Laser Light-Scattering Characterization of the Molecular Weight Distribution of Dextran

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ABSTRACT: Laser light scattering (LLS) including the angular dependence of the absolute integrated scattered intensity (static LLS) and of the line-width distribution (dynamic LLS) has been used to characterize the molecular weight distributions of dextran samples with different branching densities. In the process of converting a translational diffusion coefficient distribution \( G(D) \) obtained from the precisely measured intensity-intensity time correlation function into a molecular weight distribution (MWD), we encountered the following two problems: the change of the dextran conformation as a function of molecular weight and the lack of a set of narrowly distributed dextran standards. A procedure to solve these two problems simultaneously has been presented, wherein the weight-average molecular weight \( M_w \) obtained from static LLS is used to constrain the conversion of \( G(D) \) to MWD. By using this procedure, we were able to obtain a calibration of \( D \) (cm/\( s \)) = \( 1.98 \times 10^{-4} M_w^{0.667-0.9931 \log M_w} \) with a set of broadly distributed dextrans and to accomplish the calculation of MWD of dextran from the measured spectral distribution. The calculated molecular weight distributions are fairly comparable to the ones obtained from gel filtration experiments.

1. Introduction

Dextran is a high molecular weight branched polysaccharide synthesized from sucrose by bacteria. This polymer consists of anhydroglucose repeat units joined by \( \alpha \)-acetal linkages. Approximately 95% of those linkages are through carbons 1 and 6 in the main and branch chains and the rest of them are between carbons 1 and 3 at the branching point. Dextran is used as a partial substitute for blood plasma, mainly as a volume expander. Its pharmacological applications are directly related to its physicochemical properties. Normally, the dextran produced by industrial fermentation has to be partially hydrolyzed and then fractionated in order to give a dextran with a certain molecular weight distribution (MWD) which is suitable for clinical use. Therefore, the accurate determination of MWD of a given dextran is often important in its applications.

In the past, many methods, such as the classic fractionation (i.e., precipitation, extraction, ultrafiltration, etc.), size-exclusion chromatography (SEC), and ultracentrifuge, have been used to determine the average molecular weight or MWD of dextran. The fractionation normally involves a time-consuming process and its resolution is limited. In the case of SEC, the axial dispersion of dextran, coexistence of adsorption (dextran is a polar system), and calibration (using a set of narrowly distributed dextrans and to establish the relation between MWD and \( M_w \)) are the main difficulties. When using ultracentrifuge, the analysis of dextran in water is hindered by the large deviation from ideality. Only an apparent distribution has to be calculated by extrapolating the real distribution has to be calculated by extrapolating the apparent boundary spreading of velocities to infinite dilution. This extrapolation introduces some inaccuracies in the final molecular weight distribution.

Light scattering as a well-established analytical method has been extensively used to determine the weight-average molecular weight of various polymer samples including dextran. At the present time, due to the advances of laser as the light source, photomultiplier, correlator, and computer in the past 20 years, we are able to measure not only the average scattering intensity (static light scattering) but also the fluctuations of the scattered light (dynamic light scattering). Various computer programs have been developed to make a Laplace inversion of the measured correlation function in order to give an approximated characteristic line-width distribution, \( G(\Gamma) \) which can be further reduced to a translational diffusion coefficient distribution \( G(D) \) or even to a molecular weight distribution (MWD) if the calibration between \( D \) and \( M \) is known. As an absolute analytical method, using laser light scattering (LLS) to determine MWD has certain advantages over the other analytic techniques. For example, the calibration between \( D \) and \( M \) is independent of the particular LLS instrument.

The present work serves two purposes. One is to determine MWD of dextran for the first time by only using LLS. The other is to present a LLS data analysis procedure of using a set of broadly distributed samples to establish a calibration between \( D \) and \( M \) for some special polymers, such as dextran, whose \( D \) cannot be scaled to \( M \) as \( D = k_D M^{-\alpha_0} \) with only two scaling constants \( k_D \) and \( \alpha_0 \).

2. Basic Theories

Static Light Scattering. The angular dependence of the excess absolute time-averaged scattered intensity, known as the excess Rayleigh ratio \( R_w(\theta) \), was measured. For a dilute polymer solution at concentration \( C \) (g/mL) and scattering angle \( \theta \), \( R_w(\theta) \) can be approximately expressed as

\[
K C \frac{R_w(\theta)}{M_w P(\theta)} \approx \frac{1}{M_w P(\theta)} + 2A_2 C
\]

where \( K = 4\pi^2 n^2 (\delta n / \delta C)^2/\left(N_A \lambda_0^6 \right) \) and \( q = (4\pi n / \lambda_0) \sin (\theta/2) \) with \( N_A \), \( n \), and \( \lambda_0 \) being Avogadro's number, the solvent refractive index, and the wavelength of light in vacuo, respectively. If the root-mean-square z-average radius, \( \langle R_g^2 \rangle^{1/2} \), is smaller than \( q^{-1} \), \( 1/P(\theta) \) is approximated as \( 1 + 1/3 \langle R_g^2 \rangle q^2 \). By measuring \( R_w(\theta) \) at a set of \( C \) and \( \theta \), we can determine \( M_w \), \( \langle R_g^2 \rangle^{1/2} \), and \( A_2 \) from a Zimm plot which incorporates \( \theta \) and \( C \) extrapolations on a single grid. Figure 1 shows a typical Zimm plot of dextran in water at 25 °C.

Dynamic Light Scattering. An intensity-intensity time correlation function \( G^{(2)}(\delta t, \theta) \) in the self-heating mode...
past, various methods have been used to solve this problem: such as measuring \( D \) and \( M \) of many narrowly distributed standards;\(^{21}\) using \( G(D) \) and \( M \) of at least two broadly distributed samples and assuming polymer conformation is not a function of \( M \);\(^{16}\) estimating the calibrating constant from other experimental results (for example, from polymer conformation, solvent quality, and viscosity data);\(^{22}\) and combining the elution volume distribution \( (C(V)) \) of a broadly distributed sample from SEC with both \( G(D) \) and \( M_w \) from laser light scattering.\(^{15}\)

After having the calibration, \( G(D) \) can be transferred to MWD according to the following principles: as \( C \to 0 \) and \( \theta \to 0 \), based on eqs 1 and 3, we have

\[
\int_0^\infty G(D)\,dD = \gamma \int_0^\infty F_n(M)\,M^2\,dM \tag{5}
\]

where \( \gamma \) is a normalization constant and \( F_n(M) \) is a number distribution. Equation 5 can be rewritten as

\[
\int_0^\infty G(D)\,dM = \gamma \int_0^\infty F_n(M)\,M^2\,dM \tag{6}
\]

By comparing both sides of eq 6, we have

\[
F_w(M) = F_n(M) M \propto \frac{G(D)}{M}\,dD \quad \text{or} \quad F_n(M) \propto \frac{G(D)}{M^2}\,dM \tag{7}
\]

where \( F_w(M) \) is a weight distribution and all proportional constants have been omitted since they are irrelevant to both distributions. For a given calibration between \( D \) and \( M \), we can first calculate both \( M \) and \( dD/dM \) and then \( F_w(M) \) or \( F_n(M) \) according to eq 7.

3. Experimental Methods

**Preparation of Solutions.** The dextrans (T10, T40, T70, T500, and T2000) obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) were used without further purification. T3500 was prepared by fractionating T2000 in a standard procedure\(^{3}\) except that of T2000. These dextrans were prepared by fractionating material synthesized from sucrose by the bacterial species *Leuconostoc mesenteroides* strain B512. The branching points of dextran produced in this way are about 5% of the degree of polymerization.\(^{3}\) Doubly distilled, deionized water was used as solvent. The water content in these samples has been determined to be \(~10\%\), which was taken into account when we calculated the final dextran concentration, which ranged from 0.1 to 4 g/L depending on \( M_w \).

**Laser Light Scattering.** A commercial LLS spectrometer (ALV/SP-86, Langen in Hessen, Germany) was used with an argon ion laser (Coherent INNOVA 300, operated at wavelength 488 nm and 300 mw) as the light source. All measurements were performed at 25.0 \( \pm 0.1 \)°C.

4. Results and Discussion

Our laser light scattering results together with the gel filtration results supplied with the samples by Pharmacia Fine Chemicals are summarized in Table I. We will discuss the results of T2000 and T3250 later. It can be seen that the agreements between \( M_w \) obtained by these two different methods are rather satisfactory, except for T70. Our repeated measurements and the experiments in our other laser light scattering laboratory confirm that \( M_w \) of T70 is \(~65000\). The second virial coefficient of dextran decreases sharply as the molecular weight increases. This
suggestions that high molecular weight dextrans have more densely filled conformations because their branching densities are higher. The molecular sizes of T10, T40, and T70 are so small that the scattering intensities are virtually independent of the scattering angle. Therefore, it is impossible to determine the exact values of \( \langle R_g^2 \rangle_1/2 \) by using laser light scattering. Due to the branching, the measured \( \langle R_g^2 \rangle_1/2 \) and \( J \) values of dextrans are smaller than the ones of a linear flexible polymer with a similar number of Kuhn segments in good solvent.\(^{23,24} \) The values of \( \langle R_g^2 \rangle_1/2 \) and \( \bar{D} \) are similar to those listed in the literature.\(^{10,25} \)

Figure 3 shows six translational diffusion coefficient distributions of six dextran samples measured in water at 25 °C, where \( C \to 0 \) and \( q \to 0 \). (\( q \) is momentum transfer.)

<table>
<thead>
<tr>
<th>Table I. Static and Dynamic Light Scattering Results of Dextrans</th>
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<tbody>
<tr>
<td>( M_w/(10^3) )</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>0.99</td>
</tr>
<tr>
<td>1.38</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>9.10</td>
</tr>
<tr>
<td>0.13</td>
</tr>
<tr>
<td>1.3</td>
</tr>
</tbody>
</table>

Gel Filtration Data Provided by Pharmacia Fine Chemicals

\( M_w/(10^3) \) | T10 | T40 | T70 | T500 | T2000 | T3250 |
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>0.97</td>
<td>3.99</td>
<td>7.03</td>
<td>48.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.62</td>
<td>1.60</td>
<td>1.85</td>
<td>2.69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Translational diffusion coefficient distributions of six dextran samples measured in water at 25 °C, where \( C \to 0 \) and \( q \to 0 \). The first problem is that the branching density of dextran increases as a function of molecular weight, resulting in more compact molecules, which means \( D \neq k_d M^{-a_d} \) or the plot of \( \log D \) versus \( \log M \) is not a straight line. The second problem is that a set of narrowly distributed dextran standards is not available so that we were not able to use the measured \( \bar{D} \) and \( M_w \) as \( D \) and \( M \), respectively, in the calibration.

Figure 4 shows a log–log plot of \( D \) versus \( M_w \), where \( D \) was calculated from \( G(D) \) in Figure 3. However, the data points do not follow a straight line. The question is whether this curvature is due to polydispersity and experimental uncertainty or due to the change of branching density as a function of molecular weight. In order to answer this question, let us look at some intrinsic viscosity ((\( \eta \)) data, which are related to hydrodynamic size in a similar way as \( D \), because the plot of \( \log \eta \) versus \( \log M \) should be more curved if the curvature is intrinsic.\(^{26} \) The broken line in Figure 4 represents a least-squares fit of \( \log D = \log(6.51 \times 10^{-2}) - 0.876 \log M_w + 4.02 \times 10^{-2} (\log M)^2 \). The solid line represents a calibration between \( D \) and \( M_w \), where \( \log D = \log(1.98 \times 10^{-4}) - 0.857 \log M_w + 2.01 \times 10^{-2} (\log M)^2 \). The dashed line shows how intrinsic viscosity \( \eta \) changes as a function of \( M_w \), calculated from the data listed in ref 8. After realizing that the curvature in the plot of \( D \) versus \( M_w \) is intrinsic, we decided to use the following empirical equation to fit our data in Figure 4:

\[
\log D = \log k_D - \alpha_D \log M + \alpha_D'' (\log M)^2
\]

(8)

The broken line in Figure 4 represents a least-squares fitting of eq 8 with \( k_D = 6.51 \times 10^{-3}, \alpha_D = 0.876, \) and \( \alpha_D'' = 4.02 \times 10^{-2} \) where bars over these parameters mean that they are obtained from \( D \) and \( M_w \) instead of from \( D \) and \( M \). It is interesting to note that \( \log \eta \) is a linear function of \( M_w \) and \( M \), and \( \eta(\log M) \) is very close to Flory's prediction.\(^{26} \)

By using eqs 7 and 8 with \( k_D, \alpha_D, \alpha_D'' \) obtained from \( D \) and \( M_w \), we were able to calculate \( F_w(M) \) and \( F_n(M) \). In order to have a direct comparison with our static light scattering results, we need to calculate \( M_w \), \( M_n \), \( M_w', \) and \( M_n' \) directly, according to their definitions,

\[
(M_w)_\text{calc} = \frac{\int_0^{\infty} F_w(M) M dM}{\int_0^{\infty} F_w(M) dM} = \frac{\int_0^{\infty} G(D) dD}{\int_0^{\infty} G(D)/M dD}
\]

(9)

and

\[
(M_n)_\text{calc} = \frac{\int_0^{\infty} F_n(M) M dM}{\int_0^{\infty} F_n(M) dM} = \frac{\int_0^{\infty} G(D)/M dD}{\int_0^{\infty} G(D)/M^2 dD}
\]

(10)

The weight- and number-average molecular weights calculated with \( k_D, \alpha_D, \alpha_D'' \) are listed in Table II. It is not a surprise to find that \( M_w \) and \( M_w'/M_n' \) calculated in this way are smaller than the ones obtained by using static LLS and gel filtration because we have used \( k_D, \alpha_D, \alpha_D'' \) instead of \( k_D, \alpha_D', \) and \( \alpha_D'' \), respectively. It is known that \( k_D, \alpha_D' \) calculated from a set of broadly distributed samples are usually different from \( k_D, \alpha_D', \) and \( \alpha_D'' \) obtained from a set of monodisperse standards (or very narrowly distributed samples).\(^{10} \) On the basis of this failed trial, we decided to solve the above two problems simultaneously by using the following principle:

For \( N \)-number samples, we have \( N \)-number measured \( M_w \) and \( G(D) \), denoted as \( M_w', \) and \( G(D) \), where \( i = 1-N \).
Table II. Calculated $M_w$ and $M_w/M_s$ of Dextrans from $G(D)$

<table>
<thead>
<tr>
<th>T10</th>
<th>T40</th>
<th>T700</th>
<th>T2000</th>
<th>T3250</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_w/(10^3)$</td>
<td>0.89</td>
<td>3.24</td>
<td>5.16</td>
<td>30.6</td>
</tr>
<tr>
<td>$M_w/M_s$</td>
<td>1.46</td>
<td>1.51</td>
<td>1.63</td>
<td>2.20</td>
</tr>
</tbody>
</table>

using eq 8 with $k_D = 6.51 \times 10^{-4}$, $a_D'' = 0.659$, and $a_D' = 0.0402$

using $\log D = \log k_D - a_D' \log M + a_D'' (\log M)^2$

where $k_D = 1.90 \times 10^{-4}$, $a_D'' = 0.659$, and $a_D' = 2.09 \times 10^{-2}$

$M_w/(10^3)$ | 0.96 | 4.10 | 6.75 | 46.6 | 233 | 312 |
| $M_w/M_s$     | 1.59 | 1.63 | 1.77 | 2.38 | 6.16 | 2.07 |

using eq 8 with $k_D = 1.90 \times 10^{-4}$, $a_D'' = 0.659$, and $a_D' = 0.0209$

Figure 5. Typical plot of ERROR versus $k_D$ with different $a_D''$ but a fixed $a_D' = 0.659$, where the overall minimum is located at $a_D'' = 2.09 \times 10^{-2}$ and $k_D = 1.90 \times 10^{-4}$.

By assuming a set of $k_D$, $a_D'$, and $a_D''$ in eq 8 and using eq 9, we are able to calculate $N$-number $(M_w)_\text{calc}$ denoted as $(M_w)_i$, where $i = 1-N$. In principle, $(M_w)_i$ should equal $M_w$ if $k_D$, $a_D'$, and $a_D''$ are correctly chosen. Therefore, our object is to find a set of $k_D$, $a_D'$, and $a_D''$ which can minimize the ERROR defined as

$$\text{ERROR} = \frac{1}{N} \sum_{i=1}^{N} \left[ \frac{M_w - (M_w)_i}{M_w} \right]^2$$

(11)

It is clear that this procedure is an $M_w$-constrained analysis. In this way, by using eq 8 instead of $D = k_D M^{-a_D}$, we have taken into account the conformation change as a function of molecular weight; and by using $k_D$, $a_D'$, and $a_D''$ instead of $k_D$, $a_D'$, and $a_D''$, we have avoided the polydispersity problem.

Figure 5 shows a typical plot of ERROR versus $k_D$ with different $a_D''$ but a fixed $a_D' = 0.659$. It can be seen in Figure 5 that there is a minimum ERROR for each given $a_D''$ and there is an overall minimum for a fixed $a_D''$. Figure 6 shows a similar plot of ERROR versus $k_D$ with different $a_D'$ but a fixed $a_D'' = 2.09 \times 10^{-2}$. There is also an overall minimum for a fixed $a_D''$. Therefore, by combining Figures 5 and 6, we know there exists a set of $k_D$, $a_D'$, and $a_D''$ which corresponds to an overall minimum point on the ERROR surface. Numerically, we were able to find this overall minimum at $k_D = 1.90 \times 10^{-4}$, $a_D' = 0.659$, and $a_D'' = 2.09 \times 10^{-2}$, which defines an calibration between $D$ and $M$. The continuous line in Figure 4 represents such a calibration. Its obvious deviation from our measured $D$ and $M$, clearly shows how serious error could be introduced in practice if we would use $D$ and $M$ measured from a set of broadly distributed samples instead of $D$ and $M$.

However, from the experimental point of view, this overall minimum point is not well-defined because there is always some experimental noise in both $M_w$ and $G(D)$. The important question is how much error this uncertainty will introduce into the final molecular weight distributions.

Figure 7 shows three cumulative weight distributions of dextran T500 calculated with three different sets of $k_D$ and $a_D'$ but a fixed $a_D'' = 2.09 \times 10^{-2}$.

Figure 8 shows a comparison between $F_w(M)$ calculated from $G(D)$ and $F_w(M)$ obtained by using gel filtration (supplied by Pharmacia Fine Chemicals, Uppsala, Sweden). Two distributions in Figure 8 are comparable in spite of a slight difference in the distribution width. This difference is understandable because the scattered light intensity is proportional to $M^2$ so that small molecules in a broad distribution cannot be “seen” by the detector. Therefore, for a broadly distributed sample, MWD ob--
In the calibration process, we have shown the problems of the change of the branching density as a function of molecular weight and the lack of a set of dextran standards can be solved by using a new $M_w$-constrained analysis. This analysis procedure should be valuable for characterizing other special systems where the polymer conformation changes as a function of molecular weight or a set of narrowly distributed standards with different molecular weights is not available.

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References and Notes

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25. Ebert, K. H.; Broeche, M. Biopolymers 1967, 5, 423.