Using pulse field gradient NMR diffusion measurements to define molecular size distributions in glycan preparations

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Glycans comprise perhaps the largest biomass in nature, and more and more glycans are used in a number of applications, including those as pharmaceutical agents in the clinic. However, defining glycan molecular weight distributions during and after their preparation is not always straightforward. Here, we use pulse field gradient (PFG) 1 H NMR self-diffusion measurements to assess molecular weight distributions in various glycan preparations. Initially, we derived diffusion coefficients, D, on a series of dextrans with reported weight-average molecular weights from about 5 kDa to 150 kDa. For each dextran sample, we analyzed 15 diffusion decay curves, one from each of the 15 major 1 H resonance envelopes, to provide diffusion coefficients. By measuring D as a function of dextran concentration, we determined D at infinite dilution, D∞, which allowed estimation of the hydrodynamic radius, RH, using the Stokes–Einstein relationship. A plot of log(D∞) versus log(RH) was linear and provided a standard calibration curve from which RH is estimated for other glycans. We then applied this methodology to investigate two other glycans, an α-(1→2)-α-Rhamnopyranosyl-α-(1→4)-α-galacturonosyl with quasi-randomly distributed, mostly terminal β(1→4)-linked galactose side-chains (GRG) and an α-(1→6)-α-galacto-β(1→4)-α-mannan (Davanat), which is presently being tested against cancer in the clinic. Using the dextran-derived calibration curve, we find that average RH values for GRG and Davanat are 76 ± 6 × 10−10 m and 56 ± 3 × 10−10 m, with GRG being more polydispersed than Davanat. Results from this study will be useful to investigators requiring knowledge of polysaccharide dispersity, needing to study polysaccharides under various solution conditions, or wanting to follow degradation of polysaccharides during production.

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1. Introduction

Of the three major groups of biopolymers in nature (consisting of nucleic acids, polypeptides, and polysaccharides), polysaccharides are the most chemically and structurally diverse compounds. For example, while nucleic acids and polypeptides only form linear polymers, polysaccharides can also form branched polymeric structures in various ways, with each glycosidic linkage making a new stereogenic center. Moreover, polysaccharides like their brethren can be phosphorylated, sulfated, methylated, acetylated, etc. In all, this makes the net of possible combinations and permutations much greater than that of nucleic acids and polypeptides. It is in fact their overall heterogeneity, combined with their ability to randomly self-associate, that has impeded their study and structural characterization.

Various biophysical approaches have been used to characterize and to understand physicochemical properties of polysaccharides in solution. Given that the molecular structures of large glycans cannot be determined to high resolution, that is, by X-ray and NMR, these have usually been investigated in terms of their hydrodynamic behavior in solution by measuring viscosity and diffusion properties to provide information, for example, on their solvent and intermolecular interactions, size, and overall shape. Ultracentrifugation and light scattering have been the primary methods normally used in these endeavors, for example, on glycogen, hyalurionate, carrageenans, and chitosans. Small angle X-ray scattering has been used as well, for example, on heparin, as have rheological techniques, for example, with starch under various solution conditions to assess viscosities and derive interaction energies for structural insight. Other novel approaches use size-exclusion chromatography and multi-angle laser light scattering (SEC-MALS) for molecular weight determination of galactomann-
and confocal fluorescence recovery after photobleaching (FRAP) to study diffusion and macromolecular organization of network forming FITC-labeled agglutac, a high molecular weight proteoglycan containing chondroitin sulfate and keratin sulfate chains, as well as to study the diffusion of FITC-labeled dextrans. Still another versatile technique is pulse field gradient (PGF) NMR, which relies on variations in the strength of the applied magnetic field to dephase resonances depending on their diffusion properties, for example, with solutions and/or gels of hyaluronate and dextran. The PGF NMR technique is quite versatile and can be used with solutions, for example, at various pH values, ionic strengths, and temperatures, as well as with pre-gel solutions transitioning into gel states.

In the present study, we used the PGF NMR diffusion approach to derive translational diffusion coefficients for three polysaccharides: dextran, CRG, and Davanat. We first investigated five weight-average molecular weight \( M_w \) fractions of dextran with reported weight-average molecular weights of about 25, 49, 81, and 148 kDa. For each fraction, we derived diffusion coefficients, \( D \), as a function of the glyccan concentration and determined \( D \) at infinite dilution, \( D_{\text{inf}} \). Plots of \( \log D_{\text{inf}} \) versus \( \log M_w \) for the dextrans and lactose are linear, and a plot of \( \log D_{\text{inf}} \) versus \( \log R_h \) using the Stokes-Einstein relationship provides a standard correlation plot from which \( D \) values of other glycans may be related to the distribution of molecular sizes. We exemplify this approach here using the glycans CRG and Davanat, whose repeating motifs, along with that for dextran, are illustrated in Figure 1. The motif for CRG is mainly conjectural, as rhamnogalacturonans have irregular structures that have yet to be determined completely.

Davanat, isolated from Guar gum (Cyamopsis tetragonoloba) and subjected to a controlled partial depolymerization, is a galactomannan whose backbone is composed of \( \alpha-(1\rightarrow4) \)-linked \( \beta-D \)-mannopyranosyl units to which single \( \alpha-D \)-galactopyranosyl residues are attached via a \( \alpha-(1\rightarrow6) \)-linkage, with an average repeating unit of 17 \( \beta-D \)-Man residues and ten \( \alpha-D \)-Gal residues, and an average polymeric molecule containing approximately 12 such repeating units. Thus the ratio of mannose to galactose in this compound is 1.7. CRG, produced from crude citrus pectins, is a galacto-rhamnogalacturonate of irregular structure, with a backbone composed mainly of \( \alpha-(1\rightarrow2) \)-D-rhamnosyl-\( \alpha-(1\rightarrow4) \)-D-galacturonosyl, with quasi-randomly distributed \( \beta-(1\rightarrow4) \)-linked galactose side-chains, mostly at terminal positions. Both CRG and Davanat have been produced by controlled partial depolymerization of considerably larger polysaccharides (up to several million Daltons), and as such it is important to know their size distributions.

2. Experimental

2.1. Glycan preparation

The isolation and purification procedure for Davanat when starting from a commercial Cyamopsis tetragonoloba guar gum flour, was used essentially as previously reported. Briefly, the procedure has five principal steps: (1) aqueous extraction of galactomannans, (2) controlled partial depolymerization, (3) recovery as an insoluble copper complex, (4) recovery from the copper complex, and (5) repeated ethanol precipitations. The final yield of Davanat was typically 50% by weight of guar gum flour. The purification procedure results in a pure Davanat as a white powder with a solubility in water of more than 60 mg/mL, and a mannose:galactose ratio of 1.7. \( ^1 \text{H} \) NMR and \( ^{13} \text{C} \) NMR spectra of Davanat are described by Platt et al.

For CRG, the size reduction protocol uses controlled conditions (temperature, reaction time, and catalysts) with hydrogen peroxide and L-ascorbate to catalytically hydrolyze glycosidic linkages in the polysaccharide backbone and oligosaccharide side chains into smaller polysaccharide molecules (US Patent pending). This chemical modification is aimed at limited depolymerization of the backbone of the polymer, mainly the \( \alpha-(1\rightarrow2) \)-D-rhamnosyl-\( \alpha-(1\rightarrow4) \)-D-galacturonosyl sections, accompanied by de-methylation and de-acetylation of carboxylic groups of the polymer, which also decreases hydrophobicity, and hence increases aqueous solubility. Briefly, the crude commercially available citrus pectin is provided as solid crude powder, typically used in food industry, and is dissolved in distilled water to pH 10 with 3 M NaOH. After incubation at 50 °C for 30 min, 20% ethanol is added and the partially purified polysaccharide is precipitated to remove proteins and pigments. The polysaccharide is then dissolved to 20 g/L in water, followed by addition of N-trifluoroacetic acid to a final concentration of 0.5 M for controlled de-polymerization, which cleaves the galacto-rhamnogalacturonans backbone to the desired size. After incubation for 24 h at 60 °C, the solution pH is adjusted to 4, cooled to 4 °C, and centrifuged to remove insoluble matter. The supernatant is then neutralized to a final pH of 8.0 with 1 M NaOH, and 20% ethanol is added to recover soluble polysaccharide. The resulting soluble branched polysaccharide product (CRG) is washed with 70% ethanol or with 100% acetonitrile to provide a final dry powder. Both Davanat and CRG were reduced in molecular weight compared to their starting materials in order to increase solubility and expose galactose residues for potentially better interactions with Gal-1.

A series of dextran samples of weight-average molecular weights of 5.2, 23.8, 48.6, 80.9, and 147.6 kDa were purchased from

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Figure 1. The chemical motifs for repeat units in dextran, CRG, and Davanat are shown. That for CRG is conjectural, and can hardly be considered a ‘repeating’ unit; rather, it represents a unit that can vary along the polysaccharide chain. The 3-letter code for saccharide units is used, where Glc, Gal, Man, Aca, Rha, GalAc stand for glucose, galactose, mannose, arabino, rhamnose, and galacturonate, respectively. The ‘Me’ stands for methyl, as some of the carbohydrate groups in galacturonate units can be methylated.
Sigma–Aldrich and were used as received without further purification. For these dextran preparations, the manufacturer gives peak molecular weights and number-average molecular weights as 4.4, 21.4, 43.5, 66.7, 123.6 kDa, and 3.3, 18.3, 35.6, 55.5, 100.3 kDa, respectively. These values were derived using gel permeation chromatography. Throughout this text, we refer to these dextrans using their weight-average molecular weights, rounded to the nearest whole integer value.

2.2. Pulse field gradient NMR self-diffusion measurements

For NMR measurements, compounds were dissolved in 0.6 mL of unbuffered D2O, and the pH was adjusted by adding microliter quantities of NaOD or DCI.

Pulse field gradient (PFG) NMR self-diffusion measurements were made on a Varian INOVA-600 using a GRASP gradient unit, as previously described.24,25 NMR spectra for measurement of diffusion coefficients, D, were acquired using a 5 mm triple-resonance probe equipped with an actively shielded z-gradient coil. The maximum magnitude of the gradient was calibrated using the manufacturer's standard procedure based on the frequency spread of the applied gradient and was found to be 75 G/cm, which was consistent with the value obtained from analysis of PFG data on water using its known diffusion constant.25 The linearity of the gradient was checked by performing diffusion measurements on water over different ranges of the gradient. The PFG longitudinal eddy-current delay pulse-sequence was used for self-diffusion measurements.26

All NMR experiments were carried out at 30°C. Raw data were converted and processed by using NMRPipe27 and were analyzed by using NMRView.27

For unrestricted diffusion of a molecule in an isotropic liquid, the PFG NMR signal amplitude, A, normalized to the signal obtained in the absence of gradient pulses, is related to D by

\[ A = \exp[-\gamma^2 g^2 d^4 (D - \delta/3)] \]  

(1)

where γ is the gyromagnetic ratio of the observed nucleus; g and δ are the magnitude and duration of the magnetic field gradient pulses, respectively, and A is the time between the gradient pulses.28 For these experiments, δ = 3 ms, g = 1–75 G/cm, \( A = 58.6 \) ms, and the longitudinal eddy-current delay \( T_2 \) = 100 ms. Each diffusion constant, D, was determined from a series of 12 one-dimensional PFG spectra acquired using different g values. Diffusion coefficient measurements were calibrated by performing the PFG NMR self-diffusion measurements on standard dextran samples having weight-average molecular weights of 5, 24, 49, 81, and 148 kDa.

3. Results and discussion

3.1. PFG NMR self-diffusion measurement of a series of dextrans

We first investigated a series of dextrans, with reported weight-average molecular weights of 5, 24, 49, 81, and 148 kDa, as well as the disaccharide lactose (0.36 kDa), and performed NMR pulse field gradient diffusion measurements as a function of the glycans concentration. \(^1\)H NMR spectral trace for the 81 kDa dextran sample (6.3 mg/mL) is shown in Figure 2. Resonances are labeled according to \(^1\)H NMR assignments previously reported.29 Even though these dextran samples comprise glycans of various molecular sizes and have monomer saccharide units in different structural positions (e.g., terminal, backbone, and side-chain), their NMR spectra are essentially the same because the chemical environment of any given monomer saccharide unit is not that different from any other. Nevertheless, PFG-induced dephasing at a given resonance position is quite sensitive to the presence of different size molecular species. The inset in Figure 2 exemplifies this by showing the differential gradient-induced dephasing at 5 positions (A–E) within the H6 resonance envelope of the 81 kDa dextran sample. Resonance intensities are a function of the gradient strength as defined by Eq. 1 (see Section 2), with the most intense resonance occurring at the start of the measurement when the gradient strength is weakest. As the gradient strength is increased, resonances become more dephased and therefore they decrease in intensity. More importantly, note that dephasing (intensity decrease) is not the same at all positions through the H-6 resonance envelope, which it would be if there were only one molecular size species present.

This is more evident in Figure 3A which plots the logarithm of the fractional change in intensity of A–E (intensity divided by the initial intensity at zero gradient) versus gradient strength. Slopes of the resulting decay curves which effectively yield diffusion coefficients, D (see Eq. 1, Section 2), vary significantly for A–E. If we simply fit these data points with a straight line, we find that D values range from \( 0.32 \times 10^{-6} \text{ cm}^2/\text{s} \) (A), \( 0.35 \times 10^{-6} \text{ cm}^2/\text{s} \) (B), \( 0.47 \times 10^{-6} \text{ cm}^2/\text{s} \) (C), \( 0.28 \times 10^{-6} \text{ cm}^2/\text{s} \) (D), to \( 0.43 \times 10^{-6} \text{ cm}^2/\text{s} \) (E). These differences reflect differences in molecular sizes within this dextran sample. Moreover, some of the decay curves (i.e., B, C, and E) are non-linear, also indicating the presence of more than one molecular weight species at that particular chemical shift position (i.e., A–E). Because of this, we analyze decay curves from a number of resonances in each sample to provide a reasonable range of D values that we can then relate to molecular size.

When decay curves are curvilinear, we can estimate the extrema of D values from a given decay curve by assessing the slope of initial and final groups of points on the curve. For example, the dashed line through the final 8 points on decay curve C in Figure 3A illustrates this for the 'slow' component of the full decay curve, whereas the initial four points define the 'fast' decay component. In this instance, we have D values of \( 0.6 \times 10^{-6} \text{ cm}^2/\text{s} \) for the fast component and of \( 0.44 \times 10^{-6} \text{ cm}^2/\text{s} \) for the slow component. Given that we are nearly at the limitation of gradient strength for our spectrometer, additional points on the decay curves could not be acquired. Nevertheless, D values derived from the slow component provide good and reasonable values, as linear fits using the final 8 data points yield \( R^2 \) values greater than 0.9. In addition, we
3.2. Correlation of self-diffusion of dextrans with their molecular sizes

In order to correlate $D$ values of these dextran polymers with their molecular sizes, we determined $D$ values as a function of dextran concentration, and extrapolated $D$ to infinite dilution, yielding $D_{\text{inf}}$. For each dextran sample, we measured decay curves for the 15 most intense resonances. For example, for the 49 kDa dextran sample, $D$ values were derived and are plotted as $D$ versus concentration (mg/mL) in Figure 3B. Nonlinear decay curves were deconvoluted to yield fast and slow decay components as discussed above. However, only $D$ values from the slow components were used in the following analysis, primarily because contributions from fast components were only about 10% of the decay curve, making determination of those $D$ values less accurate.

Poly saccharides generally interact with each other to form networks in solution, and $D$ values are quite sensitive to these intermolecular interactions which contribute significantly to solution viscosity. $D$ is related to the viscosity-average molecular weight of the polymer, $M_v$, through the Mark–Houwink relationship, by the following equation which has been derived in its simplest form for a spherical particle:

$$D = \left(\frac{RT}{6\pi \eta_s N_o}\right)[10^m N_o/3K M_p^{1/2}]$$

(2)

where $R$ is the gas constant, $T$ is temperature in Kelvin, $\eta_s$ is the viscosity of the liquid medium, $N_o$ is Avogadro’s number, and $K$ (cm$^2$ g$^{-1}$) and $\alpha$ are Mark–Houwink constants determined experimentally over a range of molecular weights and polymeric structures. For example, $\alpha$ is zero for a hard sphere, 2 for a rigid rod, 1 for a semi-coil, 0.5 in a Flory theta solvent, and 0.8 in a thermodynamically good solvent. For our purpose, we need only to consider that $D \propto [1/M_p^{-1}]^{1/2}$, and although this proportionality is truly valid only for a sphere, we can generalize it to $D \propto 1/M_v^\alpha$, $M_v$ is the viscosity-average molecular weight, and ‘$D$’ is some power to which $M_v$ is raised. In this regard, the function has the phenomenological form:

$$y = a(1/x^\alpha)$$

(3)

where ‘$a$’ and ‘$b$’ are fitting parameters, and we used this equation to fit our $D$ versus concentration (mg/mL) curves in Figure 3B and derive $D_{\text{inf}}$ from the Y-intercept. The fits shown in Figure 3B as dashed lines represent the data quite well, with $D_{\text{inf}}$ values ranging from $0.42 \times 10^{-6}$ cm$^2$/s to $0.58 \times 10^{-6}$ cm$^2$/s for this 49 kDa dextran sample. It should be noted that we derive essentially the same results by plotting log $D_{\text{obs}}$ versus concentration in mg/mL and extrapolating to the Y-intercept at zero concentration.

On all five dextran samples, we determined $D_{\text{inf}}$ for the 15 major resonances from each sample. Figure 4A plots log $D_{\text{inf}}$ as a function of the log weight-average molecular weight as provided by the manufacturer. As should be evident from the above, there are multiple values for $D_{\text{inf}}$ at any one given average molecular weight due to dextran size heterogeneity within each sample. Around each group of $D_{\text{inf}}$ values, we drew a rectangle whose base indicates the molecular weight range provided by the manufacturer for each dextran sample. If the correlation between these $D_{\text{inf}}$ values and the reported weight-average molecular weight range were perfect, we would be able to draw a straight line through the diagonals of each of these rectangles. Deviations result from our analysis not capturing all possible $D$ values in each dextran sample and/or from the manufacturer’s reported weight-average molecular weight distributions being somewhat off. Nevertheless, the apparent correlation (i.e., straight line) is rather good. In fact, the $D$ value for the 0.36 kDa disaccharide lactose ($5.5 \times 10^{-6}$ cm$^2$/s, not shown in the plot) also lies on this straight line.

For a number of flexible polymers, Schmidt and Burchard demonstrated that plots of $D$ versus weight-average molecular...
weight ($M_w$) follow a power law relationship having the form $D = \alpha(M_w)^\beta$. The inset in Figure 4A shows a nonlinear least squares fit ($R^2 > 0.99$) to our data using this equation, resulting in $\alpha = 11 \times 10^{-3}$ and $\beta = -0.5$. These values are similar to others that have been reported in the literature for various dextran solutions. A few examples are $\alpha = 5.5 \times 10^{-3}$ and $\beta = -0.43$ for dilute solutions of 20 kDa to 2000 kDa dextran, and $\alpha = 2.7 \times 10^{-3}$ and $\beta = -0.37$ using a fluorescence photobleaching technique on solutions of 20 kDa to 2000 kDa FITC-labeled dextran samples. The FITC-labeling fluorescence approach used solutions of dextran in the concentration range of 0.0125 to 0.2 mg/mL, whereas we are using dextran concentrations in the 3-50 mg/mL range. While differences in $\alpha$ and $\beta$ fitting parameters among these studies may be explained in part by differences in dextran concentrations, it may also be explained by differences in branching or polydispersity of dextran samples.

The $D_{n}$ versus $M_w$ plot (Fig. 4A) also reflects the random coil nature of these polysaccharides. The Stokes-Einstein equation $D = k_BT \eta_0/6\pi R_N$ relates $D$ to the macromolecular hydrodynamic radius, $R_N$, which in turn is generally accepted as being proportional to a power, $x$, of the apparent molecular weight, $M_{app}$. This relationship can be used to compare $D$ values for two molecules (Eq. 4) under the same solution conditions:

$$D_1/D_2 = (M_{app1}/M_{app2})^x$$

Typically, $x = 1/3$ for a compactly folded molecule, and $x = 1/2$ for a random coil state. Because the Stokes-Einstein relationship has been derived specifically for a hard sphere, the actual molecular shape and the shape of the molecular aggregate will affect $D$, but usually only by about 10% when comparing two molecules having equal total volumes. By using Eq. 4, $D_{app}$ values for our glycan standards versus their molecular weights (Fig. 4A) can be approximated with $x = 1/2$, indicating that these glycan, as expected, are random coil polymers.

For comparison, Figure 4A also plots $D$ values for several globular proteins (6 kDa BPTI, 8.6 kDa ubiquitin, 13.6 kDa ribonuclease, 14.5 kDa galactin-1 monomer and its dimer [Nesmelova et al., unpublished data]). The inset in Figure 4A, which plots the average of these diffusion coefficients versus the average molecular weight, has been fit with a curve as described in the text. (B) $D_{app}$ values for these dextrans, as well as for the disaccharide lactose, are plotted versus log hydrodynamic radius, $R_H$, calculated using the Stokes-Einstein relationship as discussed in the text. The linear fit shown has a regression coefficient $R^2 = 0.99$.

3.3. Size distributions of DAVANAT and GRG

We next used the PFG NMR diffusion method to investigate molecular size distributions in samples of two different glycan: GRG and Davanat. Even though GRG and Davanat are polysaccharides like dextran, they are structurally different from dextran and from each other. GRG is a highly branched, galactorhamnoglycan...
lacturonate of irregular structure, with a backbone that is composed primarily of \(\alpha-(1 \rightarrow 2)-\alpha\)-rhamnosyl-\(\alpha-(1 \rightarrow 4)-\alpha\)-galacturono- syl sections, and the weight-average molecular weight and molecular size distribution of this GRG preparation are unknown. The galactomannan Davanát has a 17 \(\beta\)-(1 \rightarrow 4)-\(\alpha\)-mannopyranosyl 10 \(\alpha\)-(1 \rightarrow 6)-\(\alpha\)-galactopyranosyl residue repeat fragment (Fig. 1), with reports of its \(M_w\) being 48–55 kDa. Both GRG and Davanát have been prepared by controlled partial depolymerization, as explained in Section 2.

\(^1\)H NMR spectra of both polysaccharides are shown in Figure 5A (GRG) and 5B (Davanát). The more diverse and seemingly complex \(^1\)H NMR spectrum of GRG is consistent with the presence of more varied types of saccharide units within its polymeric structure, compared to Davanát whose \(^1\)H NMR spectrum is more similar to

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Figure 5. \(^1\)H NMR spectral traces for GRG (A) and Davanát (B) are shown, and resonances are labeled alphabetically, with letters corresponding to curves shown in the plots shown below these spectra, and with \(D_{\text{rel}}\) values given in Table 1. Sets of diffusion decay curves are plotted as the log of the change in resonance intensity versus gradient strength for GRG (C) and Davanát (D). The slope effectively yields \(D\) as discussed in the text. Note that some of the decay curves are nonlinear, indicating a distribution (two or more) of \(D\) values under that particular resonance. Dashed lines are drawn through the slow components of these decay curves, as discussed in the text. \(D_{\text{rel}}\) values are plotted versus concentration (mg/mL) for GRG (E) and Davanát (F). As discussed in text, dashed lines represent fits of the data to the equation \(y = \alpha x^{\beta}\) to \(D_{\text{rel}}\) from the Y-intercept. \(^1\)H resonance assignments for another galactomannan reported by Rahmanberdyyeva and Shashkov (2005) have been given under the spectrum of Davanát. Man (1 \rightarrow 4 and 1 \rightarrow 6 Gal-linked) and Gal stand for mannose and galactose units.
that of dextran. Resonance assignments for preparations of galactomannans from Gleditsia delavayi and G. aquatica have been reported by Rakhmanova and Shevchik. However, because Dovanat is a highly processed galactomannan, these assignments do not correspond exactly and unambiguously with chemical shifts of resonances in the $^1H$ spectrum of Dovanat. Nevertheless, we have indicated them under the Dovanat spectrum (Fig. 5B), for easy reference. Although unambiguous or specific assignments for $^1H$ resonances of CRG and DAVANAT are unknown, these are not really necessary for purposes of this study. Figure 5C and D gives several representative diffusion decay curves derived from gradient-induced dephasing of some resonances of CRG and Dovanat, respectively. While some of these decay curves appear linear, most are at least somewhat curvilinear, with slower decay components (indicated by dashed lines) usually dominating by 80–90%, as estimated from Y-intercepts. Curvilinear decay curves indicate the presence of at least two (or more) significantly different sized glycans, whose resonances overlap at that chemical shift position.

Figure 5E and F plots $D_{obs}$ versus concentration (mg/ml) for the major resonances from CRG and Dovanat, respectively, as labeled alphabetically in their respective spectra in Figure 5A and B. Each set of data was fit using Eq. 2 as discussed above, and the dashed lines through the data points show the actual curve fits, with $R^2$ values being greater than 0.9. The Y-intercept provides estimates of D values at infinite dilution. For either glycan, $D_{obs}$ values ranged from about $0.2 \times 10^{-6} \text{ cm}^2/\text{s}$ to $1.55 \times 10^{-6} \text{ cm}^2/\text{s}$, and tended to be expectedly smaller for the larger CRG. These values are listed out in Table 1 for all or most of the resonances shown in the $^1H$ spectra of Figure 5.

Using these $D_{int}$ values, we estimated both $R_0$ and weight-average molecular weights ($M_w$) using the dextran calibration curves (Fig. 4) as listed out in Table 1. For CRG, although $R_0$ ranges from $17 \times 10^{-10} \text{ m}$ to $134 \times 10^{-10} \text{ m}$, most values fall in the range of $46 \times 10^{-10} \text{ m}$ to $92 \times 10^{-10} \text{ m}$, with a simple average of $76 \pm 6 \times 10^{-10} \text{ m}$. For the smaller Dovanat glycan, the range of $R_0$ is much narrower with a simple average of $56 \pm 3 \times 10^{-10} \text{ m}$, and most values falling in the range of $53 \times 10^{-10} \text{ m}$ to $67 \times 10^{-10} \text{ m}$. If we assume that CRG and Dovanat behave similarly in solution as the dextrans, we find that weight-average molecular weight ($M_w$) values estimated from Figure 4A range from 3 kDa to 300 kDa for CRG and from 10 kDa to 80 kDa for Dovanat, with simple average values of $128 \pm 35 \text{ kDa}$ and $59 \pm 8 \text{ kDa}$, respectively (Table 1). Table 1 also provides estimates of the fractional contribution from each resonance derived by integrating resonances in the NMR spectra of each glycan. The average values given in parentheses were determined using these fractions as weighting factors. In either case, average values determined either way are nearly the same.

Although we have no values in the literature with which to compare our values for CRG, we do for Dovanat, at least in terms of the weight-average molecular weight. Our weight-average molecular weight for Dovanat (59 kDa) compares favorably with values reported previously (48–55 kDa) using the SEC-MALLS technique. Using the PFG NMR diffusion approach, however, we also now have information on how dispersed these glycan preparations are. Clearly, CRG is more highly poly-dispersed than Dovanat, which is expected based on the chemistry of these polysaccharides.

In summary, we have shown that PFG NMR is a useful approach to assess diffusion coefficients and to estimate molecular weights of various glycans without the need to have highly fractionated samples. The PFG NMR technique can be used with solutions, for example, at various pH values, ionic strengths, and temperatures, as well as with pre-gel solutions transitioning into gel states. As interest in carbohydrate research is increasing, this methodology should be quite useful to investigators needing to have knowledge of polysaccharide dispersity in solution, and to follow degradation of polysaccharides during chemical production.

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