Mathematical Discretization of Size-Exclusion Chromatograms Applied to Commercial Corn Maltodextrins

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Abstract

Discretization of a size-exclusion chromatography (SEC) chromatogram is shown here to be an important calculation for characterizing the distribution of a polydisperse polymer, especially when the polydispersity is large. Commercial poly-glucose maltodextrins are known to have such a polydispersity. A mathematical discretization method with Gaussian peaks centered on each individual degree of polymerization is proposed and is performed on the entire SEC chromatogram for three different grades of corn maltodextrins. Because SEC and high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD) are based on different separation mechanisms, they can be considered orthogonal techniques, and HPAEC–PAD was therefore used to validate the SEC discretization procedure. Because this validation proved satisfactory for all commercially available oligomers, the discretization is extended to all of their SEC chromatograms. Comparing the number-average molar weight and the weight-average molar weight before and after the mathematical discretization verifies that such a mathematical treatment does not denaturate the chromatogram. This approach tentatively leads to a more exhaustive characterization of a broadly polydisperse sample, such as maltodextrins, than was previously available, as it (*i*) gets rid of the apparent, chemically irrelevant, continuous molar weight distribution obtained by raw SEC and (*ii*) addresses the current detection and quantitation limits of the HPAEC–PAD technique without any sample treatment.

Introduction

Size-exclusion chromatography (SEC) is a powerful tool for polymer sample characterization, as it gives access to average molar weights and polydispersity estimations and shows a graphical representation of the molar weight distribution. Because of the well-known low resolution attained by current SEC columns, highly polydisperse polymers produce an apparently continuous distribution of molar weights, irrespective of the species' real weights, which cannot differ by less than a monomer weight (1). This apparent continuous distribution is caused by diffusion phenomena of the sample inside the column, which causes unavoidable chromatographic peak broadening, which are, as of today, the major limitation of SEC.

Maltodextrins are, by definition, a polydisperse mixture of glucose-oligomers obtained by degradation of native starch, which therefore contains any oligomeric size from the single monomer to degrees of polymerization (DP) over 10,000. In recent studies, efforts have been made to draw relationships between the molar weight distributions and physical properties of such materials. For example, the intrinsic viscosity is related to the chromatogram of mass distribution through the well-known Mark–Houwink–Sakurada equation, in which the distribution is characterized using the viscosimetric average molar weight for broad mass distributions (2) or weight average molecular mass (M_w) for narrow mass distributions (3,4). The radius of gyration and persistence length of maltodextrin samples was expressed as a function of the number average molar weight (M_n) (5). Furthermore, using calorimetry, it has been demonstrated that the heat capacity for the rubber to glass transition of maltodextrin–water systems was directly correlated to the M_n (6), whereas the glass transition temperature of dry maltodextrin samples was related to the chromatogram of mass distribution characterized by a single average molar weight (2). The determination of this characteristic average molar weight necessitates the determination of the molar fraction of each chemical species present in the maltodextrin sample, from the smallest molar weight (glucose) to the largest molar weight expressed.

Furthermore, a discretized molar weight distribution profile, in which only the molar weights corresponding to chemical species really present in the sample appear, would not only be more chemically meaningful but also provide the additional information of the total number of moles in the polymer sample.

Another chromatographic technique, namely high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) (7) is applicable to maltodextrins. This technique offers unrivalled resolution, as baseline-resolved peaks corresponding to definite oligomeric units are obtained,

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but it is unfortunately limited to low molar weight species (e.g., < DP20). For a quantitative determination, its working range is even more limited, as it requires appropriate high-purity standards for calibration, which are commercially available only up to DP7. Extension of this working range has been the aim of many recent works, and among these, a quantitation of the oligomers up to DP77 has been realized by introducing an amyloglucosidase post-column reactor to the system (8,9). However, although interesting, this refinement still only delivers a partial image of maltodextrins because DPs in the range of 77–10,000 are still ignored in the case of maltodextrins. Besides, because this analysis is partial, it does not allow the total number of molecules in the sample to be determined and, therefore, does not allow the determination of individual molar fractions for each oligomeric species.

In the present paper, an alternative approach to sample derivation procedures is discussed, either pre- or postcolumn, based on a mathematical discretization of the SEC chromatogram adapted to broad molecular weight distribution of oligomers and polymers having identical repeating units.

Experimental

Samples

Maltodextrins of different hydrolysis degrees (2DE, 10DE, and 19DE) were supplied by Roquette Frères (Lestrem, France). Molar weight distributions were determined by SEC of a 1-g/L solution of the different maltodextrin samples. Quantitation of DP1 to DP6 oligomers was realized with HPAEC–PAD.

Molar weight distribution (SEC)

System

The chromatographic system consisted of three Shodex (JM Science, Grand Island, NY) (8 × 300 mm) OH-Pak columns (SB-802.5, SB-803, and SB-804) put in series after a SB-G (6 × 50 mm) guard column, with claimed exclusion limits for pullulans of 10^4, 10^5, and 10^6 Da, respectively. The eluent was a 0.05M solution of sodium triazide (NaN_3, 99.99%) (Aldrich, Steinheim, Germany), at a flow rate of 0.5 mL/min. The instrument was an Agilent 1100 tower, consisting of a G-1311A quaternary pump, a G-1313A autosampler, and equipped with a G-1322A vacuum degasser (Agilent, Waldbronn, Germany). The injected volume was 100 µL. All measurements were made at room temperature. Detection was made with a Merck RI-71 differential refractometer (K_{RI} = 0.5468) (Merck, Darmstadt, Germany). Reproducibility of the method appeared to be acceptable (standard deviation on retention time at peak maximum was 0.04%, on total chromatogram area < 3% for five injections).

Calibration

The calibration was performed with pullulan and maltooligomer standards of molar weights ranging from 342 to 788,000 Da fit with a classical third order polynomial (R^2 = 0.9997), which appeared only slightly distorted compared with a simple first order regression as used for the discretization. The first order regression also gives a satisfactory fit (R^2 = 0.9860).

Samples

Maltose (5911.1000, Merck), maltotriose (> 95%, M-8378, Sigma, St-Louis, MO), maltotetraose (98%, 35102, Acros Organics, Geel, Belgium), maltopentaose (98.5%, 23.324.44, Acros Organics), maltohexaose (> 90%, 63416, Fluka, Buchs, Switzerland), and P-82 shodex pullulan standards (10401, Showa Denko, Düsseldorf, Germany) of 5,900, 11,800, 22,800, 47,300, 112,000, 212,000, 404,000, and 788,000 g/mol were used for completing the calibration.

Quantitation of DP1 to DP6 (HPAEC)

System

The device was a Dionex Corporation (Sunnyvale, CA) DX500 liquid chromatograph equipped with an ED40 electrochemical detector and made of a Dionex AS3500 autosampler, LC20 column enclosure, GP40 gradient pump, and PC10 pneumatic controller. The injection loop was 50 µL. The column consisted of an analytical CarboPac PA-100 (Dionex) (4 × 250 mm), which was run without a guard column. The acquisition software was Peaknet 5.1 (Dionex).

The detection waveform of the pulsed amperometric detector was that recommended for glucose oligomers by the device manufacturer, namely 50 mV from 0 to 0.4 s, 750 mV from 0.41 to 0.6 s, followed by an electrode cleaning step at –150 mV from 0.61 to 1 s. The signal integration occurred between 0.2 and 0.4 s.

The eluents used were a 1M sodium hydroxyde solution, 1M sodium acetate solution, and water, at a flow rate of 1 mL/min. Eluent program consisted in a linear gradient from an initial 90% water, 0% sodium acetate towards 50% water, 40% sodium acetate in 49 min, followed by a return to the initial conditions in 7 min. A level of 10% sodium hydroxyde was maintained constant during the entire elution.

Calibration

The calibration was made from dilutions of a stock solution of 0.1 g in 100 mL of the following sugars: glucose, maltose, maltotriose, maltotetraose, maltopentaose, and maltohexaose (DP1–6). Dilutions were made in order to obtain 5, 12.5, 25, 50, and 100 ppm solutions. An independant calibration curve was made for every sugar.

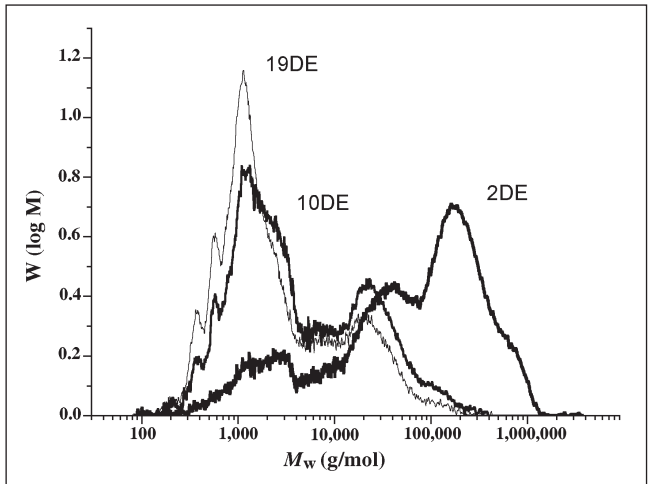

Figure 1. SEC chromatogram of 19DE, 10DE, and 2DE maltodextrins.

Sample solutions of 500 ppm were injected for the 10DE and 19DE maltodextrins, though 5,000 ppm were needed for the 2DE because of the larger portion of compounds under the limit of detection. Sample and calibration solutions were filtered through 0.45-µm syringe filter tips.

Samples

Sodium hydroxyde solution (50%, 7067, J.T. Baker, Deventer, Holland), sodium acetate (99%, 71185, Fluka, Buchs, Switzerland), glucose (99%, 49159, Fluka), and maltose (99%, 71185, Sigma, Steinheim, Germany) were used in addition to the already mentioned maltotriose to maltohexaose standards.

Results

Maltodextrins contained a broad distribution of molar weights, as expected. Figure 1 gives an idea of the polydispersity of the maltodextrins presented in this work. The chromatograms presented in Figure 1 were used as raw data, without smoothing in order to not bias the following calculations. The area under these chromatograms was normalized to unity.

Conventional approaches (such as Peakfit from Systat, Point Richmond, CA and Igor Pro from Wavemetrics, Lake Oswego, OR) use the secondary derivative method to localize the expressed molecular species. Exploitation of the size exclusion chromatograms when using these techniques reveals only 10 to 18 species for these three maltodextrin samples, whereas 500 to more than 1000 different expressed DPs are expected. Even if the reconvolution of the localized peaks gives a perfect fit, these treatments are not suitable for application of specific theoretical developments (2).

Therefore, the discretization of the chromatogram was carried out by a deconvolution into a summation of symmetrical Gaussian peaks centered on each DP according to chromatographic rules.

$$W_i = \frac{H_i}{M_i} \frac{1}{\int_0^\infty \frac{H}{M} d\log M} \qquad \text{Eq. 1}$$

here, W_i is the weight fraction of molecules, M_i is the molar weight, and H_i is the height of the signal from the baseline in the chromatogram. The factor under the integral is the normalization factor (e.g., the total amount of molecules in the chromatogram). Instead of showing up as narrow bands, diffusion phenomena occurring in chromatography force peaks to spread around the true elution volume in a Gaussian distribution, and the intensity observed results from the accumulation of neighboring peak contributions. The peak height response (H) can be expressed as in equation 2 [i.e., in a Gaussian form as a function of the elution volume (V)].

$$H = H_m \exp\left[-\left(\frac{V - V_m}{\sigma}\right)^2\right] \qquad \text{Eq. 2}$$

Here, σ is the standard deviation, and the subscript m is used for the position of the peak maximum.

Combining equation 2 with equation 1 and assuming a linear relationship between the elution volume V in equation 2 and log M (which corresponds to a first order calibration instead of the typical third order commonly used for SEC), equation 3 results in:

$$W_i = \frac{H_i}{M_i} \frac{1}{\int_0^\infty \frac{H}{M} d\log M} \exp\left[-\left(\frac{f(M) - f(M_i)}{\sigma_i}\right)\right] \qquad \text{Eq. 3}$$

$f(M)$ is the best fit for the SEC standard calibration curve between molar weights of the standards and their elution volumes. σ_i is the peak width. Optimization was done by successive iterations until a classical minimal least squares test between the calculated and the measured chromatogram was minimized. The best result was obtained for σ_i constant and equal to 0.09 for all the chromatograms tested. However, it should vary with the experimental setup. Besides, the purpose of this work is not to characterize the variability of the SEC parameters by modifying the experimental setup, but efforts are concentrated at valorizing the information contained in the resulting chromatogram.

The reconstructed chromatograms are presented in Figure 2 for the same samples as those in Figure 1 and show an apparently

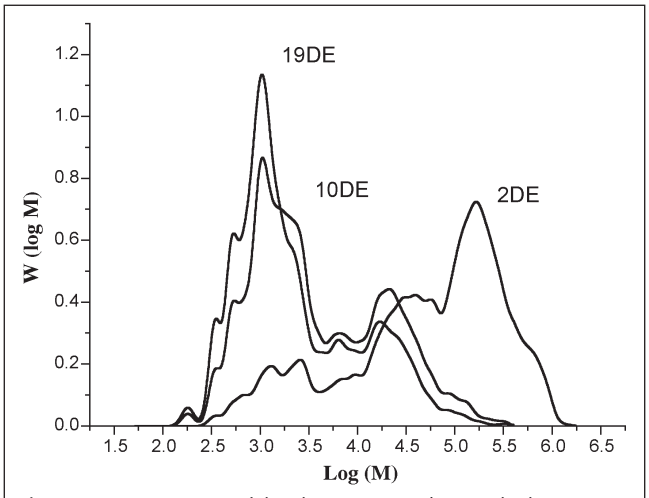

Figure 2. Reconstruction of the chromatograms by a multiple Gaussian summation.

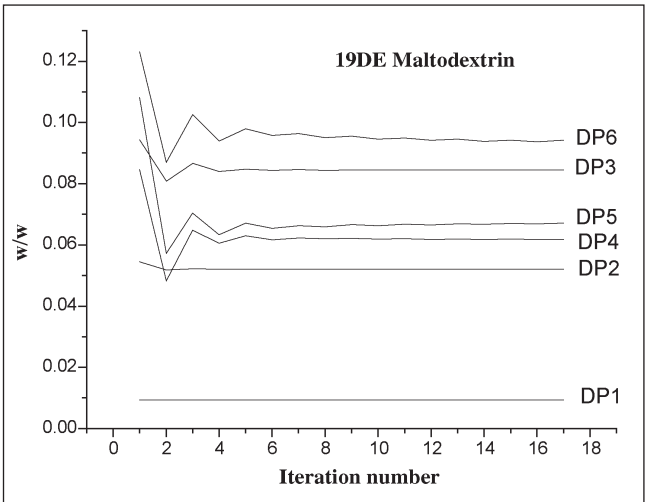

Figure 3. Variation of the peak area for DP1 to DP6 with the number of iterations for the 19DE maltodextrin during the least squares minimization.

smoothed curve because of the averaging of the distribution included around a given DP value by one average point only. For all chromatograms and all measured data point, the maximum absolute difference between experimental and calculated values was below 10^{-4} unit of amplitude. In order to generate the reconstructed chromatograms, several iterations are needed, however, it is surprising that the convergence is reached quickly and is stable for DP ≤ 10. Examples of the areas of the calculated Gaussian peak (representing the weight fraction of the considered DP) are shown in Figure 3 for DP1 to DP6 of the 19DE maltodextrin sample. As the area only oscillates between ± 4% of the final value within the first iterations of DP1 to DP6, the method seems satisfactory. The weight fractions obtained by the SEC discretization method are presented in Table I for the three maltodextrin samples.

The number of iteration required for stabilization increases with the number of chemical species present in the sample and was 17, 25, and 37 for the 19DE, 10DE, and 2DE, respectively.

Quantitation of DP1 to DP6 from the maltodextrin has been carried out with the HPAEC–PAD chromatograms for validation purposes as shown in Figure 4, and results for the three maltodextrins are summarized in Table II.

Table I. Quantitation Resulting from the Discretization of the SEC Chromatogram for DP1 to DP6*

		DP1	DP2	DP3	DP4	DP5	DP6
19DE	Area (%, w/w)	0.94	5.21	8.45	6.18	6.71	9.41
	SD† (%, w/w)	0	0.1	0.12	0.17	0.33	0.51
10DE	Area (%, w/w)	0.63	2.76	5.39	4.36	3.23	8.42
	SD (%, w/w)	0	0	0.08	0.16	0.28	0.67
2DE	Area (%, w/w)	$3.5.10^{-5}$	0.049	0.949	1.21	0.59	1.37
	SD (%, w/w)	0.02	0.0249	0.008	0.035	0.036	0.082

* Values are given in weight fractions.
† SD = standard deviation.

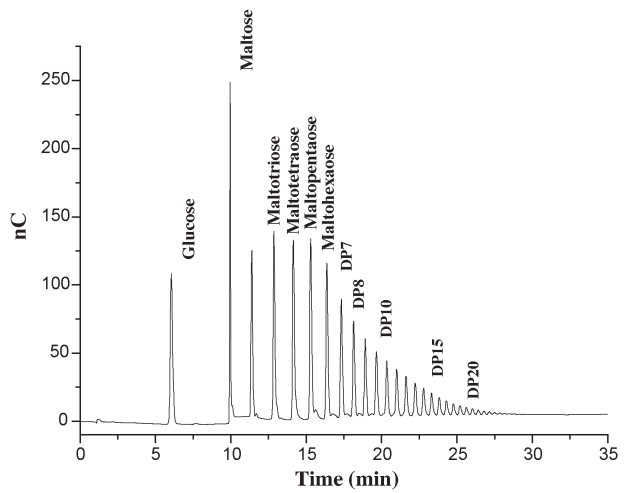

Figure 4. A typical HPAEC–PAD chromatogram for maltodextrin 10DE. DP20 is the limit of detection.

Table II. Quantitation Resulting from the HPAEC–PAD Results for the Quantitation of DP1 to DP6*

		DP1	DP2	DP3	DP4	DP5	DP6
19DE	Area (%, w/w)	0.93	5.30	7.83	5.66	7.11	8.51
	SD† (%, w/w)	0.02	0.2	0.11	0.12	0.07	0.10
10DE	Area (%, w/w)	1.42	2.44	3.74	2.98	4.16	5.21
	SD (%, w/w)	0.02	0.20	0.14	0.08	0.14	0.12
2DE	Area (%, w/w)	0.07	0.46	0.66	0.56	0.71	1.02
	SD (%, w/w)	0.01	0.01	0.03	0.01	0.01	0.02

* Values are given in weight fractions.
† SD = standard deviation.

Discussion

A comparison between the quantities mathematically derived from SEC data and the quantities measured by HPAEC–PAD is shown in Figure 5 for DP1 to DP6. A good correlation exists between the two sets of values. The slope of the regression is 1.05, close to 1.00 for perfect accordance. Because SEC and HPAEC are orthogonal techniques, this result suggests that the mathematical discretization of the SEC chromatogram could be extended towards larger DP numbers that cannot be directly quantitated by HPAEC–PAD.

A verification of the accuracy of this extension of the discretization to higher DP can be made through the average molar weights. The full SEC distribution is indeed characterized by the M_w and M_n. To validate the discretization method, the characteristic average molar weights derived from the discretized SEC chromatogram need to match those directly derived from the raw chromatograms. Figure 6 shows that the average molar weight values by raw and discretized SEC chromatograms are in excellent agreement.

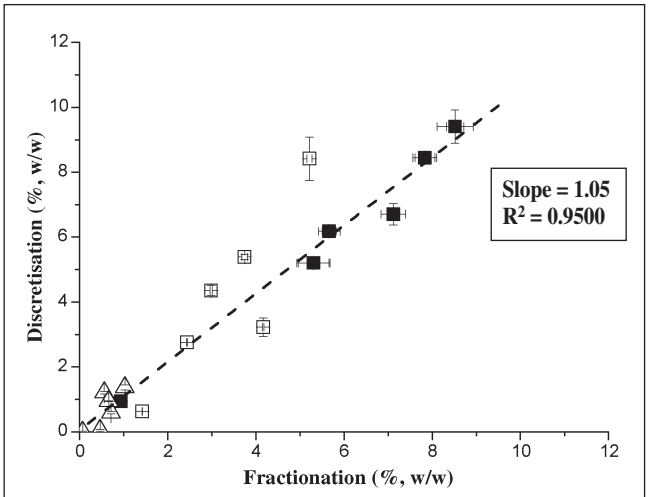

Figure 5. Comparison between the fractions obtained by mathematical discretization of the SEC chromatogram and the fractions measured by HPAEC–PAD for DP1 to DP6. Solid squares = 19DE, open squares = 10DE, and open triangles = 2DE.

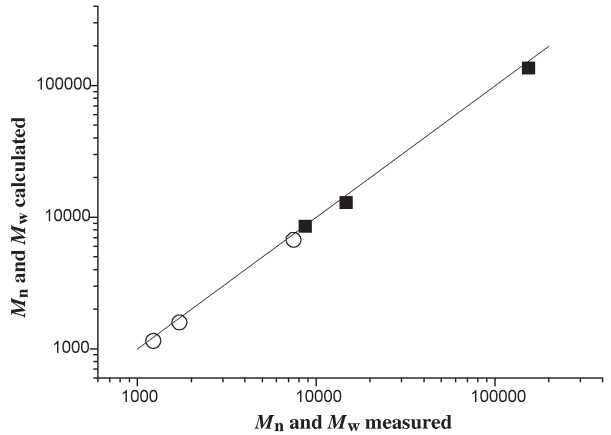

Figure 6. Comparison between the average molar weights obtained from the discretized and the raw SEC chromatograms. Open circles are number average molar weights (M_n) and solid squares are weight average molar weights (M_w). The line is the perfect accordance to guide the eyes.

Conclusion

As verified by HPAEC–PAD, the discretization of the SEC chromatogram delivers accurate weight fractions for DP1 to DP6 present in maltodextrin samples. When extending this calculation to the entire SEC chromatogram, the only parameters that can be compared with the untreated chromatogram for verification purposes are M_n and M_w. Because these were found to be similar before and after the treatment, we conclude that the discretization procedure was coherent. It allowed the determination of the total number of molecules present in the sample and, therefore, of the molar fractions of every individual oligomer present in addition to the weight fractions. Also, this approach bypasses the limits of detection and quantitation of HPAEC–PAD, which were too low to allow a proper characterization of broadly polydisperse materials such as maltodextrins. Finally, this approach delivered a chemically relevant description of the SEC chromatograms of such polydisperse systems and allowed less dependency on the SEC column choices and analysis conditions. Such an SEC discretization approach is original compared with available commercial software (such as PeakFit from Systat and Igor Pro from Wavemetrics), as they generally use Gauss-Lorentz functions and treat the chromatograms as distributions of peaks that do not necessarily reflect the real chemical composition of the sample but rather subjective groups of molar weights. With these approaches, the peak number cannot reflect the real number of chemical species present in the sample, unless the total number of chemical species is known *a priori*. Our approach is, therefore, both more objective as no *a priori* knowledge is required and less time-consuming, as it directly delivers a molar weight distribution that reflects the real chemical composition of the sample, the number of moles present in solution, and the molar fraction of each individual oligomer in the sample in a single analysis. This discretization of the raw chromatographic results was necessary to link the molecular weight distribution of various maltodextrin grades to their glass transition temperature (2). Further verifications by comparison of the total number of moles should be done, as well as extensions of this model to other polydisperse samples of varying monomer sizes to assess the limits of the peak deconvolution algorithm.

Acknowledgments

Dedicated to Dr. F. Naef on the occasion of his 65th birthday.

References

1. D.R. White, Jr., P. Hudson, and J.T. Adamson. Dextrin characterization by high-performance anion-exchange chromatography-pulsed amperometric detection and size-exclusion chromatography-multi-angle light scattering-refractive index detection. *J. Chromatogr. A* **997(1-2):** 79–85 (2003).
2. F. Avaltroni, P.E. Bouquerand, and V. Normand. Maltodextrin molecular weight distribution influence on the glass transition temperature and viscosity in aqueous solutions. *Carbohydr. Polym.* **58(3):** 323–34 (2004).
3. M.U. Beer, P.J. Wood, and J. Weisz. A simple and rapid method for evaluation of Mark-Houwink-Sakurada constants of linear random coil polysaccharides using molecular weight and intrinsic viscosity determined by high performance size exclusion chromatography: application to guar galactomannan. *Carbohydr. Polym.* **39(4):** 377–80 (1999).
4. T. Sato, T. Norisuye, and H. Fujita. Double-stranded helix of xanthan: dimensional and hydrodynamic properties in 0.1 M aqueous sodium chloride. *Macromolecules* **17(12):** 2696–2700 (1984).
5. K. Aeberhardt, J.-Y. de Saint Laumer, P.-E. Bouquerand, and V. Normand. Ultrasonic wave spectroscopy study of sugar oligomers and polysaccharides in aqueous solutions: The hydration length concept. *Int. J. Biol. Macromol.* **36(5):** 275–82 (2005).
6. P.D. Orford, R. Parker, S.G. Ring, and A.C. Smith. Effect of water as a diluent on the glass transition behaviour of malto-oligosaccharides, amylose and amylopectin. *Int. J. Biol. Macromol.* **11(2):** 91–96 (1989).
7. Y.-J. Wang and L. Wang. Structures and properties of commercial maltodextrins from corn, potato, and rice starches. *Starch/Stärke* **52:** 296–304 (2000).
8. K.-S. Wong and J.-L. Jane. Quantitative analysis of debranched amylopectin by HPAEC-PAD with a postcolumn enzyme reactor. *J. Liq. Chromatogr. & Rel. Technol.* **20:** 297–310 (1997).
9. K.-S. Wong and J.-L. Jane. Starch chain length analysis by using and anion-exchange chromatography system equipped with an enzyme reactor and a PAD detector. *J. Chromatogr. Library* **66:** 403–22 (2002).

Manuscript received July 22, 2005;
revision received November 23, 2005.