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Laser light-scattering studies of pachyman

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Abstract

Three fractions of a pachyman sample extracted from poria cocos sclerotium in both Me_2SO and a 0.5 N NaOH aqueous solution were investigated by static and dynamic laser light scattering (LLS). In static LLS, the angular dependence of the excess time-average scattering intensity led to the weight-average molecular weight M_w, while in dynamic LLS, the Laplace inversion of a precisely measured intensity–intensity correlation function resulted in a translational diffusion coefficient distribution $G(D)$. A combination of static and dynamic LLS results enabled us to establish a calibration of D (cm^2/s) $= 3.6\times10^{-4}\,M^{-0.674}$. Using this calibration, we were able to convert each $G(D)$ into a corresponding molar mass distribution $F(M)$. We found that the ratio of the radius of gyration to the hydrodynamic radius was greater than 2, indicating that the pachyman chains have an extended conformation in Me_2SO. Our results also revealed the formation of large pachyman aggregates with a loose structure in aqueous NaOH solution.

Keywords: Pachyman; Polysaccharide; Light scattering; Fractionation; Aggregation

1. Introduction

Polysaccharides, a type of important biopolymer, have been used in the food industry and in medicine for a long time. Recently, polysaccharides have attracted much more attention due to their biological activities and special solution properties [1–7]. Poria cocos has long been used in Chinese traditional medicine as a diuretic and anti-aging drug. Pachyman (a special kind of polysaccharide extracted from poria cocos sclerotium) and its derivatives have various biological activities, such as mitogenic/complement-activating activities [8], antimutagenic activity [9], and antitumor activity [10,11]. The biological activities of a given polysaccharide are often affected by its molar mass, degree of branching and chain conformation [12–14], but there are some contradictions in the literature concerning the relationship between its structure (or molar mass) and biological activity [15]. Therefore, a precise determination of its molecular parameters is the first step towards a better understanding of its biological properties. As biopolymers, polysaccharides in general have more complex structures and chain conformations

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than normal synthetic polymers [16]. Polysaccharides can act as random coils, rod-like chains, helices, or aggregates in different solvents [17–24]. The conformation of polysaccharides has been studied by different methods, such as NMR spectroscopy, molecular dynamics studies, atomic force microscopy (AFM), and laser light scattering (LLS). Static LLS as an absolute and well-established analytical method is often used to determine the weight-average molar mass (M_w) and to measure the average radius of gyration $\langle R_g \rangle$. Recently developed dynamic LLS enables us to measure the average hydrodynamic radius $\langle R_h \rangle$. The ratio of $\langle R_g \rangle / \langle R_h \rangle$ is directly related to the chain conformation. A newly developed analytical method of combining static and dynamic LLS results in not only an average molar mass, but also a molar mass distribution.

2. Experimental

Sample preparation.—Pachyman was extracted from poria cocos sclerotium in aq 0.5 N NaOH. The fractionation was made in a mixture of acetone (as precipitant) and 0.5 N NaOH at room temperature. Three pachyman fractions used in this study are designated as FR-1, FR-2 and FR-3 hereafter according to their depositing sequence. A relatively concentrated stock solution was prepared by dissolving the appropriate amount of pachyman in the solvent, and, after near-complete dissolution, it was centrifuged at 15 000 rpm for 8 h to remove trace amounts of insoluble substances. A series of solutions with different desired concentrations were obtained by a successive dilution of such a clarified stock solution. Finally, each solution was further clarified with a 0.1 or 0.2 μm Waterman filter, depending on the chain size.

Laser light scattering (LLS).—In static LLS, the excess absolute scattered light intensity (also known as the Rayleigh ratio, $R_{vv}(\theta)$) of a polymer solution at a relatively low angle (θ) and low concentration (C) is related to the weight-average molar mass (M_w) by [25]

$$\frac{KC}{R_{vv}(q)} \approx \frac{1}{M_w}\left(1 + \frac{1}{3}\langle R_g^2 \rangle_z q^2\right) + 2A_2C \qquad (1)$$

where $K = 4\pi^2 n^2 (dn/dC) 2/(N_A \lambda_0^4)$ and $q = (4\pi n/\lambda_0) \sin(\theta/2)$, with N_A, n, and λ_0 being Avogadro's number, the solvent refractive index and the wavelength of light in vacuum, respectively. $\langle R_g^2 \rangle_z^{1/2}$ (or written as $\langle R_g \rangle$) is the z-average radius of gyration and A_2 is the second virial coefficient. By measuring $R_{vv}(q)$ at different C and q, we can determine M_w, $\langle R_g \rangle$ and A_2 from the Zimm plot. In dynamic LLS, an intensity–intensity time correlation function $G^{(2)}(t,q)$ in the self-beating mode is normally measured [26,27]. The Laplace inversion of $G^{(2)}(t,q)$ can lead to a linewidth distribution $G(\Gamma)$, which can be further reduced to a translational diffusion coefficient distribution $G(D)$ if the relaxation is diffusive.

A commercial LLS spectrometer (ALV/SP-150) was used, which was equipped with an ALV-5000 multi-tau digital time correlator and a 400-MW ADLAS DPY425II solid-state laser ($\lambda = 532$ nm) as the light source. The primary beam is vertically polarized. All the LLS measurements were performed at 25.0 ± 0.1 °C. The specific refractive index increment dn/dC was determined by a novel differential refractometer that was incorporated into the LLS spectrometer [28], wherein the same laser light source is used for both the LLS spectrometer and the refractometer, so that the wavelength correction of dn/dC was avoided.

3. Results and discussion

Fig. 1 shows a plot of $\langle \Gamma \rangle$ versus q^2 for pachyman FR-1 in Me_2SO at 25 °C where

$$\langle \Gamma \rangle = \int_0^\infty G(\Gamma)\Gamma \, d\Gamma$$

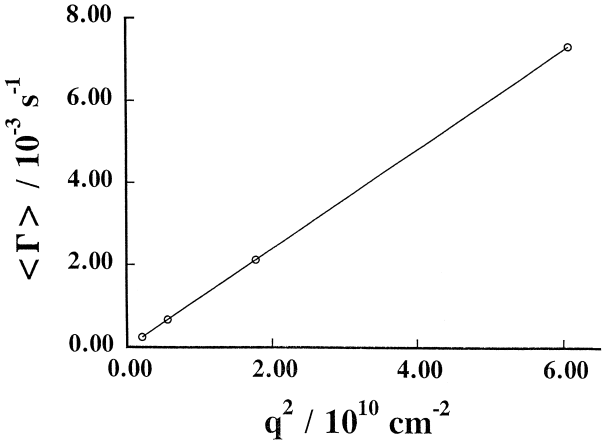

Fig. 1. q^2-dependence of $\langle \Gamma \rangle$ for pachyman FR-1 in Me_2SO at 25 °C, where $C = 1 \times 10^{-3}$ g/mL.

The linear q^2-dependence of $\langle\Gamma\rangle$ indicates that the relaxation processes measured in dynamic LLS is diffusive and the slope leads to $\langle D\rangle$, i.e. $\langle\Gamma\rangle/q^2 = D$. It is worth noting that in the concentration range used, $\langle D\rangle$ is nearly independent of the pachyman concentration. Therefore, $G(\Gamma)$ can be transformed into $G(D)$ by $\Gamma/q^2 = D$. Further, the average hydrodynamic radius $\langle R_h\rangle$ is related to $\langle D\rangle$ by $\langle R_h\rangle = K_B T/(6\pi\eta\langle D\rangle)$. Both the static and dynamic LLS results of the three pachyman fractions are summarized in Table 1. The positive values of A_2 indicate that Me_2SO is a good solvent for pachyman at 25 °C. Three fractions of pachyman have no significant difference in M_w, contradicting the expectation of a decrease of M_w from FR-1 to FR-3. Table 1 also shows that the three pachyman fractions have different $\langle R_g\rangle/\langle R_h\rangle$ ratios. It has been predicted and proved, that for a given polymer/solvent system, $\langle R_g\rangle/\langle R_h\rangle$ depends on the chain architecture, conformation and polydispersity, but not on the molar mass [31]. For example, for a coil chain in a good solvent, $\langle R_g\rangle/\langle R_h\rangle = 1.5$. The relatively large values (2.0–2.7) of $\langle R_g\rangle/\langle R_h\rangle$ in Table 1 indicate that pachyman has an extended chain conformation in Me_2SO. The increase of $\langle R_g\rangle/\langle R_h\rangle$ shows that the chain extension and rigidity increase in the order of FR-1, FR-2, and FR-3.

Fig. 2 shows that the three pachyman fractions in Me_2SO at 25 °C have a unimodal translational diffusion coefficient distribution G(D). Using the previously established method of combining static and dynamic LLS results (M_w and $G(D)$) of two or more broadly distributed samples [21] we got a calibration between D and M, i.e. D (cm^2/s) $= k_D M^{-\alpha D}$ with $k_D = 3.6\times10^{-4}$ and $\alpha_D = 0.674$. (It should be noted that this calibration is only an approximation.) The high value of α_D (~0.674) further indicates that pachyman has an extended chain conformation in Me_2SO at 25 °C. Using this set of k_D and α_D values, we were able to convert each G(D) into a corresponding differential weight distribution of molar mass $F_w(M)$ shown in Fig. 3 and calculate both the weight-average molar mass

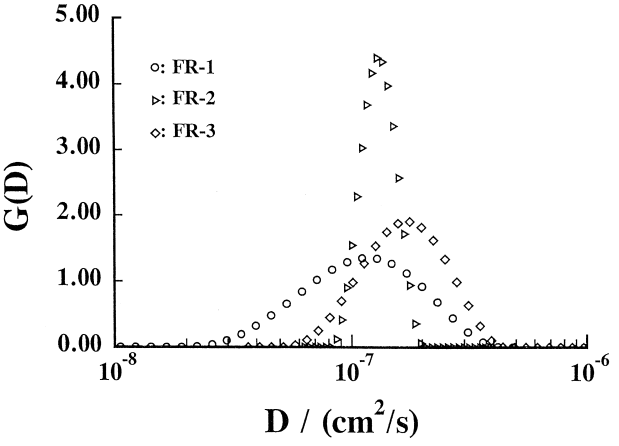

Fig. 2. Translational diffusion coefficient distributions G(D) of three pachyman fractions in Me_2SO at 25 °C, where $C = 1\times10^{-3}$ g/mL and $\theta = 15°$.

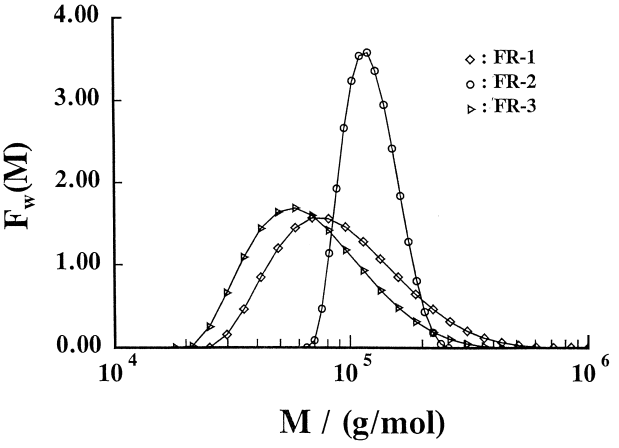

Fig. 3. Differential weight distributions of three pachyman fractions calculated from G(D) in Me_2SO, where $C = 1\times10^{-3}$ g/mL and $\theta = 15°$.

$(M_w)_{calcd}$ and polydispersity index $(M_w/M_n)_{calcd}$, which are also summarized in Table 1. It is not clear at the present stage why FR-2 has a much narrower $F_w(M)$ than FR-1 and FR-3.

Further, we studied the three pachyman fractions in 0.5 N NaOH because (1) pachyman was extracted from poria cocos sclerotium in aqueous solution; and (2) its applications often involve its aqueous solution. Our results showed that pachyman in aqueous solution displayed a much larger

Table 1
Summary of static and dynamic LLS results of three pachyman fractions in Me_2SO at 25 °C

Sample	M_w ($\times10^{-5}$ g/mol)	A_2 ($\times10^4$ mol mL/g^2)	$\langle R_g\rangle$ (nm)	$\langle D\rangle$ ($\times10^8$ cm^2/s)	$\langle R_h\rangle$ (nm)	$\langle R_g\rangle/\langle R_h\rangle$	$(M_w)_{calcd}$ ($\times10^{-5}$ g/mol)	$(M_w/M_n)_{calcd}$
FR-1	0.92	5.7	15	14.3	7.66	2.0	0.90	1.4
FR-2	1.06	5.5	22	13.0	8.43	2.6	1.07	1.1
FR-3	0.67	1.6	17	17.8	6.16	2.7	0.67	1.3

Table 2
Summary of static and dynamic LLS results of pachyman fractions in 0.5 N NaOH at 25 °C

Sample	$M_{w,app}$ ($\times 10^{-5}$ g/mol)	$A_{2,app}$ ($\times 10^{5}$ mol mL/g^2)	$\langle R_g \rangle_{app}$ (nm)	$\langle D \rangle_{fast}$ ($\times 10^{8}$ cm^2/s)	$\langle D \rangle_{slow}$ ($\times 10^{8}$ cm^2/s)	$\langle D \rangle_{app}$ ($\times 10^{8}$ cm^2/s)	$\langle R_h \rangle_{app}$ (nm)	$\langle R_g \rangle_{app}/\langle R_h \rangle_{app}$
FR-1	4.57	5.6	48.2	22.3	4.8	7.9	31	1.6
FR-2	1.11	1.4	37.7	13.2	2.8	7.7	26	1.5
FR-3	2.38	−13.4	38.3	31.8	6.5	9.6	25	1.5

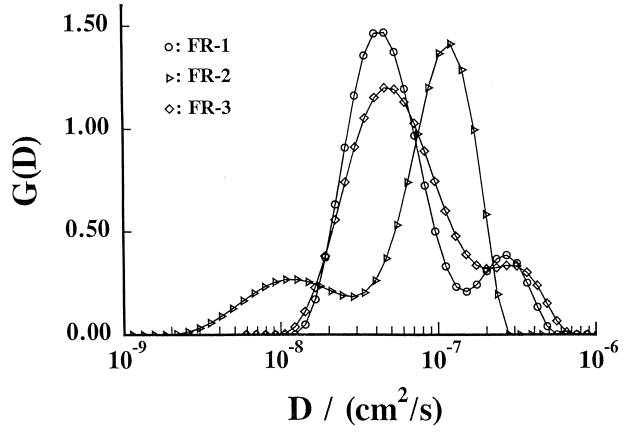

Fig. 4. Translational diffusion coefficient distributions G(D) of three pachyman fractions in 0.5 N NaOH at 25 °C, where $C = 1\times10^{-3}$ g/mL and $\theta = 15°$.

apparent M_w and $\langle R_g \rangle$ (see Table 2). The small and even negative A_2 indicates that for pachyman, water (aqueous NaOH) is not a good solvent. The solubility increases in the order FR-3, FR-2 and FR-1, similar to the case of pachyman in Me_2SO. The higher apparent M_w indicates the aggregation of individual pachyman chains in aqueous solution.

Fig. 4 shows that pachyman in aqueous solution has a bimodal distribution, clearly showing the chain aggregation. The peak with a higher average $\langle D \rangle$ represents individual pachyman chains, while the peak with a lower $\langle D \rangle$ can be attributed to some slow-moving large pachyman aggregates. The apparent average hydrodynamic radii obtained from the cumulative analyses are also listed in Table 2. The apparent $\langle R_g \rangle/\langle R_h \rangle$ ratios of all the pachyman fractions are ~1.5, indicating that the pachyman aggregates formed in aqueous solution have a relatively loose structure.

4. Conclusions

Me_2SO is a good solvent for pachyman at room temperature, where pachyman exists as individual chains with an extended-chain conformation. For pachyman in Me_2SO, its translational diffusive coefficient (D) can be scaled to its molar mass (M) by D (cm^2/s) $= 3.6\times10^{-4}\,M^{-0.674}$. Using this scaling, we can estimate its molar mass distribution from its corresponding $G(D)$. It is worth noting that this scaling is independent of a particular spectrometer. In 0.5 N NaOH, the pachyman chains have a tendency to form larger aggregates with a relatively loose structure.

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