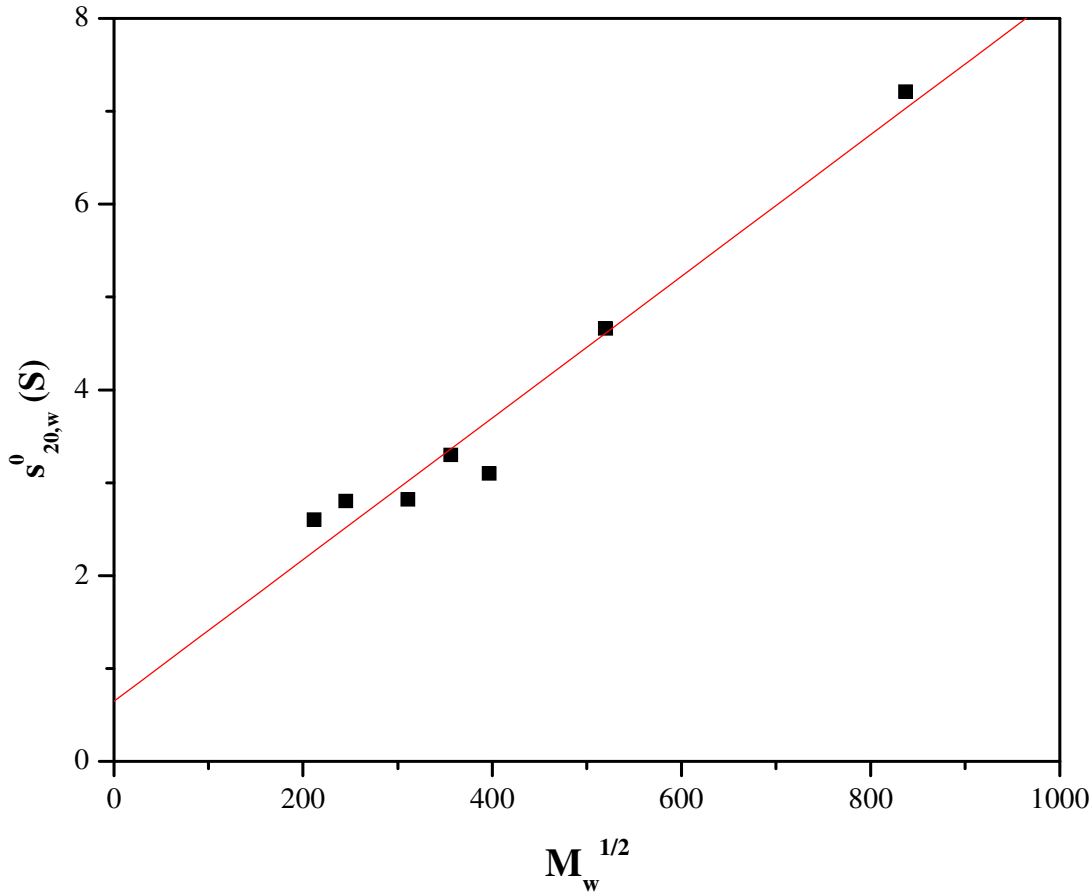


Figure 7

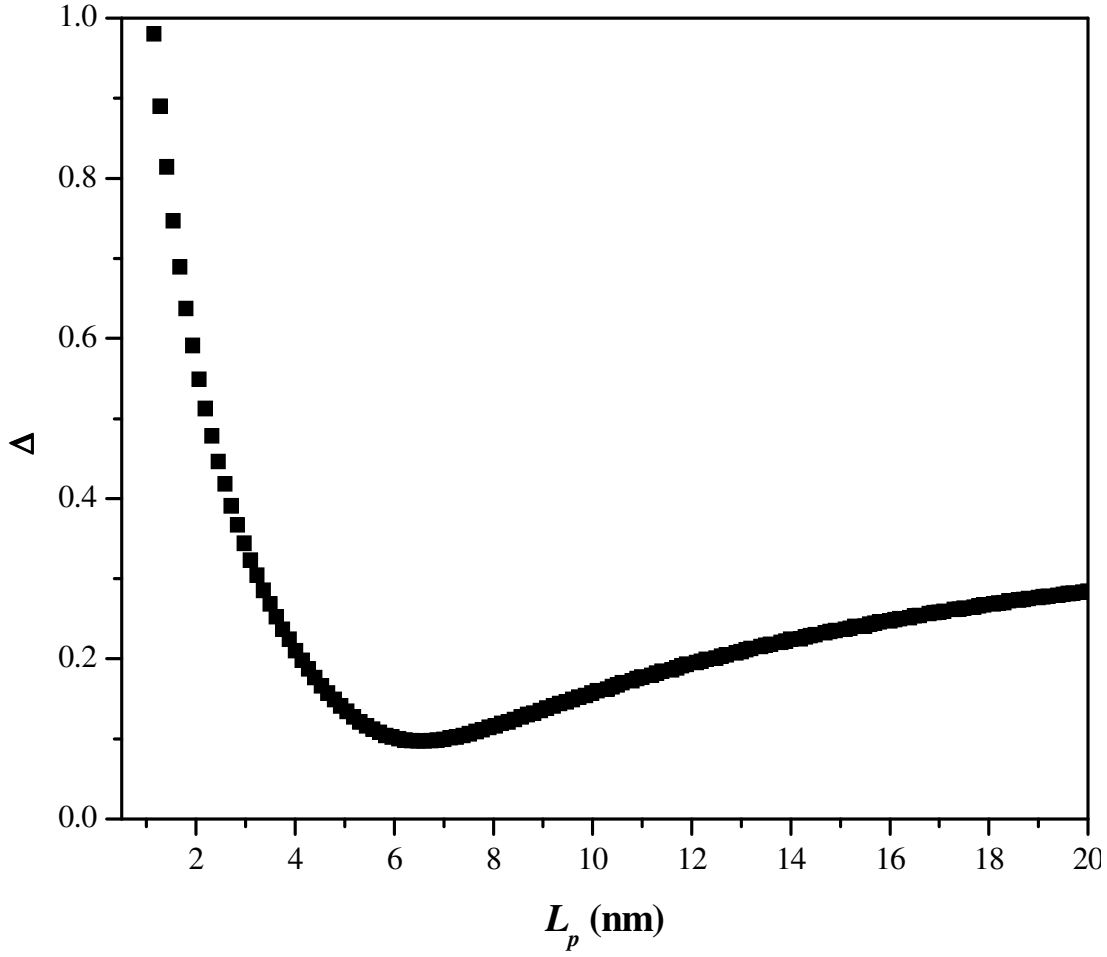
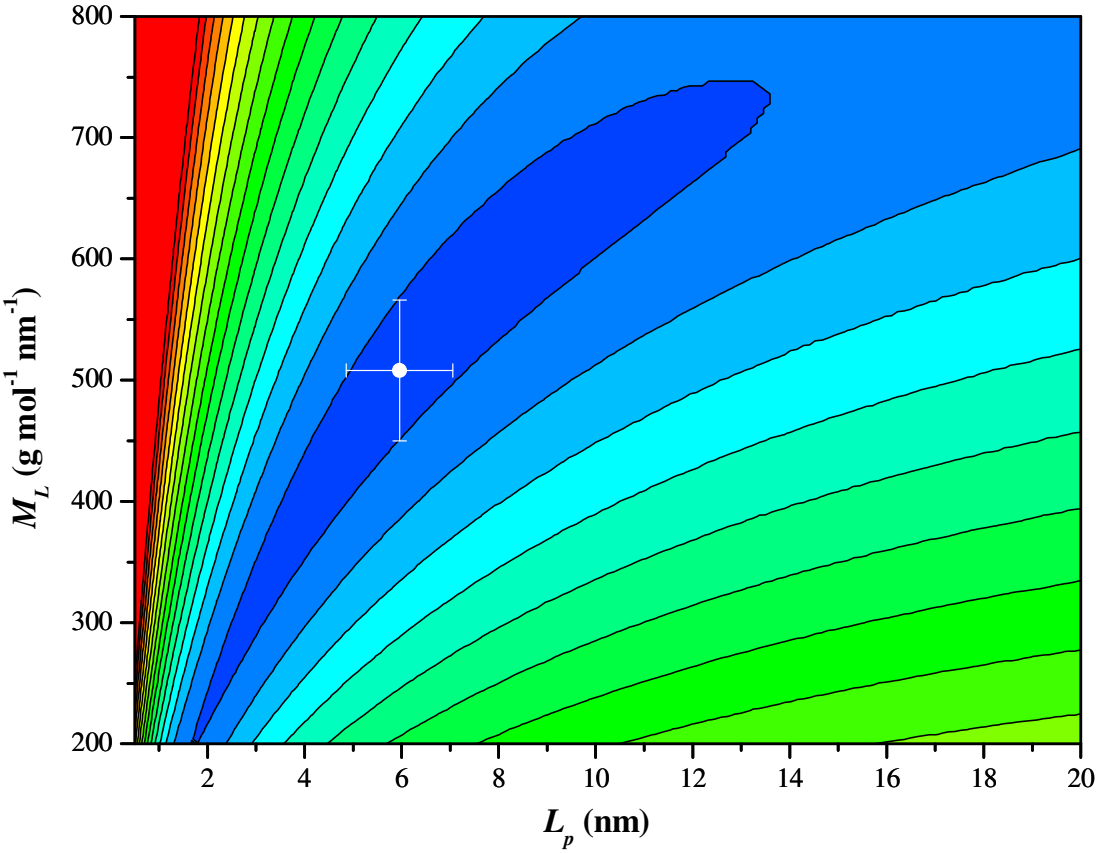


Figure 8


800
700
600
500
400
300
200
2
4
6
8
10
12
14
16
18
20
M_L (g mol^{-1} nm^{-1})
L_p (nm)