# Rheological and Light Scattering Properties of Flaxseed Polysaccharide Aqueous Solutions

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Polysaccharides isolated from flaxseed meals using ethanol consisted of a soluble (~7.5% w/w) and an insoluble fraction (2% w/w). The soluble fraction was dialyzed in various salt concentrations and characterized using viscometry and light scattering techniques. Observations using a size-exclusion column coupled to a multiangle laser light scattering (SEC-MALLS) revealed three molecular weight fractions consisting of a small amount (~17%) of large molecular weight species ($1.0 \times 10^6$) and a large amount (~69%) of small molecular weight species ($3.1 \times 10^5$ Da). Dynamic light scattering measurements indicated the presence of very small molecules (hydrodynamic radius ≈ 10 nm) and a very large molecular species (hydrodynamic radius in excess of 100 nm); the latter were probably aggregates. The intrinsic viscosity, $[\eta]$, of the polysaccharide in Milli-Q water was 1030 ± 20 mL/g. The viscosity was due largely to the large molecular weight species since viscosity is influenced by the hydrodynamic volume of molecules in solution. The Smidsrod parameter $B$ obtained was ~0.018, indicating that the molecules adopted a semi-flexible conformation. This was also indicated by the slope (~0.56) from the plot of root-mean-square (RMS) radius versus molar mass ($M_w$).

## Introduction

Flaxseed contains approximately 35% lipid, 30% proteins, and 35% fiber, 7−10% of which is soluble fiber whose composition varies by cultivar and geographic location.[1] Extraction of oil (approximately 30% w/w) from the seeds of *Linum usitatissimum* (or flaxseed) leaves behind a large amount of flaxseed meal which is usually processed as a livestock feed.[2−4] Flaxseed meal has also been added to various food products including muffins, bread, spaghetti, snack bars, salad toppings, etc.,[5−7] but fundamental understanding of the actual functional properties of the meals in a complex food systems is not well established. In the last two decades, a number of studies have attempted to purify and characterize the proteins[8−10] and the mucilage.[11−22] Mucilage has drawn much attention mainly because of its supposed health benefits (soluble dietary fiber, reducing the risk of diabetes, and cholesterol lowering properties) [23−27] and its potential physical functional properties in food applications as a thickener and an emulsifier.[6,13,28−30] Flaxseed polysaccharide is said to resemble gum Arabic in its emulsification properties.[21] In addition, it was reported to have comparable water-binding capacity to guar gum.[18] The physicochemical characteristics of the polysaccharide have been studied over the last two decades, and it has been reported that the mucilage is a heterogeneous polysaccharide consisting of xylose, glucose, galactose, arabinose, rhamnose, fucose, and galacturonic acid.[15,21] The polysaccharide was first reported by Hunt and Jones[31] to contain a neutral fraction and an acidic fraction. Recent studies using ion-exchange and size-exclusion chromatography further revealed that the neutral polysaccharide fraction (~75%) is a galacto-arabino-xylan ($M_w \approx 1.16 \times 10^6$ Da) and the acidic portion consists of two pectin-like fractions of rhamnogalacturonan ($M_w \sim 6.5 \times 10^5$ and $1.7 \times 10^4$ Da respectively).[22] However, the molar masses of these fractions may differ depending on the genotype and climatic conditions where the crop is grown.[16] The aim of this study was to characterize the physicochemical properties of flaxseed polysaccharide isolated from the meal of flaxseed grown in New Zealand. This study employed simple isolation techniques that could be easily adapted for industrial scale-up. Using both rheological and light scattering measurements, the flow behavior and molecular parameters of the biopolymer were investigated in dilute solutions. Knowledge of the physicochemical characteristics of the polysaccharide molecules would be essential if its application as an ingredient in food systems is to be well understood.

## Materials and Methods

**Isolation of Flaxseed Polysaccharides.** The flaxseed meal used in this study was a byproduct of flaxseed oil processing using a cold extraction method and kindly supplied by Functional Whole Foods Ltd., New Zealand. The flaxseed was of the cultivar known as *Hinao*. Extraction of the mucilage was carried out by dispersing the flaxseed meal in Milli-Q water (water purified by treatment with a Milli-Q apparatus, Millipore Corp., Bedford, MA) at a water/solid ratio of 25:1 and temperature of 20 °C for 4 h under continuous agitation using a mechanical overhead stirrer (IKA, Type RW20.n, 400 rpm). The viscous mixture was centrifuged at 12 000*g* for 60 min at 25 °C (RC5C Sorvall centrifuge and GS-3 rotor, Kendro Laboratory Products GmbH, Langenselbold, Denmark). The creamy layer at the top of the supernatant was discarded, and the rest of the supernatant was subjected to ethanol precipitation (95% ethanol/mixture ratio of 3:1). The precipitate was allowed to form and left in the solvent for approximately 10 min with occasional gentle stirring. The precipitate was then recovered using a sieve to allow excess solvent to drain. The precipitate was dispersed in Milli-Q water with continuous stirring and left overnight at 20 °C. The mixture was then centrifuged (27 000*g*, 60 min, 25 °C). Both the supernatant (the soluble fraction) and the pellet (the insoluble fraction)

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were recovered. The supernatant was dialyzed (12 000 molecular weight cutoff dialysis tubing) in Milli-Q water at 20 °C with three changes of water over a 24-h period. The tubings were then transferred to solutions made up of various salt concentrations (0.00, 0.01, 0.05, 0.075, 0.1, 0.5, and 1 M) and 0.02% w/v sodium azide and left for another 24 h at 20°C. The dialyzed samples were filtered through a 0.22 $\mu$m filter and used for light scattering measurements and intrinsic viscosity measurements.

Chemical analysis for determination of total carbohydrate was adapted from the phenol-sulfuric acid method as described by Dubois et al. (1956). On milliliter of the sample was mixed with 1 mL of phenol solution (5% w/v) followed by addition of 5 mL of concentrated sulfuric acid. This mixture was left at room temperature for 30 min prior to measuring absorbance at 485 nm using a spectrophotometer (Ultrospec 2000, Amersham Pharmacia Biotech, Piscataway, NJ). The total amount of carbohydrate was determined based on a standard calibration curve prepared using glucose. All analyses using the phenol-sulfuric acid method were performed in duplicate.

**Multiangle Laser Light Scattering (MALLS).** The mobile phase or solvent used in light scattering experiments was 0.1 M NaCL solution containing 0.02% w/v sodium azide. The solution was filtered through a 0.22 $\mu$m membrane filter (Millipore) followed by a 0.025 $\mu$m membrane filter and degassed under vacuum for approximately 1 h at room temperature. All glass apparatus that was in contact with the mobile phase and EPS samples was prewashed in 5% v/v nitric acid followed by thorough rinsing with Milli-Q water to minimize contamination by dust particles. The flaxseed polysaccharide sample dialyzed in 0.1 M NaCl and filtered (0.22 $\mu$m) was loaded into the flow cell. The concentration of the EPS sample was determined using the phenol-sulfuric assay[32] after the filtration step. The chromatography mode used a high-performance liquid chromatography (HPLC) (GBC Scientific Equipment Ltd, Victoria, Australia) consisted of a HPLC pump (model LC 1150), UV detector (model LC 1200), and system organizer (model LC 1440). Separation was achieved using the Shodex SB-806 HQ HPLC column (Tokyo, Japan) as a size-exclusion column (SEC). This column separates proteins from 1 to 20 000 kDa. The mobile phase was continuously gassed with helium and passed through the HPLC column at a flow rate of 0.5 mL/min at 20 psi. The eluent from the SEC column passed through the UV detector at 280 nm, the DAWN DSP MALLS (Wyatt Technology, Santa Barbara, CA) photometer (fitted with 16 photodetectors at different angles, a helium neon laser with an operating wavelength of 632.8 nm, and a K-5 flow cell) and then through the differential refractive index (DRI) detector (Waters, model R401, Milford, MA). The polysaccharide sample (50 $\mu$L) was loaded and separated at 25 °C in the SEC column at a flow rate of 0.5 mL/min over approximately 30 min elution time. Astra software (version 4.5, Wyatt Technology Corp., Santa Barbara, CA) was used to analyze the data using the Debye plot to generate molecular parameters including the weight-average molar mass ($M_w$) and the *z*-average RMS radius ($r_z$). These principles are explained by the Rayleigh−Debye−Gans light scattering model for dilute polymer solutions[33]

$$\frac{R_\theta}{K_c} = M_w P_\theta - 2A_2 c M_w^2 P_\theta^2 \qquad (1.1)$$

where $R_\theta$ is the Rayleigh excess scattering, the excess intensity of light scattered at angle $\theta$ (i.e., the intensity due to the solute), $K$ is an optical constant, $c$ is the concentration of solutes (g/mL), $M_w$ is the weight-average molecular weight, $P_\theta$ is the scattering function, and $A_2$ is the second virial coefficient.

**Intrinsic Viscosity.** The intrinsic viscosity [$\eta$] was determined using a Cannon-Ubbelohde low-shear four-bulb capillary viscometer (Viscometer no. 75, S766, Cannon Instrument Co.). The relative viscosity ($\eta_{rel}$) of the sample was measured at 20 °C, and the respective dialysates were regarded as the solvent. The sample (10 mL) was loaded into the capillary viscometer and allowed to equilibrate for approximately 5 min at 20 °C before measuring the efflux time. Triplicate measurements were carried out for each sample dilution. The experimental data obtained were substituted in the Huggins and Kraemer functions (see eqs 1.2 and 1.3) and plotted against the polysaccharide concentration

$$\frac{\eta_{sp}}{c} = [\eta] + K'[\eta]^2 c \qquad (1.2)$$

$$\frac{\ln \eta_{rel}}{c} = [\eta] + K''[\eta]^2 c \qquad (1.3)$$

where $K'$ is the Huggins coefficient and $K''$ the Kraemer coefficient. The linear functions were extrapolated to zero concentration to obtain the intrinsic viscosity at the intercept.

**Dynamic Light Scattering.** The light scattering apparatus used in this study has been fully described previously.[34] In summary, a vertically polarized argon-ion laser (Spectra Physics 165) operating at 488 nm was used to illuminate a sample maintained in a Precision Devices goniometer. The measurements were made at scattering angles $\theta$ varying between 30° and 120°, and all measurements were performed at a constant temperature of 25 °C. The scattered light was collected using a single-mode optic fiber (Thorlabs Inc., Germany), and the intensity autocorrelation function was obtained using a correlator.com Flex 990EM-12 multiple tau correlator. The measured intensity autocorrelation functions were then analyzed using the regularization method CONTIN[35] to obtain the distribution of decay times $\Gamma$. Finally, the hydrodynamic radius $R_h$ of the biopolymer was extracted using the Stokes−Einstein relationship for the diffusion coefficient $D$[36]

$$D = \frac{1}{\Gamma q^2} = \frac{k_B T}{6\pi\eta_s R_h} \qquad (1.4)$$

with $q = (4\pi n/\lambda)\sin(\theta/2)$, the scattering wave vector, $n = 1.34$, the refractive index of water, $\eta_s = 0.78$ mPa·s, the viscosity of water at 25 °C, $k_B$ is Boltzmann's constant, and $T$ is the absolute temperature.

## Results and Discussion

**Isolation and Purification of Flaxseed Polysaccharides.** The isolation procedure was carried out at ambient temperature since contamination with proteins during extraction can be a problem particularly if high temperatures are used.[18] Flaxseed polysaccharide extracted from flaxseed meal yielded approximately 7.5% w/w soluble fraction and 2% w/w insoluble fraction. Elemental analysis showed that the freeze-dried purified soluble fraction was composed of 39.55% carbon, 6.56% hydrogen, 40.31% oxygen, and 6.34% nitrogen. The values indicate that the fraction is a polysaccharide with an empirical formula approximating $CH_2O$. The presence of nitrogen is probably derived from protein contaminants; however, it was previously reported that a small percentage (~3.5%) is an integral part of the polysaccharide structure.[30] Furthermore, a protein content of up to ~29% has been reported to be present in the extracted polysaccharide fraction from flaxseed meal,[18] and it has been suggested that composition varies with raw materials and extraction conditions.[19−21,37,38] The insoluble fraction on the other hand was composed of 43.68% carbon, 6.80% hydrogen, 31.62% oxygen, and 9.69% nitrogen.

**Size-Exclusion Column Coupled to a Multiangle Laser Light Scattering (SEC-MALLS).** The soluble fraction was analyzed by SEC-MALLS. The concentration of the polysaccharide solution was $5.4 \times 10^{-3}$ g/mL. The chromatogram (Figure 1) shows three distinct light scattering peaks, namely, peak 1, 2, and 3. The molecular parameters for the peaks were determined using Zimm plots. The weight-average molar masses ($M_w$) obtained from the Astra software were $1.0 \times 10^6$, $6.7 \times$

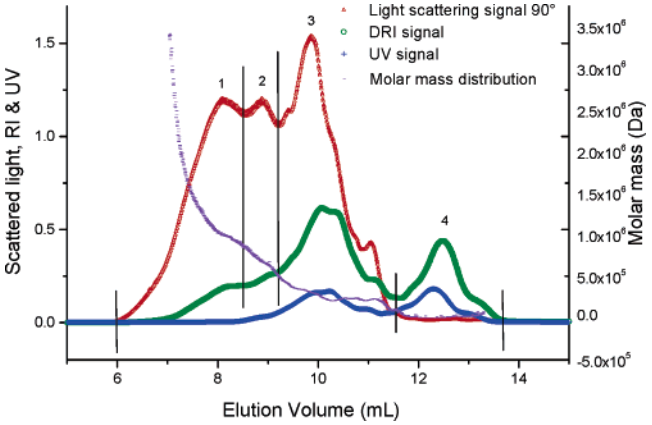


**Figure 1.** Light scattering signal at 90° (△), UV signal (+), DRI signal (○), and molar mass distribution (−) as a function of elution volume for filtered (0.22 $\mu$m) flaxseed polysaccharide solution eluted with 0.1 M NaCl mobile phase through a Shodex SB-806 HQ HPLC column. Peaks denoted as 1, 2, and 3 were selected for molar mass determination.

$10^5$, and $3.1 \times 10^5$ Da, respectively. The root mean-square radii ($r_z$) for the three respective fractions were 61.9, 49.0, and 25.3 nm, respectively. The value of d*n*/d*c* determined in this study was 0.130 mL/g. The integrated areas for peaks 1, 2, and 3 of the DRI curve (segregated by vertical lines) shown in Figure 1 are approximately 17%, 13%, and 70%, respectively. The data indicated that there is only a small proportion of the large molecular weight species (~17%) (although it is probably this fraction that is responsible for the viscosity of the polysaccharide solution). In previous studies the largest molecular weight fraction was known to be the neutral polysaccharide fraction of arabinoxylan and the smaller molecular species belonged to the acidic fractions of rhamnogalacturonan. Although these have not been validated in this study, the molecular species separated by the SEC column appeared to follow a trend similar to that reported by other researchers.[39] In addition, the molecular weight fractions were fairly close to those reported by Warren and co-workers,[22] although differences in their monosaccharide composition and molar masses could be expected for different flaxseed cultivars.[40,41] It was also noted in the UV chromatogram that the higher UV signals were observed in the smaller molecular fractions in peak 3. It is unknown at present if the proteins were part of the polysaccharide structure and/or present due to contamination during the isolation process. The good emulsification properties of flaxseed mucilage as reported[13] may suggest the presence of proteins in the molecular structure similarly to gum Arabic.

A plot of root-mean-square molecular radius (RMS) as a function of molar mass over the range from $4 \times 10^5$ to $5 \times 10^6$ Da for the flaxseed polysaccharide solution in 0.1 M NaCl is shown in Figure 2. Note, below $4 \times 10^5$ Da the technique was too insensitive to obtain reliable RMS radii. Generally, a slope of ~0.7 suggests a relatively stiff rodlike conformation, a value of ~0.6 suggests a flexible random coil conformation in a good solvent, a value of ~0.5 suggests a flexible random coil conformation in a theta solvent, and a slope of ~0.3 implies a compact structure resembling the shape of a sphere. The gradient (~0.56) obtained from the plot (Figure 2) suggests that the molecular species may adopt a slightly stiff random coil conformation.[42]

**Dynamic Light Scattering.** Figure 3 shows the autocorrelation function for flaxseed aqueous solution ($1.5 \times 10^{-3}$ g/mL) measured at $\theta = 90°$. The corresponding relaxation rate distributions obtained by CONTIN clearly show two main

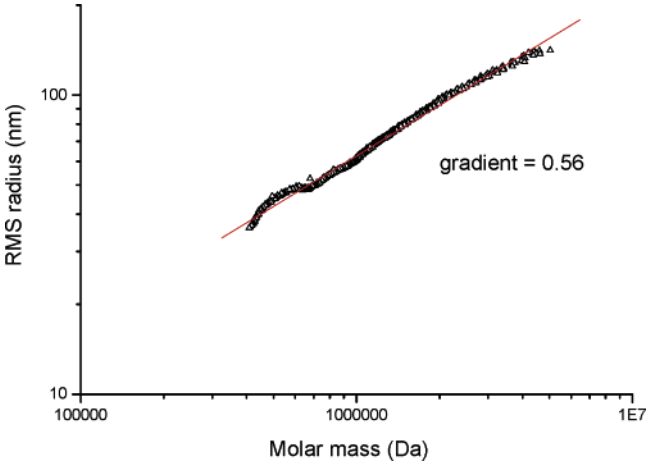


**Figure 2.** Molar mass as a function of root-mean-square (RMS) radius plot for flaxseed polysaccharide solutions in 0.1 M NaCl.

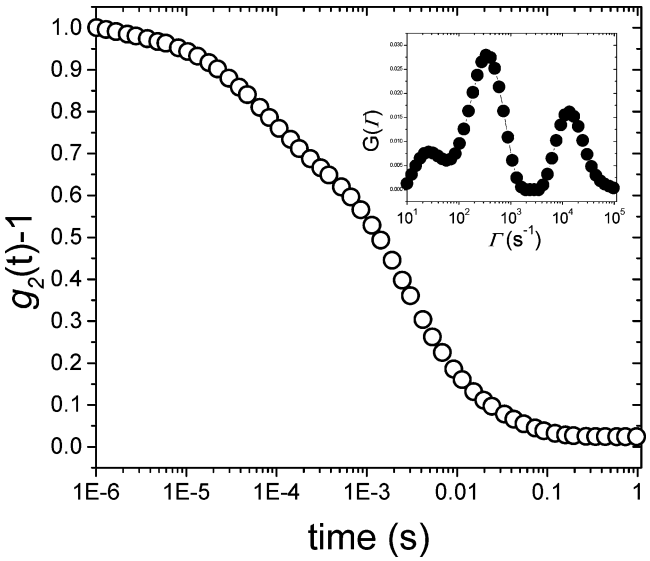


**Figure 3.** Autocorrelation function as function of correlation time and (inset) resulting gamma distribution G(Γ) as a function of Γ measured at a scattering angle $\theta = 90°$ for 0.15% (w/v) flaxseed in aqueous solution.

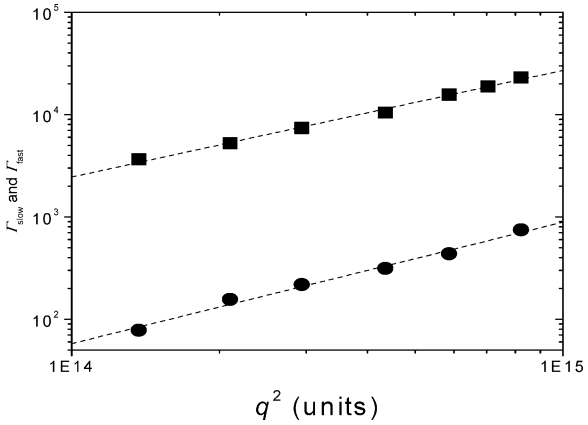


**Figure 4.** Relaxation rates for the slow mode $\Gamma_{slow}$ (●) and fast mode $\Gamma_{fast}$ (■) as a function of the square of the scattering wave vector $q^2$ for 0.15% (w/v) flaxseed in aqueous solution.

modes, designated as slow mode $\Gamma_{slow}$ and fast mode $\Gamma_{fast}$. Furthermore, these two relaxation rates varied linearly with the square of the scattering wave vector $q$ (Figure 4), indicating that these two modes are diffusive. The corresponding hydro-

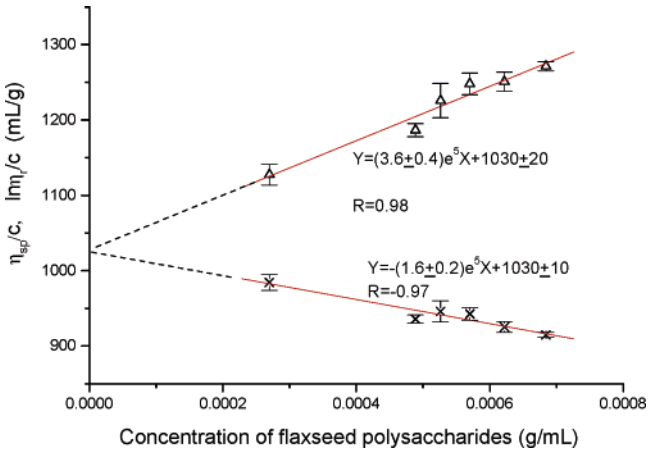


**Figure 5.** Huggins ln $\eta_r$/c (△) and Kraemer $\eta_{sp0}$/c (X) plots for flaxseed polysaccharide in Milli-Q water containing 0.02% w/v sodium azide. Intrinsic viscosity (1030 ± 20 mL/g) was estimated by extrapolation (dashed) of linear fits (solid lines) to the intercepts. Error bar represents mean ± standard error, $n = 4$. Huggins constant ($K'$) = 0.34 ± 0.05; Kraemer constant ($K''$) = −0.15 ± 0.02.

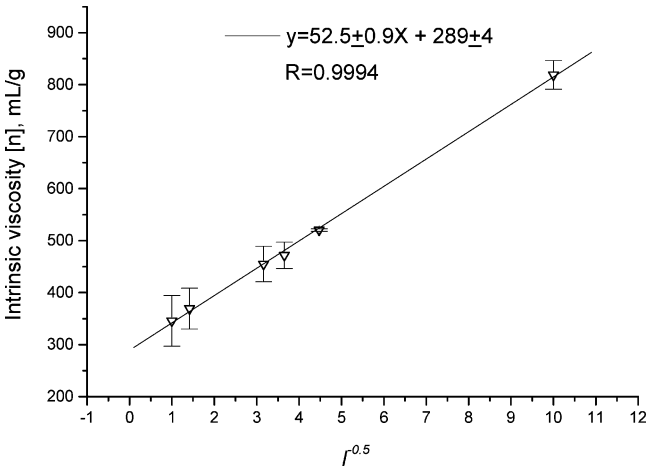


**Figure 6.** Intrinsic viscosity at infinite ionic strength determined by extrapolation of the linear plot. $S = 52.51$. Error bar represents mean ± standard error, $n = 2$.

dynamic radii were found to be approximately 10 and greater than 100 nm using eq 1.4. Measurements performed on more dilute solutions yielded similar values, indicating that the hydrodynamic radii were insensitive to polysaccharide concentration.

There are significant differences between the dynamic light scattering and SECMALLS observations. Dynamic light scattering yielded two main populations, while SEC-MALLS yielded three. This is likely due to the separation by the HPLC column, allowing better resolution of the different flaxseed populations by static light scattering. However, it is likely that the value of the hydrodynamic radius (10 nm) measured by dynamic light scattering corresponds to the small molecular weight species (peak 3) detected by SEC-MALLS. In addition, since the molecular weight of peak 3 is $3.1 \times 10^5$ and the corresponding hydrodynamic radius is 10 nm, this could be an indication that these molecules are relatively stiff.

The slow mode detected by dynamic light scattering could be aggregates, which are measured by DLS but to a lesser extent by SEC-MALLS, as the concentration used in SEC-MALLS is much smaller than in DLS measurement. In addition, during SEC-MALLS measurements the sample underwent separation during elution through the column.

**Intrinsic Viscosity.** The Kraemer and Huggins plots obtained by capillary viscometry for flaxseed polysaccharide in Milli-Q water at 20 °C are shown in Figure 5 together with linear fits to the data. The Huggins constant $K'$ is a measure of hydrodynamic interactions between macromolecule pairs[43,44] and usually has values between 0.3 and 0.8 with values of 0.3−0.4 for a polymer in good solvents and 0.5−0.8 for polymers in theta solvents. For values above 0.8, aggregation of the macromolecules is likely to occur.[45] For a random coil polymer, $K' - K'' = 0.5$.[43] Our data shows that the flaxseed polysaccharide resembled a random coil conformation in Milli-Q water with $K' - K'' = 0.49 \pm 0.07$. The [$\eta$] of the polymer in Milli-Q water was 1030 ± 20 mL/g. The intrinsic viscosity was likely influenced by the large molecular species despite being present in a small quantity (∼17%). Flaxseed polysaccharide's intrinsic viscosity is higher than gum arabic (∼600 mL/g)[46] and lies between 1379 and 925 mL/g for locust bean gum and guar gum, respectively,[47] but lower than xanthan gum (∼1680 mL/g).[48] In comparison to other values reported for flaxseed polysaccharide, the [$\eta$] value obtained here in 1 M NaCl solution was ∼345 mL/g, which is lower than the values reported by Cui and Mazza[15] that ranged from 434 to 658 mL/g.

Since flaxseed polysaccharide is known to contain acidic groups, the polyelectrolyte effect of the polysaccharide fraction would be expected. Hence, with increasing ionic strengths, the intrinsic viscosity decreased. This is because the conformation of the macromolecules was affected by the presence of salt on the negatively charged groups on the polymer molecular structure. With increasing concentration of counterions, intramolecular repulsion between equal-sign charges on the macromolecule backbone decreases, causing the hydrodynamic volume to decrease as the chain becomes more compact[45] and therefore a decrease in [$\eta$]. Figure 6 shows that the intrinsic viscosity increases almost linearly with $I^{-1/2}$ according to the following equation

$$[\eta] = [\eta]_\infty + SI^{-1/2} \tag{1.6}$$

where $[\eta]_\infty$ is the intrinsic viscosity at infinite ionic strength, $I$ is the ionic strength of the solution, and $S$ is a constant.

From the plot the intrinsic viscosity at infinite ionic strength, $[\eta]_\infty$, for which the electrostatic repulsions are completely screened can be determined by extrapolating to the intercept at infinite ionic strength.[49] The intrinsic viscosity at infinite salt concentration, $[\eta]_\infty$ obtained by extrapolation, was 289 mL/g. This can then be related to the intrinsic viscosity of a neutral polymer of similar chain length and molecular geometry.[50] The constant $S$ can be used as a quantitative measure of chain stiffness between different polyelectrolytes. However, this is only possible when comparisons of different polymers are made at a constant molecular weight. The constant $S$ is directly proportional to the molecular weight of polymer and depends on the nature of the counterions.[51,52] Smidsrod and Haug (1971) suggested an empirical parameter $B$ which relates to chain flexibility. The parameter $B$ can be determined easily by obtaining the value of $S$ from the isoionic dilution procedures of a polymer and measuring its intrinsic viscosity (molecular weight does not need to be known) at one particular ionic strength (0.1 M NaCl). The expression is written as

$$S = B([\eta]_{0.1})^{\nu}$$

where the exponent $\nu$ generally varies very little between 1.2 and 1.4 and $[\eta]_{0.1}$ is the intrinsic viscosity at 0.1 M ionic strength. Therefore, using the average value of 1.3 for the

exponent $\upsilon$, $B$ can be obtained directly by measurement of the $[\eta]$ of a polymer at different ionic strengths. The parameter $B$ has been found to correlate well with theoretical parameters of chain flexibility,[53] which has been reported the to be inversely proportional to persistence length for all polyelectrolytes. A low value of $B$ is associated with stiff polymer backbones.[51] In this study, the parameter $B$ for flaxseed polysaccharide obtained was ~0.018 based on an average value of $\upsilon \approx 1.3$. The parameter $[\eta]_{0.1}$ is the intrinsic viscosity at 0.1 M ionic concentration, expressed as dL/g. The $B$ value indicates that the flaxseed polysaccharide was between a flexible and a semi-flexible polymer compared to xanthan gum molecules. The latter is known to adopt a stiff conformation and has a $B$ value of ~0.005,[54] whereas carboxymethylcellulose known to be a flexible chain has a $B$ value of ~0.045−0.065.[55]

## Conclusions

Using three different techniques, viscometry and static and dynamic light scattering, it was found that flaxseed polysaccharide is made of slightly stiff random coil molecules. This conclusion is supported by the slope of 0.56 of the plot of radius of gyration against molar mass and by the Smidsrod parameter $B$ of 0.018 obtained by viscometric measurements. Furthermore, dynamic light scattering has shown that flaxseed polysaccharide is partly made of molecules with small hydrodynamic radii (10 nm) which would indicate that they are stiff random coil molecules. Both SEC-MALLS and dynamic light scattering showed that there is a large amount of small molecular weight species, which resulted in a lower intrinsic viscosity, compared to other polysaccharides generally made of much larger molecules. From SEC-MALLS measurement, one of the fractions of the flaxseed polysaccharide appears to contain protein, as reported previously in the literature.

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