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The Measurement of Mass and Size Distributions, Conformation, and Branching of Important Food Polymers by MALS Following Sample Fractionation

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SUMMARY: Three distinctive food polymers (ultra high molar mass amylopectin, guar gum, and hemi-cellulose) were chosen as model samples to illustrate the use of multi angle light scattering (MALS) detection in conjunction with size exclusion chromatography (SEC) separations for the characterization of such macromolecules. By combining SEC and MALS, absolute molar mass, rms radius and their distributions can be measured readily without reference to any molar mass standards and without the need to make structural assumptions. In addition, the conformation and branching of those polymers can be derived also.

Introduction

The characterization of food polymers in terms of their solution properties plays an important role in their incorporation and performance in various food products for which they are selected. Among their properties, some of the most significant are the average molar mass and size as well as their distributions. All of these quantities are determinable in an *absolute* sense, *i.e., derived from first principles*, or in other words, without reference to any size or mass standards nor to any semi-empirical formulas or relationships. In solution, there are only three methods for making absolute measurements of molar mass. They are membrane osmometry, sedimentation equilibrium, and light scattering. The last has by far the greatest range of application and yields, in addition, the so-called root mean square (rms) radius of the measured molecules. The rms radius is often referred to in the literature by the misnomer *radius of gyration*.

Historically, light scattering measurements have referred to measurements made over a range of scattering angles with respect to the direction of an incident illuminating beam [1-3]. Over the

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past decade, the term multi angle light scattering or MALS has been used to reinforce the meaning and procedure of this classical approach and differentiate it from the so-called low angle (laser) light scattering (LALLS)[4,5] measurement: a means whereby the molar mass alone may be extracted from a light scattering measurement performed at a single, very small scattering angle. In recent years certain *pseudo* light scattering devices have been introduced. These include devices making measurements at two angles and at a single 90° angle; the latter measurement generally being combined with semi-empirical viscometric measurements. They are called *pseudo* light scattering because they are not absolute and require calibration in each solvent by reference to so-called known molar mass standards. In addition to requiring monodisperse standards for such calibration in each solvent (for some solvents such standards often do not exist), these *pseudo* light scattering techniques further require an *a priori* knowledge of the molecular conformation of the unknown polymers.

In order to determine the distributions of molar mass and size, the sample must be solvated and subsequently fractionated by size exclusion chromatography (SEC), field flow fractionation (FFF), or other liquid chromatography methods. Most food polymers are separated by SEC[6,7] though recently separation by sedimentation FFF[8] has become an attractive alternative since there are negligible shearing forces associated with FFF. As MALS measurements produce the weight average molar mass and light scattering average rms radius, during fractionation one can derive these quantities at each elution slice[3] and, assuming nearly monodisperse fractions in each slice, the distributions of these quantities and their other moments (number and *z*-average). Since both molar mass and size at each fractionated slice are measured simultaneously but independently, the conformation of a polydisperse polymer can be obtained readily from the slope of a log-log plot of molar mass and rms radius. By adding a MALS detector to an SEC or FFF system, not only can the tedious column calibrations be eliminated, but also more accurate results can be obtained due to the absence of assumptions regarding molecular conformation and the independence of the results on chromatographic conditions. In the event that the unfractionated sample is measured using the so-called Zimm plot method[1], the weight average molar mass, the light scattering average rms radius, and the second virial coefficient may be derived. In this type of measurement, potential degradation of the polymers by the chromatographic columns or fractionation channel can be avoided, but no distribution data can be obtained.

Theory

Equation 1 below presents the standard relation between the measured quantities and those derived in the limit of vanishingly small concentration[1)] which is generally the case under the conditions used in SEC or FFF separations. The definitions of the quantities are readily found in many of the references[1-3)], and are only briefly summarized here.

$$\frac{K^*c}{R(\theta)} \approx \frac{1}{MP(\theta)} + 2A_2c \qquad (1)$$

The weight average molar mass is M and A_2 is the second virial coefficient. The excess Rayleigh ratio, $R(\theta)$, is measured at concentration c and light scattering angle θ. It represents the excess scattering per steradian from the solution above that of the pure solvent into the angle θ, all divided by the incident light intensity I_0. The constant $K^* = 4\pi^2(dn/dc)^2n_0^2/(N_A\lambda_0^4)$ where N_A is Avogadro's number, dn/dc is the refractive index increment, n_0 is the solvent refractive index, and λ_0 is the vacuum wavelength of the incident light. The mean square radius, $<r_g^2>$, is derived from the initial slope of the excess Rayleigh ratio. The scattering form factor, $P(\theta)$, is given by the general form

$$P(\theta) = 1 - \alpha_1 \sin^2(\theta/2) + \alpha_2 \sin^4(\theta/2) - \ldots \qquad (2)$$

where

$$\alpha_1 = \left(\frac{4\pi n_0}{\lambda_0}\right)^2 \langle r_g^2\rangle/3 \quad \text{and} \quad \langle r_g^2\rangle = \frac{\sum_i r_i^2 m_i}{\sum_i m_i} = \frac{1}{M}\int r^2 dm$$

The sums are over all mass elements m_i of the molecule. The distance of mass element m_i from the molecular center of mass is r_i.

The weight average molar mass, M, is obtained from Eq. 1 in the limit as c and θ approach zero. Note that in typical chromatographic separations, the final concentrations of the fractionated samples entering the flow cell of the MALS detector are very low, since the samples have undergone significant dilution within the SEC columns or FFF channel. Therefore the term containing the second virial coefficient in Eq. 1 is generally negligible relative to the first term on the right side of the equation and may be omitted.

Experimental

A Model 2690 chromatograph (Waters, Milford, MA) was used to deliver reproducible flow. A DAWN-DSP MALS detector and an Optilab DSP differential refractometer (both from Wyatt Technology Corp., Santa Barbara, CA) were used to monitor the effluent from the SEC columns. The MALS detector was also used alone to perform batch measurements.

Three distinct samples are presented here and considered exemplars of the MALS technique and deductions. The first sample is an unfractionated amylopectin from processed waxy maize starch supplied by USDA. The starch was solvated and analyzed in a mixture of 90% DMSO and 10% water. Six solutions were prepared at different concentrations ranging from 0.1 to 1 mg/mL. The *dn/dc* value was found in the literature as 0.074 mL/g 10). The MALS measurement was carried out in batch mode with scintillation vials. The second sample is guar gum provided by China National Petroleum Corp. The guar gum was separated with two linear SEC columns, TSK GMPWXL (TosoHaas, Montgomeryville, PA) and an aqueous mobile phase with phosphate buffered saline. In order to avoid viscous fingering effects11), guar gum sample was analyzed at a relatively low concentration, approximately 0.4 mg/mL. The injection volume was 100 μL. The final sample is a hemi-cellulose separated by using a TSK G6000PWXL column. The mobile phase was water with 50 mM sodium nitrate. The sample concentration and injection volume were similar to those of guar gum.

Results and Discussion

Figure 1 shows a Zimm plot of the unfractionated amylopectin from processed waxy maize starch in 90% DMSO with water. The angular dependence in the Zimm plot was fitted using the Berry formulation12) which fits $\sqrt{K^{*}c/R(\theta)}$ against $\sin^{2}(\theta/2)$. The derived weight average molar mass, rms radius, and second virial coefficient from this plot were $(1.0 \pm 0.1)\ \mathrm{x}10^{9}$ g/mol, 460 ± 20 nm, and $(6 \pm 9)10^{-7}$ mol mL/g^{2}, respectively. These values are in good agreement with the ones reported in a recent publication 10).

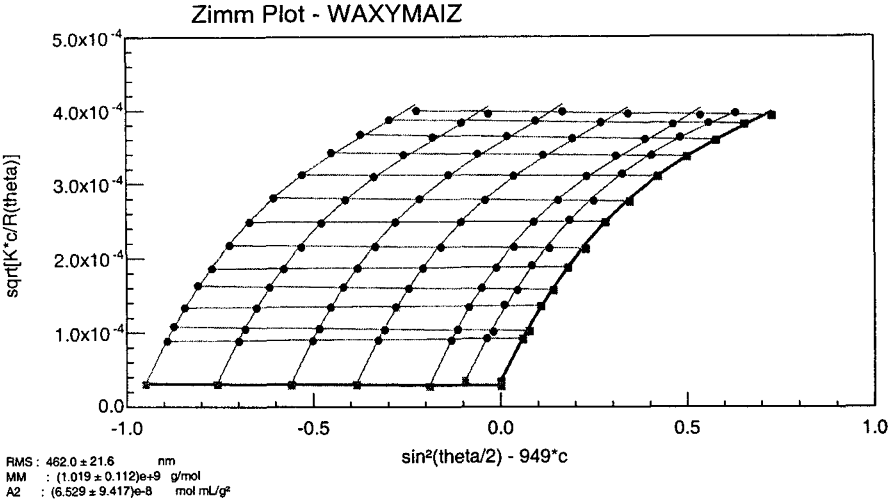

Figure 1. Zimm plot of amylopectin from waxy maize starch. The angular dependence was fitted using the Berry formulation.

The plot of molar mass (calculated from the measured excess Rayleigh ratio at each elution slice) *versus* elution volume (the so-called "calibration" curve of an SEC column) for the guar gum sample is shown in Fig 2. Superimposed thereon is the signal from the differential refractive index detector. The noisy appearance of the calculated molar mass at both peak front and tail is due to low signal-to-noise ratio from either the refractormeter (at peak front) or the MALS detector (at peak tail). In order to enhance the accuracy and reproducibility of the data, a polynomial (often linear under ideal SEC conditions) was used to fit the logarithmic molar mass *versus* elution volume. A least squares linear fit of the data based on the errors associated with each calculated molar mass was used to produce the superimposed line in Fig. 2.

Figure 3 shows the superimposed conformation plots for the hemi-cellulose and guar gum samples. These least squares weighted plots were generated using the calculated molar mass and corresponding rms radius for each elution slice. The slope of the guar gum sample (the curve further to the right) is calculated as 0.57, which corresponds to a random coil structure, whereas the slope of the hemi-cellulose is only 0.40, indicative of a highly branched structure[3)].

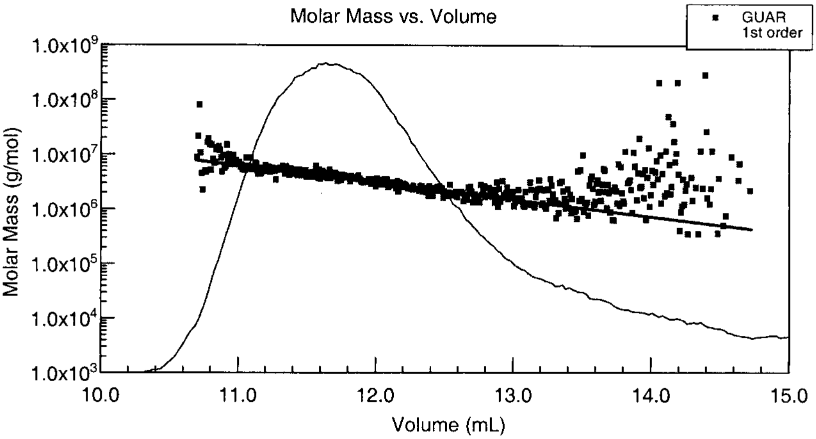

Figure 2. Calculated molar mass *versus* elution volume for guar gum sample. The solid line is the linear fit to the experimental error-weighted measured molar masses.

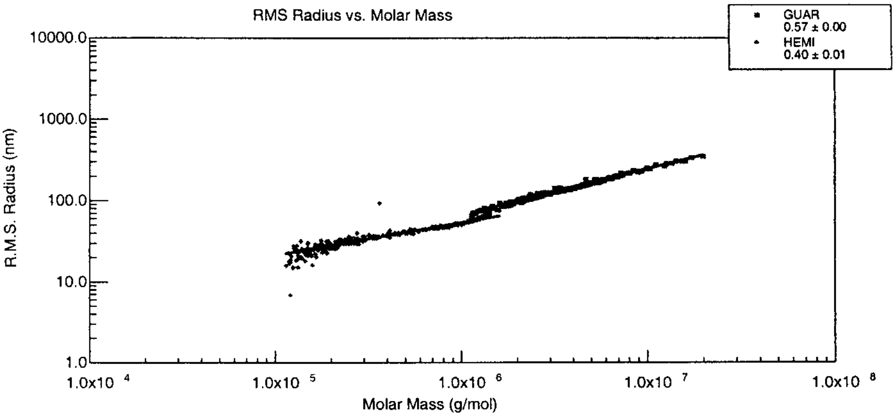

Figure 3. Conformation plots for hemi-cellulose and guar gum.

The calculated differential weight fraction distributions, dW(M)/dlog$_{10}$M, for the hemi-cellulose and guar gum samples are plotted against the logarithm base 10 of the molar mass in Fig. 4. Such distributions represent the most powerful means for comparing samples in terms of their mean molar mass and polydispersity. The distributions are generally plotted on a semi logarithmic scale to conform to the expected linear separation produced by SEC columns. For such linear separations, the resultant distributions with the log$_{10}$ M scale reversed would appear the same as the traces from a concentration detector.

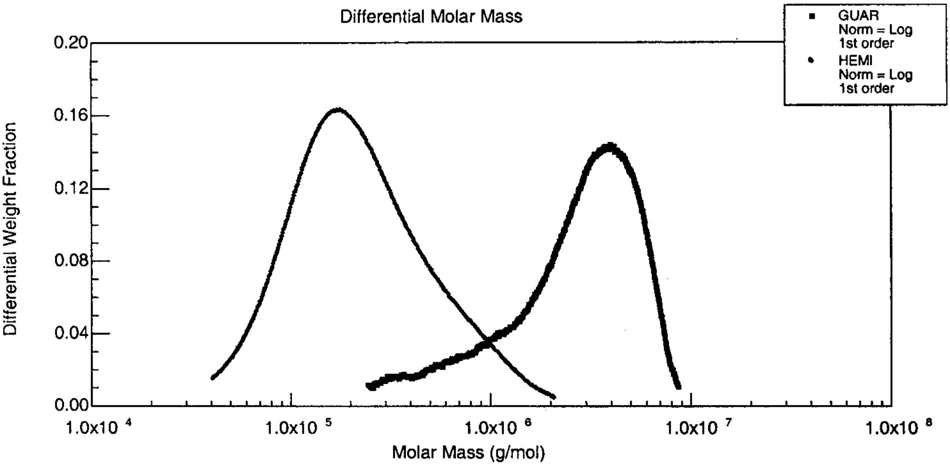

Figure 4. Differential weight fraction distributions for the hemi-cellulose and guar gum samples whose conformations are shown in Fig. 3

Conclusions

The examples above are typical of food polymers separated by SEC (or FFF) and then followed by MALS measurements. For such macromolecules, the combination of MALS with various fractionation techniques permits the absolute characterization of their molar mass and size distributions. In an unfractionated sample, the weight average molar mass, second virial coefficient, and light scattering average square radius may be obtained over an extremely broad range of molar masses by conventional MALS techniques.

As a powerful fractionation procedure, FFF is only now being explored [8, 13-20]. The long delay in trying such methods, especially for very large molecules subject to shearing in conventional SEC columns, relates more to the historic reluctance [20] of laboratories to combine such techniques with MALS rather than any shortcomings of the separation techniques themselves. The trouble with FFF from a historic point of view relates to the theoretical basis of these techniques which claim to be able to determine hydrodynamic size directly from the channel set-up conditions and the subsequently measured times of elution. Such determinations have been found to be lacking in a variety of aspects. The determination of molar mass, as opposed to hydrodynamic radius, from such theory suffers from the same problems as conventional, calibration dependent SEC. The addition of MALS detection following fractionation by FFF has made a major impact on the analytical results achievable. Much more significant data will certainly become available within the near future as such techniques be further exploited.

Many applications to the analysis of macromolecules of importance as food additives by the use of MALS are being reported frequently. A weekly updated bibliography may by found on the World Wide Web pages: http://www.wyatt.com.

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