

**Strategies for Food Quality Control
and
Analytical Methods in Europe**

CHARACTERIZATION OF COMPLEX POLYSACCHARIDES BY SIZE EXCLUSION CHROMATOGRAPHY WITH VISCOSITY DETECTION

Summary

Complex polysaccharides obtained from plants and microbes are finding increased application in the food industry as additives to improve functional properties of processed foods. High performance size exclusion chromatography (SEC), concentration and viscosity detection, and gaussian curve fitting have successfully elucidated the chemical and physical behavior of pectin in solution. This method has now been applied to gum tragacanth, gum locust bean, carboxymethylcellulose (CMC), alginates, gum arabic, and apple pectin. Weight average intrinsic viscosities were determined directly from areas under the concentration and pressure curves. In addition, global and component R_g 's and molecular weights (MW in kda, kilodaltons) have been determined from universal calibration with dextrans and pullulans. SEC with concentration and viscosity detection has good potential for either rapid identification of gums or for determining physical properties critical to quality control of polysaccharides in the food industry.

Introduction

Recent studies of size exclusion chromatography (SEC) of pectin using both concentration and viscosity detection have suggested that pectin chromatograms can be analyzed in terms of a few gaussian components (1-3). Global values for radius of gyration (R_g), intrinsic viscosity (η), and molecular weight (MW) can be calculated from component properties determined by universal calibration (3). We have extended this analysis to some polysaccharide gums of interest to the food industry with the goal of applying SEC with concentration-viscosity detection to uniquely characterize these biopolymers in small volumes of dilute aqueous solution. In addition, such analysis could also be useful in following subtle changes in polysaccharides in solution that are caused by heating, micro-waving, aging, chemical modification, enzymatic degradation, or adulteration.

Methods

Waters μ -Bondapak E-HighA, E-1000, and SynChrom Synchronapak GPC-100 columns were used in series at 35° C. with 0.05 M NaNO_3 mobile phase at a nominal flow rate of 0.5 ml/min. The SEC system used DP (viscosity) and RI detectors in series and was calibrated with pullulan (narrow) and dextran (broad) standards of known R_g , η , and MW (3). Every R_g , η , and MW value in this report is a weight average. The band-spreading parameter, sigma, was determined by fitting a narrow pullulan peak to one gaussian curve. This sigma was then used in subsequent fittings for gums. Peak positions for component elution volumes were initially separated by 2x sigma. The final peak position of each component was converted to a value for R_g and, for a viscosity peak, the value for (MW x η) through universal calibration (3). The ratio of viscosity component height to concentration component height was related to η by a factor determined with pullulan standards. MW was then calculated from the experimentally determined values for component R_g and component intrinsic viscosity. Equation {1} was fitted to the component data for 3 alginates, CMC and gum tragacanth.

$$[\text{MW}][\eta] = [84.6][R_g]^{2.68}$$

Results and Discussion

One resolved concentration chromatogram for gum locust bean is reconstructed in Fig. 1. The viscosity chromatogram always resolved into fewer matching components since later eluting concentration component material did not exhibit detectable viscosity. Because R_g depends on component peak height position, an accurate flow rate is critical for determination of K_{av} . The calculated properties of the polysaccharides analyzed are in the Table.

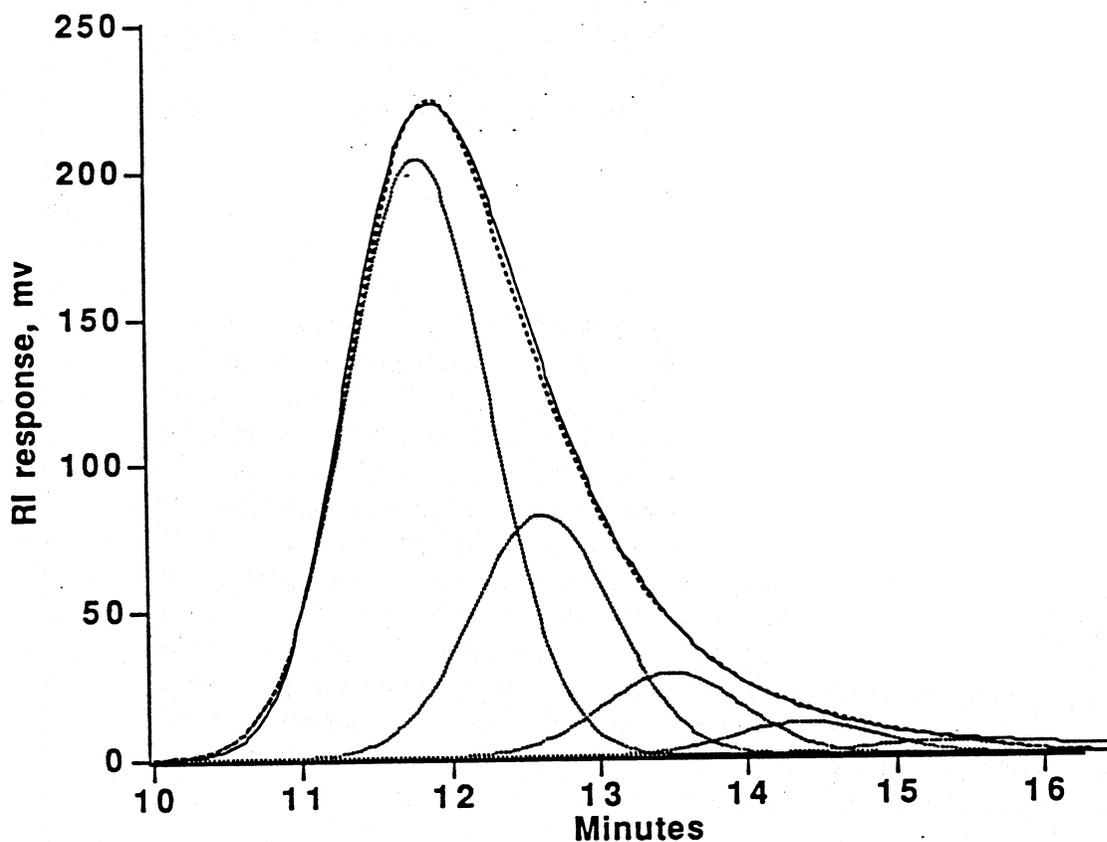


Fig. 1: Fit (long dashed line) of 5 gaussian components (short dashed line) with sigma values of 0.445 min. to size-exclusion concentration chromatogram (solid line); 35° C., 0.05 M NaNO₃ mobile phase, gum locust bean.

Gum Tragacanth: The SEC chromatogram was resolved into 6 concentration and 4 viscosity components (Table). A MW of 413 kda, global eta of 7.2 dl/g and global R_g of 46.8 nm were obtained. Gum tragacanth is a complex, acidic polysaccharide with high molecular weight (840 kda) and high viscosity reported for one purified fraction (4). Extended stiff chains of this fraction have dimensions of 450 nm by 2 nm (4). Commercial gum tragacanth is usually blended to provide specified properties (5).

Table. Number of component gaussian peaks from SEC of polysaccharides and calculated weight averages for R_g, eta, and molecular weight.

Polysaccharide	Number of RI	components DP	R _g nm	Eta dl/g	MW kilodaltons
Gum Tragacanth	6	4	46.8	7.2	413
Gum Locust Bean	5	3	47.2	9.9	297
Carboxymethylcellulose	6	4	35.0	12.2	134
Alginate IV	5	3	31.3	17.6	79
Alginate VI	5	3	31.9	10.0	99
Alginate VII	5	3	26.9	6.4	99
Apple Pectin	5	3	16.3	1.36	119
Gum Arabic	3	1	13.8	0.13	526

Gum Locust Bean: 5 Concentration components, shown in Fig. 1, and 3 viscosity components were obtained (Table). An eta of 9.9 dl/g at 35° C. is at the low end of the range of eta from 9.9 to 15.7 dl/g at 25° C. reported for a series of galactomannans (6). The MW of 297 kda (Table) agrees with one literature value of 310 kda (6). Our results with gum locust bean, which is known to vary in properties, are consistent with MW and eta values obtained for purified galactomannans (6). As shown in Fig. 1, gum locust bean appears to contain a small amount of R_g (2.5 to 5.0 nm)-component-5. A small component also appears in pectin chromatograms (2). Galactomannans have an extended β-mannan conformation that promotes high viscosity without gel formation (6). The presence of a small component fraction may derive from short chain galactomannan oligomers (MW < 2000) in this gum.

Carboxymethylcellulose (CMC): 6 Concentration components and 4 viscosity components were found (Table). The MW of 134 kda is within the range of 100-500 kda for CMC and the eta of 12.2 dl/g establishes that the CMC tested has high viscosity (7). The food industry could benefit by application of SEC with viscosity detection to the quantitative characterization of CMC in terms of R_g, eta, and MW, as well as the polydispersities of these properties. The R_g and eta values for CMC in the Table reflect the known extended, inflexible conformation associated with β-glucans.

Sodium Alginates: The commercial alginates analyzed have decreasing η_{sp} in the order IV > VI > VII (17.6 to 6.4 dl/g, Table). This decrease appears to be directly related to decreasing R_g rather than to MW (Table). Freshly extracted sodium alginates from various algae have η_{sp} from 12 to 16 dl/g, 0.2 M NaCl, 25° C. , and MW's from 143-351 kda (8). Commercial alginates have MW's from 32 to 200 kda (9). Further processing, such as freeze-drying, reduces both η_{sp} and MW (8). In this case MW was determined by light scattering and by sedimentation, with good agreement, and η_{sp} was determined by standard capillary viscosity methods (8). The major strength of SEC with viscosity detection is that R_g , η_{sp} , and MW can be co-determined in a few hours with a minimum of 2 to 10 ml of dilute (0.5 to 2%) solution. The sensitivity is limited by the viscosity.

Apple Pectins: The 5 concentration components and 3 viscosity components found for apple pectin is consistent with results obtained with other pectins (3). The η_{sp} of 1.36 dl/g at 35° C. (Table) is within η_{sp} values of from 1.2 to 2.4 dl/g reported for apple pectins at 28 to 40° C. (10). The results with apple pectin quantitatively show that commercial apple pectin has less viscosity and is lower in molecular weight than grapefruit pectins (3). SEC with viscosity detection offers a rapid and flexible means of quantitative comparison of pectins from different sources and subjected to different treatments (2).

Gum Arabic: The SEC chromatogram was resolved into 3 concentration and 1 viscosity components (Table). A MW of 526 kda, η_{sp} of 0.13 dl/g and global R_g of 13.8 nm were obtained. Values of 500 kda for MW (11) and 0.20 dl/g for η_{sp} (25° C.) have been reported (12). The SEC results are consistent with a high molecular weight polysaccharide with a compact shape, such as a sphere or a tight, stiff spiral, that exhibits low viscosity (11). Of all the polysaccharides tested, gum arabic had the most narrow peak with the highest weight fraction (0.83) in one concentration component.

Because SEC separates polysaccharides predominantly on the basis of size, usually specified in terms of R_g , there are instances when different polysaccharides present similar R_g 's and quite different intrinsic viscosities. These conditions bring into focus unusual cases in which at constant R_g , polymers exhibit an inverse relationship between MW and η_{sp} as shown by equation (1). In order for a small, uncharged polymer to have an appreciable intrinsic viscosity, an extended, relatively inflexible conformation is required.

Conclusions

Size exclusion chromatography (SEC) with viscosity detection offers a rapid, flexible means of characterization of soluble polysaccharides used throughout the food industry. Quality control, processing changes, and detection of adulteration are some potential areas in which SEC with viscosity detection can have a significant application. Costs for determination of R_g , η_{sp} , and MW of polysaccharides by SEC in terms of time and instrumentation can be greatly reduced when compared to light scattering, sedimentation, and traditional viscometers that require serial dilutions. SEC is performed with small volumes of dilute solutions that can be pre-conditioned with guard columns. SEC systems are currently being strengthened by the development of light scattering detectors, which will provide independent determination of MW.

References

1. M. L. Fishman, K. C. Gross, D. T. Gillespie and S. M. Sondey, *Arch. Biochem. Biophys.* **274** (1989), 179
2. M. L. Fishman, Y. S. El-Atawy, S. M. Sondey, D. T. Gillespie and K. B. Hicks, *Carb. Polym.* **15** (1991), 89
3. M. L. Fishman, D. T. Gillespie, S. M. Sondey, and Y. S. El-Atawy, *Carb. Res.* **215** (1991) in press
4. N. Gralén and M. Kärholm, *J. Coll. Sci.* **5** (1950), 21
5. G. Meer, W. Meer and T. Gerard in *Industrial Gums*, R. Whistler, ed. (Academic Press, New York, 1993, 2nd ed.) 291
6. C. M. Dea, A. H. Clark and B. V. McCleary, *Food Hydrocolloids* **1** (1986), 129
7. J. B. Baldorf and J. M. Rossman in *Industrial Gums*, R. Whistler, ed (Academic Press, New York, 1993, 2nd ed.) 704
8. D. J. Wedlock, B. A. Fasihuddin and G. O. Phillips, *Food Hydrocolloids* **1** (1987), 207
9. R. H. Walter and R. M. Sherman, *Food Hydrocolloids* **2** (1988), 151
10. A.-M. Sjöberg, *Food Hydrocolloids* **2** (1987), 271
11. S. M. Blake, D. J. Deeble, G. O. Phillips and A. DuPlessey, *Food Hydrocolloids* **2** (1988), 407
12. D. M. W. Anderson and S. Rah, *Carb. Res.* **4** (1967), 298