Determination of the Absolute Molecular Weight Averages and Molecular Weight Distributions of Alginates Used as Ice Cream Stabilizers by Using Multiangle Laser Light Scattering Measurements

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High-performance size exclusion chromatography with multiangle laser light scattering detection (HPSEC-MALLS) was used for characterizing complete molecular weight distributions for a range of commercial alginates used as ice cream stabilizers. For the samples investigated, molecular weight averages were found to vary between 115 000 and 321 700 g/mol and polydispersity indexes varied from 1.53 to 3.25. These samples displayed a high content of low molecular weights. Thus, the weight percentage of material below 100 000 g/mol ranged between 6.9 and 54.4%.

Keywords: Alginates; molecular weight; multiangle laser light scattering (MALLS); ice cream

INTRODUCTION

Alginates occur as the major structural polysaccharides of marine brown algae. They constitute a family of unbranched binary copolymers of 1-4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. The physical properties of alginates depend on their uronic acid composition and on their molecular weight averages and molecular weight distributions (Gacesa, 1988).

The suppliers often propose alginates as ice cream stabilizers. Such additives are incorporated in ice cream formulations to improve the stability and eating texture of the product. During the distribution, storage in the retail chain, and consumer use, ice cream products can be exposed to considerable fluctuations in temperature. Stabilizers are claimed to protect the ice cream from such temperature shock by both retarding or reducing ice crystal growth and maintaining product integrity by inhibiting serum separation (whey off). Stabilizers can confer structural integrity to the ice cream product and thus minimize the changes of shape induced by temperature variations. Furthermore, the use of stabilizers gives a thick body to ice cream texture, and so contributes toward the creaminess of the product (Onsoyen, 1992).

To reach a better understanding of the relationship between the structure and the functionality of alginates as ice cream stabilizers, it was decided to carry out a basic study. However, before investigating the functionality of alginates in ice cream products, structural features needed to be established. The M/G ratio and the monomer sequence distribution in the copolymer were determined in a previous study (Grasdalen et al., 1979). Molecular weight is another fundamental parameter characterizing such macromolecules. The knowledge of the molecular weights of polysaccharides is of fundamental importance for the understanding of the relationships between their structure and properties in a large number of food applications (Sandford and Baird, 1983).

The most widespread technique of characterizing these structural features is size exclusion chromatography (SEC), whereby the sample molecules are separated by size as they pass through the gel-filled column. In general, the sample concentration as a function of retention volume is measured using a concentration-sensitive detector, such as a differential refractive index detector (DRI), and a calibration curve for the chromatographic system is then used to derive the molecular weight distribution of the sample. The calibration curve for a given system is ideally found by injecting a series of standard samples of known molecular weight distribution. However, for the curve to be valid, the calibration standards used should have the same conformation and chemical structure as the samples to be analyzed (Yau et al., 1979). In many cases this is neither practical nor possible, especially for new and unknown polymers. As a result, the conversion of the sample distribution, measured in terms of elution volume, to a molecular weight distribution is at best an approximation and, at worst, misleading. Light scattering provides a method of determining absolute molecular weight and size distributions directly (Debye, 1944).

In the present work, molecular weight averages and molecular weight distributions of a set of commercial alginates are investigated by using multiangle laser light scattering (MALLS) measurements.

MATERIALS AND METHODS

Materials. The alginates were commercial samples obtained from different suppliers: Pronova Biopolymer (Protanal SP5H, SF 120 L, 10/40, LF 120 L, SF 120 M, LF 200, and MIA 95), The Nutrasweet Kelco Company (Mannucol DMF), Danisco ingredients (Danisco 750), and SKW Biosystems (Satialgine S 1100).

Sample Preparation. Commercial alginates (3 mg/mL) were dispersed in 0.1 M NaCl and stirred for 60 mn at room temperature. Then, the samples were filtered through a 0.2
The excess Rayleigh ratio, \( R(\theta) \), which describes the scattering after the contribution of the pure solvent is subtracted, is the light scattered by the pure solution at an angle \( \theta \) in excess of that scattered by the pure solvent, divided by the incident light intensity; it can be expressed as:

\[
KC/R(\theta) = 1/M_w P(\theta)/2A_2
\]  

(1)

The excess Rayleigh ratio, \( R(\theta) \), which describes the scattering after the contribution of the pure solvent is subtracted, is the light scattered by the pure solution at an angle \( \theta \) in excess of that scattered by the pure solvent, divided by the incident light intensity; \( M_w \) is the weight average molar mass, \( A_2 \) is the second virial coefficient; \( K \) is an optical constant function of the specific refractive index increment \( \mathrm{dn/dc} \). Finally, \( P(\theta) \) is the form factor which describes the scattered light's angular dependence \( P(\theta) \sim \sin^2(\theta/2) \), from which the mean square radius of the molecules, \( r^2 \), may be determined.

According to eq 1, plotting \( R(\theta)/KC \) against \( \sin^2(\theta/2) \) will yield values of \( M_w \) and \( r^2 \) from the intercept and the slope. This plot is called a Zimm plot.

Molecular masses calculated from first- and second-order Debye and first-order Zimm gave comparable results. Therefore, the data were analyzed with the first-order Zimm equation for consistency and to save time.

Two important parameters needed to obtain accurate molecular weight information are \( \mathrm{dn/dc} \) and \( A_2 \). The \( \mathrm{dn/dc} \) is necessary for determining the absolute quantities of material eluting at each volume increment. This information is needed for determining the weight fractions and for extrapolation of the light-scattering data to zero concentration (eq 1, Zimm plot). \( A_2 \) describes the interaction between the solvent and the polymer chain. If the polymer–solvent interactions are large, the polymer coil will expand and the solvent is considered good (\( A_2 \) is positive). If the solvent is poor, polymer–polymer forces dominate causing the polymer chain to collapse either inter- and/or intramolecularly. This parameter is especially important when using poly electrolytes where repulsive forces play an important role. \( A_2 \) is important when calculating absolute molecular weights by defining the slope of the extrapolation in the Zimm plot from known concentration of polymer (obtained from RI measurement and \( \mathrm{dn/dc} \) to zero concentration). The \( \mathrm{dn/dc} \) used for alginate in 0.1 M NaCl is 0.150, as reported by Mackie et al. (1980). The \( A_2 \) of alginate in 0.1 M NaCl was taken from the literature as \( 7 \times 10^{-3} \, \text{mL/mol g}^{-2} \) (Martinsen et al., 1991).

Polystyrene standards, molecular weights and polydispersity indexes are generally sensitive to the peak region selected. The technique used to make peak selection consistent was as follows:

- Make peak start and end marks at positions where the signals from the concentration detector are 3–5 times that of baseline noise.
- Keep peak start and end elution volumes constant under conditions where the peak elution volumes are similar.

The reproducibility of data obtained was tested by multiple injection of the same sample. The values for the number- \( (M_n) \), weight- \( (M_w) \), and Z- \( (M_z) \) average molecular weights, polydispersity index \( (M_w/M_n) \), and peak \( M_w \) only varied from 5 to 9% between the different runs. Finally, uncertainties were estimated experimentally by Wyatt’s ASTRA program by determining the statistical fluctuation in each detector’s signal, including all photodiodes and the RI signals. For each sample, the uncertainty was below 3%.

RESULTS AND DISCUSSION

Using HPSEC, alginate chains were separated according to their hydrodynamic volume. This volume depends on the hydration shell, polysaccharide structure, and conformation. Moreover, for poly electrolytes such as alginates, the chain extension largely depends on polymer–solvent interactions. Therefore, the eluant conditions used, 0.1 M NaCl at 25 °C, were chosen to prevent aggregation, ensuring that \( M_w \) values of individual chains only are measured.

Figure 1 shows chromatograms obtained from the DAWN 90° detector and the refractometer for the sample Protanal SF 120 L in 0.1 M NaCl at 25 °C. A plot of molecular weight versus volume for the sample Protanal SF 120 L in 0.1 M NaCl at 25 °C is shown in Figure 2. As expected for a broad sample separated by the HPSEC system, the molecular weight decreased over the elution volume from \( \sim 16 \) to \( \sim 22.0 \, \text{mL} \). The amount of scatter in molecular weight data increased considerably in the range of elution volumes between \( \sim 21.5 \) and \( \sim 24.0 \, \text{mL} \). This phenomenon is probably due to the combination of low sensitivity of MALLS detector for small sized molecules, decreases in Rayleigh scatter with particle size, and low quantity of material...
present, making the system too dilute to be measured accurately (Fishman et al., 1996).

By combining RI data and the slope of the molecular weight versus volume, the differential molecular weight distribution was calculated for the sample Protanal SF 120 L in 0.1 M NaCl at 25 °C (Figure 3). This plot shows how much material is contained in any molecular weight interval. For the sample Protanal SF 120 L, the peak M_w approaches 225 000 g/mol.

Figure 4 displays the cumulative molecular weight distribution. This distribution gives, for each molecular weight, the weight fraction of material having molecular weight less than the given weight. Thus, the cumulative distribution approaches zero at low molecular weights, and unity at high molecular weights. This plot is particularly useful in determining what molecular weights are contained in the high and low molecular tails of the sample.

For sample Protanal SF 120 L (Figure 4), the low 10% weight fraction is below 53 600 g/mol, while the high 10% fraction is above 404 000 g/mol.

The molecular weight distributions of several commercial alginates currently used as ice cream stabilizers were investigated using the HPSEC-MALLS system. Relevant molecular parameters are given in Table 1, and molecular weight distribution plots (Figures 5 and 6) were calculated. These results correlate well with published data (Martinsen et al., 1991; Horton et al., 1991). As shown in Table 1 and Figures 5 and 6, M_w averages (from 115 000 to 321 700 g/mol) and M_w distributions of commercial alginates vary to a large extent. These variations can be due to the natural algal source or can be induced by seasonal, environmental, and/or post-harvesting effects or by process conditions.

Figures 5 and 6 show the wide molecular weight distributions of these samples and the large amount of low molecular weight tails. The large molecular weight distributions were corroborated by polydispersity in-

Table 1. Relevant Molecular Parameters of Several Commercial Alginates

<table>
<thead>
<tr>
<th></th>
<th>Danisco 750</th>
<th>Protanal LF 200</th>
<th>Mannucol DMF</th>
<th>Protanal SP5H</th>
<th>Protanal SF 120 L</th>
<th>Protanal 10/40</th>
<th>Protanal LF 120 L</th>
<th>Protanal SF 120 M</th>
<th>Protanal MIA 95</th>
<th>Satialgine S 1100</th>
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</thead>
<tbody>
<tr>
<td>M_n 10^{-3}</td>
<td>132.4</td>
<td>139.2</td>
<td>93.9</td>
<td>106.7</td>
<td>110.5</td>
<td>61.9</td>
<td>169.6</td>
<td>103.6</td>
<td>56.9</td>
<td>200.6</td>
</tr>
<tr>
<td>M_w 10^{-3}</td>
<td>219.5</td>
<td>212.6</td>
<td>182.7</td>
<td>187.9</td>
<td>204.6</td>
<td>115.0</td>
<td>207.7</td>
<td>221.1</td>
<td>184.9</td>
<td>321.7</td>
</tr>
<tr>
<td>M_z 10^{-3}</td>
<td>303.9</td>
<td>285.1</td>
<td>278.3</td>
<td>276.1</td>
<td>304.4</td>
<td>157.9</td>
<td>402.6</td>
<td>335.2</td>
<td>289.5</td>
<td>429.5</td>
</tr>
<tr>
<td>M_w/M_n</td>
<td>1.66</td>
<td>1.53</td>
<td>1.95</td>
<td>1.76</td>
<td>1.86</td>
<td>1.86</td>
<td>1.86</td>
<td>1.70</td>
<td>2.14</td>
<td>1.60</td>
</tr>
<tr>
<td>Peak M_w 10^{-3}</td>
<td>265.0</td>
<td>254.4</td>
<td>209.5</td>
<td>205.8</td>
<td>225.1</td>
<td>110.0</td>
<td>334.5</td>
<td>281.7</td>
<td>266.1</td>
<td>363.0</td>
</tr>
<tr>
<td>% &lt; 100 000</td>
<td>20.4</td>
<td>19.0</td>
<td>31.9</td>
<td>29.2</td>
<td>26.8</td>
<td>54.4</td>
<td>14.4</td>
<td>26.0</td>
<td>39.9</td>
<td>6.9</td>
</tr>
</tbody>
</table>
molecules were characterized by a weight percentage material below 100,000 g/mol varying between 6.9 and 54.4%. The next step of this study will attempt to establish the existing relationships between the structural features of the alginates and their ability to stabilize ice cream products.

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LITERATURE CITED


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