Use of the Extended Fujita method for representing the molecular weight and molecular weight distributions of native and processed oat beta-glucans

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Abstract

Beta 1-3, 1-4 glucans ("beta glucans") are one of the key components of the cell wall of cereals, complementing the main structural component cellulose. Beta-glucans are also an important source of soluble fibre in foods containing oats with claims of other beneficial nutritional properties such as plasma cholesterol lowering in humans. Key to the function of beta-glucans is their molecular weight and because of their high polydispersity - molecular weight distribution. An approach for polydisperse polymers was recently published based on sedimentation velocity in the analytical ultracentrifuge. This converts a distribution of sedimentation coefficient g(s) vs s plot into a distribution of molecular weight utilising the power-law or scaling relationship between the sedimentation coefficient and molecular weight, $s = \kappa_s M_w^b$ where s is the sedimentation coefficient, M_w is the weight-average molecular weight and κ_s and *b* are characteristic coefficients related to conformation. We establish and apply the power law relation for the first time to identify the (weight average) molecular weights to a series of native and processed oat beta-glucans and then, using the Extended Fujita approach evaluate their molecular weight distributions. The application of this approach to beta glucans from other sources is considered.

Keywords: beta-glucans, oat, power law, Extended Fujita approach

Introduction

Beta 1-3, 1-4 glucans, "beta glucans" are one of the key polysaccharides of the cell walls of oats and other cereals, complementing the main structural component cellulose (Mantovani, 2008; Havrlentová et al, 2016; Harding et al, 2017). Beta-glucans are also an important source of water soluble dietary fibre in oat-containing foods with claims of other beneficial nutritional properties such as promoting plasma cholesterol reduction and lowering postprandial glycaemia (Braaten et al, 1994). Besides their chemical structure (Figure 1), the key to the function of beta-glucans in the plant, and their use (or potential use) commercially or in biomedicine is their molecular weight and, because of their high polydispersity – their molecular weight distribution. In some cases, preparations of beta – glucan can be readily soluble and have fairly unimodal distributions of size. In other cases insoluble components or purities are present (see Wang et al, 2002) and unless removed can cause problems with several techniques used to characterize the physical properties of the soluble components. A recent study (Grundy et al, 2017) has considered solubility and dissolution kinetics and linked this to the biological activity of beta-glucan.

<Figure 1 near here>

An approach for polydisperse polymers was recently published (Harding et al, 2011) based on the matrix-free technique of sedimentation velocity in the analytical ultracentrifuge and converts a distribution of sedimentation coefficient g(s) vs s plot into a distribution of molecular weight utilising the power-law or scaling relationship between the sedimentation coefficient and molecular weight (see Tsvetkov et al, 1971, Smidsrød and Andresen, 1979, Harding et al, 1991)

$$s = \kappa_s M_w^{\,b} \qquad (1a)$$

where s is the sedimentation coefficient, M_w is the weight average molecular weight and κ_s and *b* are characteristic coefficients related to conformation. For example, *b* = 0.4-0.5 for a coil type of conformation, ~0.15-0.2 for a rod and ~0.67 for a sphere.

The sedimentation coefficient s depends on the size and shape of the macromolecule (Harding et al 2015) but if κ_s and *b* are known then M_w can be found from

$$M_w = (s/\kappa_s)^{1/b} \qquad (1b)$$

We establish and apply the power law relation for the first time based on work on oat beta-glucan BG90. We then for a series of beta-glucans of lower degree of purity identify the molecular weights to identify the (weight average) molecular weights to a series of and then for a series of roll-milled processed beta-glucans, use the Extended Fujita approach (Harding et al, 2011) to evaluate the molecular weight distributions of a series of roll-milled beta-glucans.

A major development in the molecular weight determination of polysaccharides occurred 3 decades ago with the introduction of the SEC-MALS method (size exclusion chromatography coupled to multi-angle light scattering) (see Wyatt, 1992; 2012; Harding, 1994; Harding & Jumel 1998), which provides separation and absolute molecular weight analysis. The first application of this method to polysaccharides – namely alginates - was in 1991 (Horton et al., 1991; Harding et al., 1991), and to glycoconjugates in 1996 (Jumel et al, 1996), and is a technique which has now become the chosen method for many polymer systems.

However, there are two major limitations which restricts the types of molecule that can be successfully analysed (i) the separation limit for the columns (usually up to a maximum of 2-3x10^6 g /mol) and (ii) non-inertness of the columns used. Some beta-glucans (see Wang & Ellis, 2014) have very large molecular weights (either native or through aggregation phenomena) that exceed the molecular weight limit, and many offer solubility problems that can affect the inertness of the columns. Issues of solubility and non-unimodality can also affect other methods such as sedimentation equilibrium in the analytical ultracentrifuge and our recently developed SEDFIT-MSTAR method (Schuck et al, 2014). The current method based on sedimentation velocity at high speeds is more suited and supra-molecular impurities are automatically removed.

The Extended Fujita approach offers a complementary alternative to SEC-MALS and sedimentation equilibrium and can be applied to situations in which other methods cannot be employed.

The extended Fujita approach of Harding, Schuck & coworkers

Fujita (1962) provided the basis for converting a (differential) distribution g (s) of the sedimentation coefficient s into a (differential) distribution f (M) of the molecular weight M for linear polymers, based on the assumption that the polymers behave as randomly coiled polymers in solution, with b = 0.5 in Eq. (1). Harding et al (2011) provided a generalisation of this method to cover any conformation type (including spheres, rods and coils). The term g(s) is defined as the population (weight fraction) of the species with a sedimentation coefficient between s and s + ds and f (M) is

defined as the population (weight fraction) of species with a molecular weight between M and dM. The transformation from g (s) vs. s to f(M) vs M is obtained as follows:

$$g(s).ds = f(M).dM \quad (2)$$

and so

$$f(M) = g(s)\,(ds/dM) \quad (3)$$

where

$$ds/dM = b.\,\kappa_s^{1/b}.s^{(b-1)/b} \quad (4)$$

Therefore to perform the transformation the conformation type or *b* needs to be known under the particular solvent conditions and at least one pair of s-M values is needed to define the κ_s from Eq. (4). Care needs to be expressed concerning thermodynamic/hydrodynamic non-ideality, but these effects can be avoided by working at low concentrations, taking advantage of the fact that sedimentation velocity experiments can be performed at concentrations as low as 0.1 mg/ml, where such effects are usually negligible. The method has now been built into the popular sedimentation velocity analysis platform known as SEDFIT (Dam & Schuck, 2004; Brown and Schuck, 2006). In the transformation a diffusion corrected variant of g(s) known as c(s) is sometimes used.

Evaluating the scaling parameters κ_s and *b* for oat beta glucans

We can now seek to establish the procedure for oat beta-glucans by establishing the κ_s and b parameters. To do that, we select a beta-glucan, which is fully soluble and has a unimodal distribution of sedimentation coefficient. One such material is a substance known as oat beta-glucan BG90, supplied by F. Proton (Swedish Oat Fibre, Bua, Sweden) that has previously well characterized chemically (see Grundy et al, 2017, where it is referred to as "BG2"). Its g(s) vs s distribution is shown in Figure 2a. We then obtain the *b* and κ_s in the following way:

<Figure 2 near here>

Defining b: we use SEC-MALS coupled to an on-line viscometer. Figure 3a shows the SEC elution concentration profile recorded using the on-line differential refractometer and one of the light scattering detectors. We then record the intrinsic viscosity $[\eta](V_e)$ versus weight average molecular weight $M_w(V_e)$ profile and from the slope of a plot of log (Figure 3b) across the main peak of elution volumes (V_e) and evaluate the viscosity power law (scaling) coefficient *a* from the slope. It should also be remarked that across the selected peak the very accurate linear fit is indicative of a pure component within this range. From Figure 3b a value of $a = 0.62$ corresponds to a flexible coil conformation. We can then use the Tsvetkov relation (Tsvetkov, Eskin & Frenkel, 1970) linking the sedimentation and viscosity power law coefficients to obtain *b*:

$b = (2\text{-}a)/3$ (5)

and obtained a value for $b = 0.46$. We then repeated the whole procedure on a different instrument in a different institution and re-assuringly obtained a similar value $b = 0.45$. This gave us a working value for $b = (0.455 \pm 0.010)$

<Figure 3 near here>

Defining κ_s. To do this, we simply use equation 1 with the weight average sedimentation coefficient s, the weight average M_w from SEC-MALS and a *b* value of (0.455 ± 0.010). The weight average sedimentation coefficient after extrapolation to zero concentration (Figure 2b) to remove non-ideality effects = (4.82 ± 0.10)S. The values for M_w from both instruments were 634,000 and 651,000 g/mol so we take M_w = (642,000±10,000) g/mol, and because of the very low concentrations after dilution on the columns, non-ideality effects can be ignored. This gives a value for κ_s under high dilution conditions (<0.15 mg/ml):

$$\kappa_s = \{s/M_w^{\,b}\} = \{4.82/642000^{\,0.455}\} = 0.01098 \quad (6)$$

Where distributions g(s) vs s cannot be obtained under high dilution conditions the s value at that concentration should be substituted into equation (6) and the appropriate κ_s value selected (Table 1). If g(s) vs s plots are obtained for other concentrations then the appropriate sedimentation coefficient value for that concentration should be found using the Gralén relation:

$$\{1/s\} = \{1/s^o\}.\{1 = k_s c) \quad (7)$$

where s^o is the value extrapolated to zero concentration (i.e. non-ideality free) and k_s is the concentration dependence or 'Gralén' coefficient (see Harding & Johnson, 1985), where (Figure 2b) $k_s = (420 \pm 40)$ ml/g. Note that the Gralén coefficient should not be confused with the power-law scaling coefficient κ_s.

<Table 1 near here>

Figure 4 shows the distribution obtained in this way for oat beta glucan BG90. One can see the broiad distribution and the large amount of high molecular weight material >500,000 g/mol.

<Figure 4 near here>

Application to roll mill processed oat beta glucans

To further illustrate application of the method we look at 5 roll-milled oat beta glucans, produced by Lantmännen Cerealia, Moss, Norway and provided by Dr. Myriam Grundy (Kings College, London): Belinda 200μm, Belinda 710 μm, Belinda 710 EtOH, Matilda 200μm and Matilda 710 μm. The sedimentation coefficient distributions were recorded at 1 mg/ml. The value of κ_s is adjusted accordingly (Table 1), and the corresponding distributions shown in Figure 5 showing the broad range of sizes reaching > 2 million molecular weight. For oat beta glucans using the *Extended Fujita* procedure (Harding et al 2011) the power law parameters *b* and κ_s from Table 1 can be used.

Simple power-law application

If simply the (whole distribution) weight average molecular weights M_w rather than distributions of f(M) vs M are sought then these can be obtained directly from the sedimentation coefficient from equation 1b. Table 2 shows values of some other oat beta glucans obtained in this way.

<Table 2 near here>

Discussion

The inherent fractionation ability of the sedimentation velocity method without the need for columns or membranes, together with the automatic removal of supramolecular impurities appears to be useful for impure or incompletely soluble materials. The Extended Fujita method has been incorporated into the SEDFIT suite of analytical ultracentrifuge algorithms, developed by Schuck & coworkers (see Harding et al 2011, Brown & Schuck, 2006) and is readily useable. The method is seen as complementary to SEC (or FFF) –MALS, which have been established as the method of choice for many polymeric molecular weight distribution analysis, and, for polysaccharides since the first analyses in 1991 (Horton et al, 1991). However, the latter methods are not useful in cases where non-inertness of the columns (SEC) or membranes (FFF) are suspected, there is poor solubility or the separation range has been exceeded.

The Calcofluor fluorescence labelled chromatography method (Ballance et al, 2015; Grundy et al, 2017) – specific for β(1-4) bonds - has also proved useful for the

analysis of low molecular weight beta glucans in the presence of non β(1-4) containing impurities although for distributions containing substantial amounts of high molar mass material that method gives only an approximate estimate for the molecular weight, because it does meet the molecular weight cut of 500,000 (Rieder et al, 2015; Grundy et al, 2017). For such systems the Extended Fujita method also provides a useful complementary approach. We are now exploring the extension of our approach to other beta glucans, most notably from barley and wheat.

Methods

Beta glucans. Oat beta-glucan BG90 was supplied by Swedish Oat Fiber AB (Väröbacka, Sweden) and solubilised according to the following method:
0.0280 g of Oat BG90 β-glucan was accurately weighed into the base of a pre-weighed 50 mL dry pyrex conical flask. 5 mL of 0.1M pH7 PBS (I=0.1M) buffer was the added to pre-wet the sample. A further 15ml aliqout of buffer was then added ensuring all solid material was washed below the liquid surface., A pre-weighed magnetic stirrer bar was added with a pre-weighed '3-port PTFE Duran bottle insert' (port 1- thermocouple wire, port 2-sealed injection, port3 –blank), designed to prevent evaporative loss. The mixture was then placed on a hot-plate magnetic stirrer at 80.0^{0}C for 1hr. A final 5ml aliquot of buffer was added again ensuring any solid material was washed below the liquid surface, and heated for a further 1hr at 80.0^{0}C until dissolved.

Oat BG90 solution was then extensively dialysed (>24h, 1 change) against the 0.10M pH7.0 phosphate-chloride buffer (Green, 1933). It was then made up to a stock concentration of 1.0 mg/ml (measured refractometrically).

Analytical Ultracentrifugation. Sedimentation coefficient distributions were evaluated using the Beckman Optima XL-I analytical ultracentrifuge (Beckman Instruments, Palo Alto, USA). A volume of 400 µl of beta-glucan solution and matching amounts of buffer were injected into appropriate channels of 12 mm double sector aluminium epoxy cells with sapphire windows. Solutions were centrifuged at 40000 rpm at a temperature of (20.0±0.1)°C. The weight average sedimentation coefficient 's' (in Svedbergs, S) for a particular component was then corrected to standard solvent conditions (the density and viscosity of water at a temperature of 20.0^{o}C). A partial specific volume of 0.63 ml/g was used.

SEC-MALS. The SEC consisted of an Postnova Analysis PN7505 degassing unit (Landsberg am Lech Germany), Shimadzu LC-10AD HPLC Pump (Shimadzu UK, Milton Keynes, UK.), fitted with a Spark-Holland Marathon Basic autosampler (Spark Holland, Emmen, The Netherlands) combined with a TSK Gel guard column (7.5 x 75mm) and TSK Gel G5000, G6000 columns (7.5 x 300mm) connected in series (Tosoh Biosciences, Tokyo, Japan). Light scattering intensity was detected using a DAWN® HELEOS™ II, light scattering photometer connected in series to an ViscoStar® II on-line differential viscometer, Optilab® rEX refractive index detector (Wyatt Technology Corporation, California, U.S.A.). The stock solution of 1.0mg/ml was filtered through a 0.45µm syringe filter (Whatman, Maidstone, England) - to remove any insoluble material or dust prior to injection - and then injected into the

autosampler. 100 µL of each solution was injected onto the columns at ambient temperature. The eluent employed was the PBS dialysate at a flow rate of 0.8 mL/min. ASTRA™ (Version 6) software (Wyatt Technology Corporation, Santa Barbara, U.S.A.) was used to estimate the weight average M_w and z-average M_z molecular weights with a 1st order Zimm extrapolation (Wyatt, 1992, 2012). Because of the low solute concentrations after dilution on the columns non-ideality effects were assumed as negligible. A refractive increment (*dn/dc*) ~ 0.146 mL/g was used.

Acknowledgement

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Table 1. Scaling *b*, κ_s values for transforming sedimentation coefficient distributions for oat beta glucans

concentration	*b*	κ_s
< 0.15 mg/ml	0.455	0.01098
0.2 mg/ml	0.455	0.01037
1 mg/ml	0.455	0.00768

Table 2. Sedimentation coefficients of oat beta glucans from the Oatwell32 series (0.2 mg/ml), and corresponding molecular weights[a]

Sample	s (S)	10^6 x M_w(g/mol)
Oat 32 extract, Rieder et al (2012) with Na_2SO_4	9.24	3.05
Oat 32 extract minus Na_2SO_4	8.68	2.70
+ 2nd peak[b]	22.5	22.8
Oat 32 extract, Beer et al	8.63	2.62
Oat 32 extract, Wang et al	6.61	1.46

[a] evaluated from equation 1b. [b]This may represent part of the supra-molecular aggregate or non-beta glucan material

Legends to Figures

Figure 1. Part of the 1-3, 1-4 β-glucan molecule

https://commons.wikimedia.org/wiki/File:Beta-1,3-1,4-glucan.png

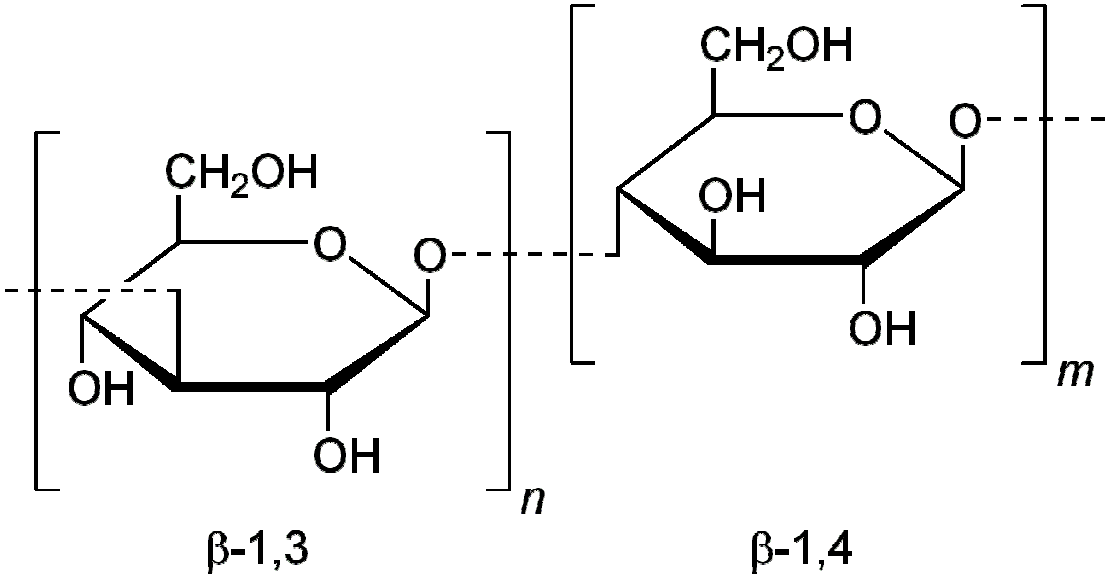
Figure 2. Analytical ultracentrifugation of oat beta-glucan BG90. (a) Sedimentation coefficient distribution plots g(s) vs s for in phosphate-chloride buffer pH = 6.8, I=0.10M, at 6 serial dilutions from 1.0 mg/ml. A rotor speed of ? was used. (b) reciprocal plot of s versus concentration, fitted to $\{1/s\} = \{1/s^o\}.\{1= k_s c)$ where k_s is the concentration dependence or 'Gralén' coefficient (Gralén, 1944; Harding & Johnson, 1985). From the fit a value of $s^o = (4.82 \pm 0.10)$S and $k_s = (420 \pm 40)$ ml/g are obtained.

Figure 3. SEC-MALS of oat beta-glucan BG90. (a) Elution profile with the beta glucan peak limits selected in gray. Blue line: refractrometric (concentration) signal. Red line (light scattering signal recorded at a scattering angle of 90^o. Both profiles normalized to a maximum of 1.0 (b) Mark Houwink-Kuhn Sakurada (MHKS) plot of intrinsic viscosity [η] values versus molecular weight M_w corresponding to elution volume values V_e within the marked limits of (a). Fit parameters shown in the inset.

Figure 4. Molecular weight distribution f(M) vs M for oat beta-glucan BG90. After transformation from the g(s) vs s distribution for c = 0.125 mg/ml (Figure 2a), with coefficients $b = 0.455$ and $\kappa_s = 0.01908$

Figure 5. Molecular weight distribution f(M) vs M for various processed (roll milled) oat beta-glucans. Loading concentration c = 1.0 mg/ml. $b = 0.455$, $\kappa_s = 0.00768$. Note the presence of significant amounts of low molecular weight material.

Fig 1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OH
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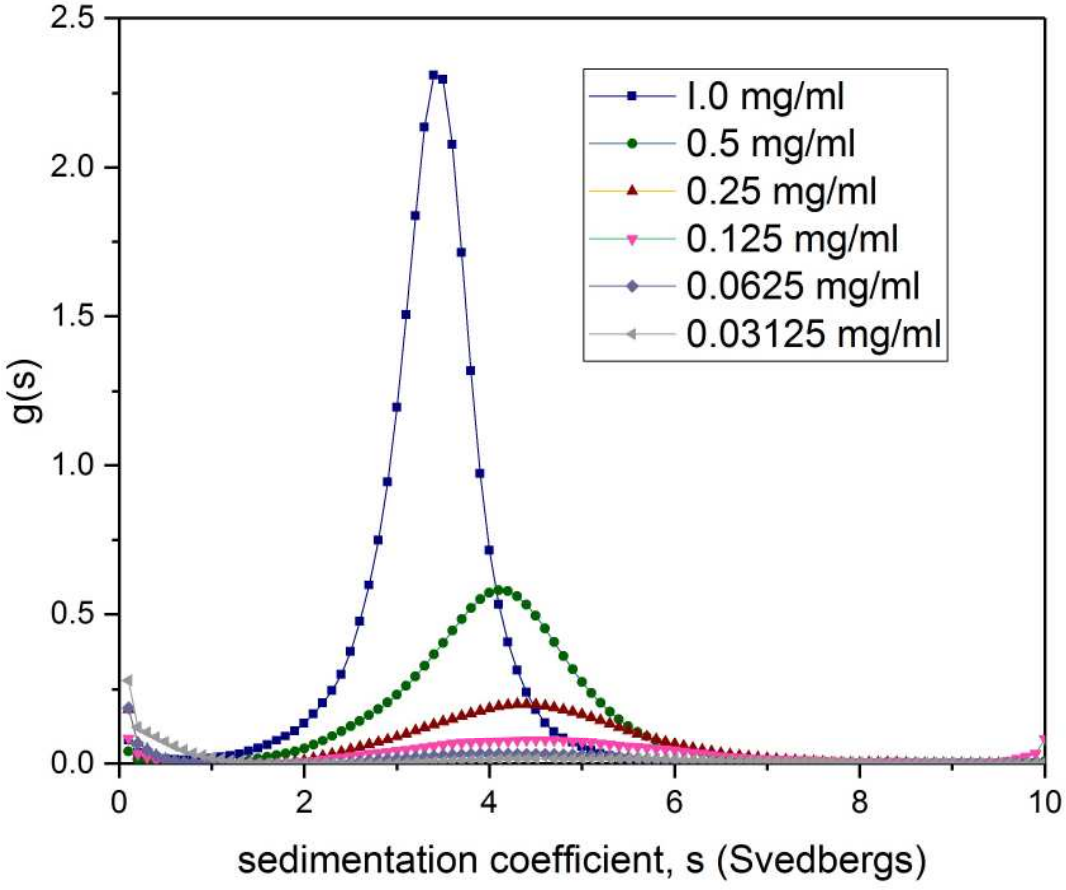
β-1,3
CH2OH
O
O
OH
OH
m
β-1,4

Fig 2a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1.5
1.0
0.5
0.0
g(s)
0
2
4
6
8
10
sedimentation coefficient, s (Svedbergs)
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0.125 mg/ml
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0.03125 mg/ml

Fig 2b

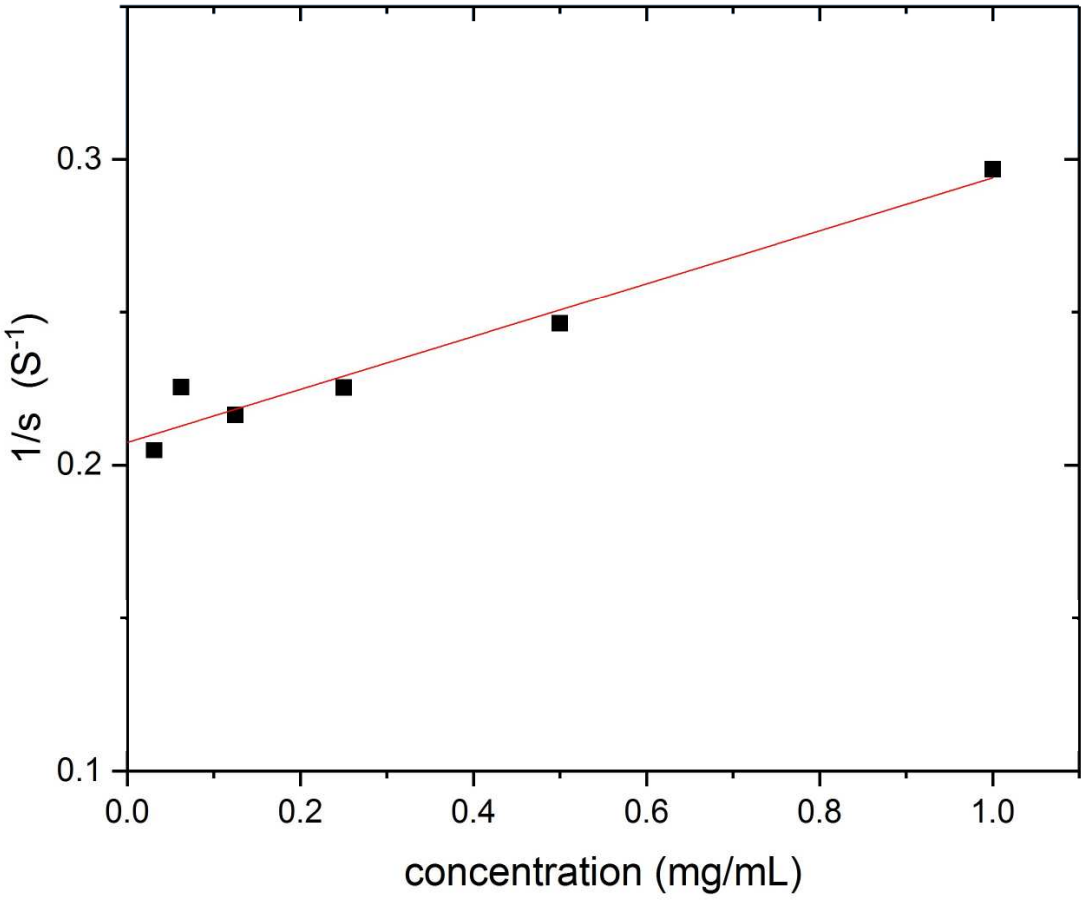
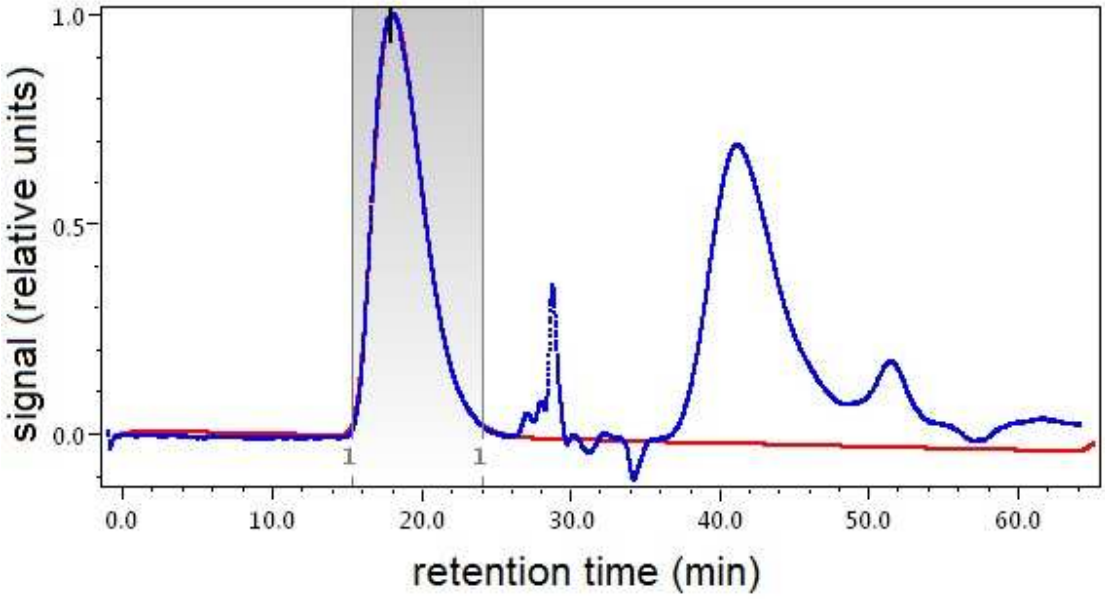


Fig 3a


signal (relative units)
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0.5
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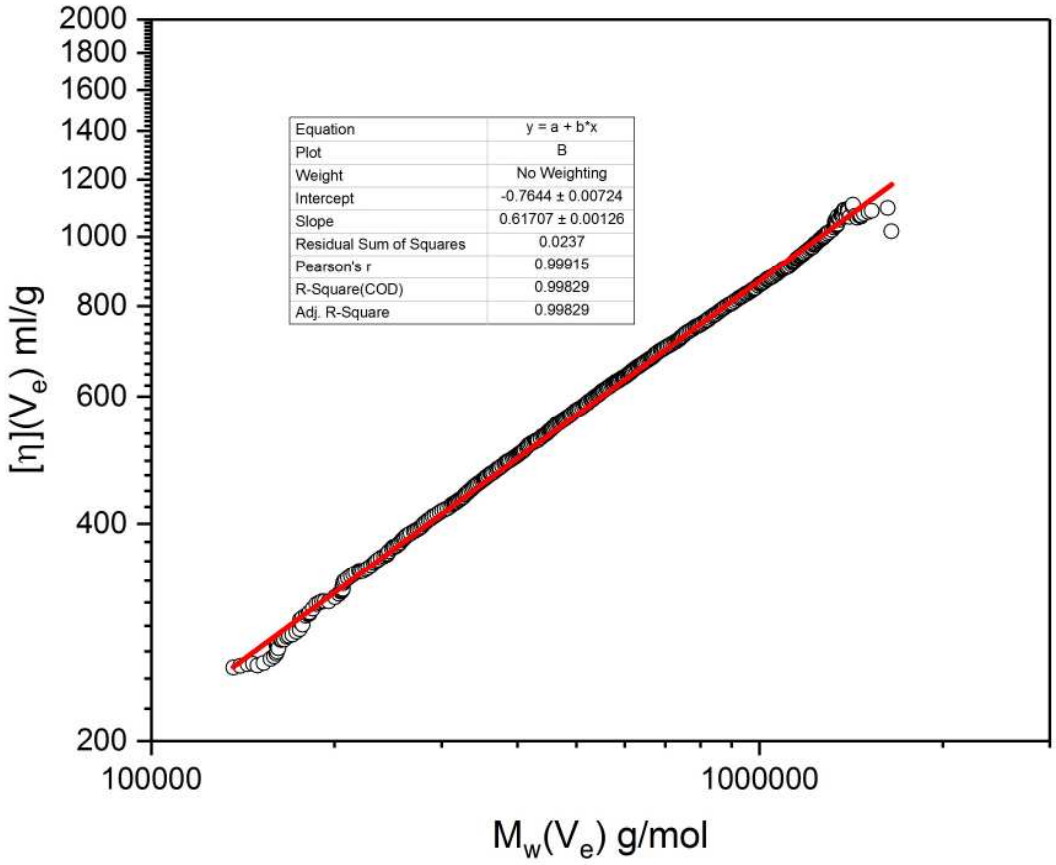
60.0
retention time (min)

Fig 3b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Plot B
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Slope 0.61707 ± 0.00126
Residual Sum of Squares 0.0237
Pearson's r 0.99915
R-Square(COD) 0.99829
Adj. R-Square 0.99829
[η](Ve) ml/g
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1600
1400
1200
1000
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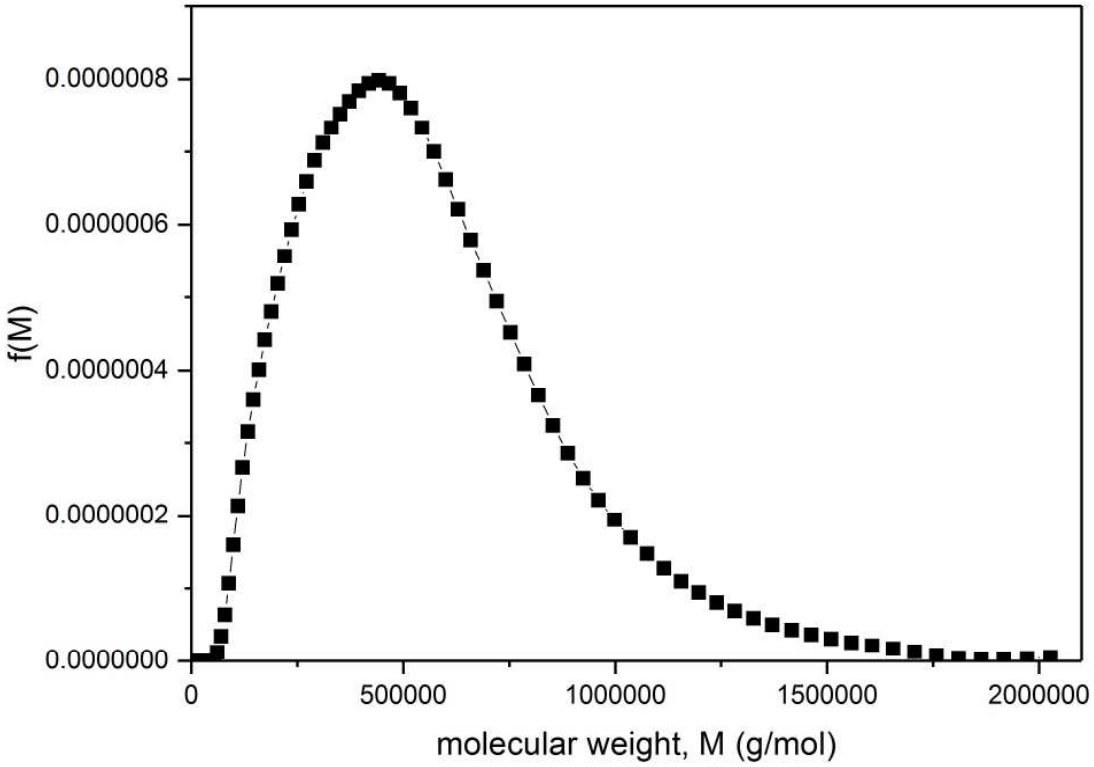
600
400
200
100000
1000000
Mw(Ve) g/mol

Fig 4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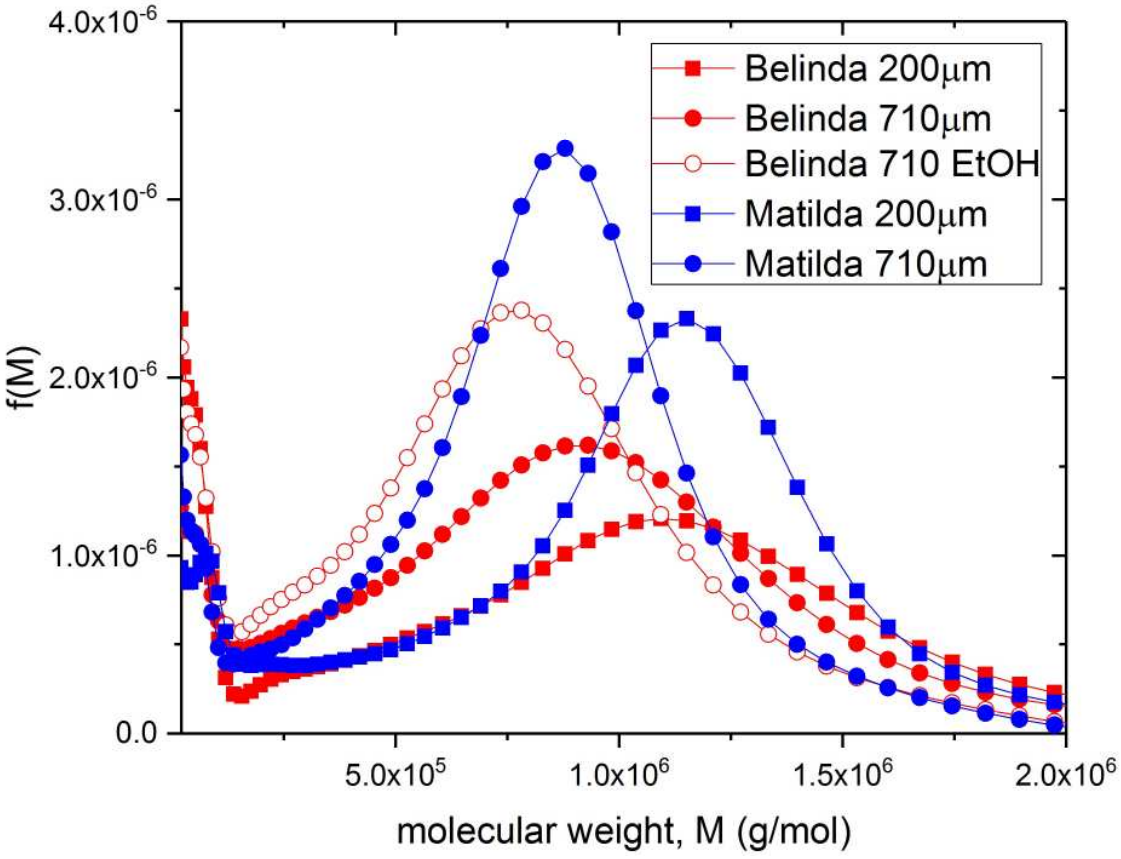
molecular weight, M (g/mol)

Fig 5


4.0x10-6
3.0x10-6
2.0x10-6
1.0x10-6
0.0
f(M)
Belinda 200μm
Belinda 710μm
Belinda 710 EtOH
Matilda 200μm
Matilda 710μm
5.0x10^5
1.0x10^6
1.5x10^6
2.0x10^6
molecular weight, M (g/mol)