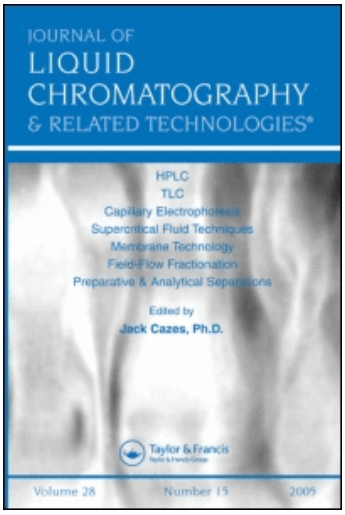

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Jianzhong Lou[a]; Marcus N. Myers[a]; J. Calvin Giddings[a]
[a] Field-Flow Fractionation Research Center Department of Chemistry, University of Utah, Salt Lake City, Utah

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SEPARATION OF POLYSACCHARIDES BY THERMAL FIELD-FLOW FRACTIONATION

JIANZHONG LOU, MARCUS N. MYERS, AND J. CALVIN GIDDINGS
Field-Flow Fractionation Research Center
Department of Chemistry
University of Utah
Salt Lake City, Utah 84112

ABSTRACT

Polysaccharides are complex polymers of great biological and industrial importance. The fractionation and determination of molecular weight distributions of these substances are required for many applications. Thermal field-flow fractionation (thermal FFF) is shown to be applicable to the separation and characterization of a variety of polysaccharides in dimethyl sulfoxide (DMSO). Samples of pullulans, dextrans, and Ficolls are readily fractionated according to differences in molecular weight. Calibration plots have been developed to obtain molecular weight distributions. More complex polysaccharides, such as starch and cellulose, have also been investigated. This study is complicated by the poor solubility of these materials. Nonetheless, starch can be successfully separated into amylose and amylopectin fractions, the latter consisting of an ultrahigh molecular weight polymer with a highly branched structure. A cationic corn starch has also been resolved into its components. These examples suggest that thermal FFF is widely applicable to polysaccharides.

INTRODUCTION

Polysaccharides are biopolymers of both biological and industrial importance. The occurrence and applications of polysaccharides are ubiquitous [1-4]. These polymers usually exhibit a wide molecular weight distribution (MWD). Many applications require the isolation, fractionation, and molecular weight determination

of these substances. For this reason, we have investigated the applicability of field-flow fractionation (FFF) to the separation of a variety of polysaccharides. Specifically, pullulans, dextrans, Ficolls, and starch polymers dissolved in dimethyl sulfoxide (DMSO) are fractionated by thermal FFF.

Pullulan, dextran, and Ficoll span a broad range of polysaccharide branching structures, and have been the subject of some extensive studies [5-7]. Pullulan is a linear polymer of glucose, produced by enzymes secreted by *Aureobasidium* bacteria. The chain is linked by one α-D-(1-6)-glycosidic bond and two α-D-(1-4)-glycosidic bonds alternating regularly. Pullulan is manufactured commercially by enzymatic fermentation processes. In medicine, pullulan solutions can be used to extend plasma supply and pullulan gel can be used to purify enzymes. In addition, pullulan can be used in diet foods as a starch substitute because pullulan cannot be degraded by *in vivo* digestive enzymes. In industry, pullulan films are used as packaging materials for drugs and coatings for electrodes [5]. Pullulan can be used as a gelling agent in food [8] and a protective coating on stainless steel during annealing [9].

Dextran is a partially branched, high molecular weight polymer of glucose, synthesized by the enzyme on the cell surface of *Leuconostoc* or *Streptococcus* bacteria. The backbone of dextran is linked through α-D-(1-6)-glycosidic bonds and the branches are linked by α-D-(1-3)-glycosidic bonds. Dextran also has many uses [6]. Dextran fractions of relatively low molecular weights (~40,000) are used in blood substitutes. Dextran crosslinked with epichlorohydrin, called Sephadex, has been widely used for the fractionation of proteins, nucleic acids, and polysaccharides. The "salting-out" effect of dextran on proteins makes dextran very useful for tests of antigen-antibody reactions. Dextran is also used to stabilize isolation media for subcellular species. It is reported that dextran can be used in veterinary work [10] and in human immunization [11].

Ficoll is a densely branched copolymer of sucrose and epichlorohydrin (1-chloro-2,3-epoxypropane). Ficoll is widely used as a material to form density gradient in various centrifugations of biological species such as blood granulocytes [7, 12, 13] and as a polysaccharide antigen for clinical tests [14]. Ficoll is also used as model for inert spheres in aqueous SEC calibrations [15].

The physical, biological, and clinical properties of these polysaccharides vary with MWD. For example, native dextrans are too high in molecular weight (up to 10^8) to be used in blood substitutes [6].

Apart from our interest in the foregoing polysaccharides, we have also investigated some more complex industrial polysaccharides such as starch, cellulose, and their derivatives. Starch contains amylose and amylopectin in different proportions depending on the plant source. About 97% of all the industrial starch is obtained from corn [16]. Cellulose occurs in cotton, wood, and other plant cell walls [17]. Amylose is a linear polymer of glucose linked by α-D-(1-4)-glycosidic bonds [16]. Amylopectin is linked by both α-D-(1-4)-glycosidic and α-D-(1-6)-glycosidic bonds, probably with random-branching structure [18]. Some researchers believe starch is a giant macromolecule, and that the appearance of amylose and amylopectin is the result of polymer degradation occurring during sample processing [16]. Cellulose is also a linear polymer of glucose but differs from amylose in that it is linked by β-D-(1-4)-glycosidic bonds [17]. Derivatives of starch and cellulose generally have backbone configurations resembling those of their parent polymers [16, 17]. Because the processing rheology and the quality of end products are correlated with MWD, techniques for determining MWDs for these polysaccharides are needed by industry for both processing equipment design and quality control.

MWD can be crudely estimated by some non-fractionation methods such as dynamic light scattering (DLS) [19], which generally requires some assumption about the distribution function, thus often eliminating the possibilities of detecting multimodal distributions or the existence of tails in MWD. In many engineering applications, the presence of a small fraction of ultrahigh molecular weight polymer can strongly influence key material properties, such as normal stresses [20], drag reduction [21], and fracture strength [22].

For the past three decades, size exclusion chromatography (SEC) has been the standard method for polymer separation and MWD determination. However, SEC is not ideally suited for separating ultrahigh molecular weight polymers, due in part to the difficulties in preparing robust packings with sufficiently large pores to allow the permeation of large macromolecules [23-24]. The possibility of the shear degradation of large, fragile macromolecules in porous media [24-27] is another shortcoming of SEC. Cationic polymers may experience sample adsorption on the packing of SEC [27-30]. SEC columns are vulnerable to clogging. Filtration is generally required prior to SEC, which can leave out tails in MWD. Other limitations of SEC for ultrahigh molecular weight applications have been described [31].

Several attempts have been made to use SEC to separate starch polymers [32-36], but the results are still being debated. In order to overcome the drawbacks of SEC, other alternatives have been considered for application to starch polymers, such as flow separation [37], thin-layer chromatography (TLC) [38], radial migration [39], multicell membrane dialysis [40], capillary hydrodynamic chromatography (CHDC) [41, 42], and continuous sedimentation-velocity separation [43].

Field-flow fractionation (FFF) is a relatively new family of elution methods that offer a promising solution to some of the problems of ultrahigh molecular weight polysaccharide separation. In FFF, separation is achieved in a ribbon-like thin channel with no packing inside. A parallel-plate Poiseuille flow is established in the channel. An external field is applied across the channel thickness to drive molecules to different velocity streams and cause separation [44-52]. First proposed in 1966 [44], FFF has now evolved to many techniques, including thermal FFF, sedimentation FFF, flow FFF, and electric FFF [51].

Of all the FFF techniques, thermal FFF has been most widely applied to polymer separations [45-48, 50, 51]. Thermal FFF offers some unique advantages for the study of polymers. Because thermal FFF utilizes an open channel, it has a minimal surface area exposed to polymer molecules. The choice of surface material (or coating) is quite freely made and therefore the problem of sample adsorption can be controlled. Polymer recovery is high. There is no need for sample filtration. Shear degradation is also minimized. Most important of all, thermal FFF can be extended into the ultrahigh molecular weight range.

Despite its broad potential for polymer analysis, much of the research on thermal FFF to date has been devoted to the separation of well-understood synthetic linear polymers. These polymers permit the use of conventional solvents and detection systems [45, 47, 50]. The existence of narrow molecular weight standards has allowed the development of calibration procedures for many of these polymers [50]. A great deal of work has been done on polystyrene standards, some with molecular weights up to 20 million daltons, by thermal FFF [47]. Very little FFF work has been performed on polysaccharides. Myers et al. first subjected a water-soluble blue dextran to thermal FFF, but observed no retention in water because the thermal diffusion effect in water was too weak. They found that the thermal diffusion of blue dextran was enhanced in a mixed solvent of water and DMSO [45]. The lack of retention in water was confirmed by Kirkland and Yau for

polysaccharides [48]. More recently, Wahlund and Litzen used flow FFF to fractionate an aqueous solution of a fluorescent dextran [49].

The objective of this work is to examine and expand the breadth of applicability of thermal FFF to the separation of polysaccharides. The solvent chosen is DMSO, although similar separations are expected in other solvents except water for polysaccharides.

The separations of starch and cellulose are complicated by their poor solubility. A preferred solvent for starch is DMSO [32-36]. However, even in DMSO, starch polymers are difficult to dissolve; all the procedures reported in the literature involve vigorous heating, stirring, and filtering. These steps may cause both mechanical and thermal polymer degradation. The sample filtration step can be bypassed using thermal FFF.

THERMAL FFF PRINCIPLES

The separation of polymers in thermal FFF is realized by the coupling of the thermal diffusion (induced by a temperature gradient) and the nonuniform flow of the carrier along the channel axis. A schematic view of differential polymer retention in a thermal FFF channel is shown in Figure 1. After a polymer sample is injected into the FFF channel inlet, the thermal diffusion, driven by the temperature gradient, and the normal diffusion, driven by the concentration gradient, are usually balanced within a fraction of a minute. As a result, an exponential equilibrium concentration profile will be formed against the cold wall (designated as the accumulation wall) for each component. Because of the nonuniformity of the flow velocity profile in the FFF channel, components with different mean elevations (designated as ℓ_1 and ℓ_2 in Figure 1) above the accumulation wall will occupy different velocity regions and will thus be swept through the channel at different velocities and emerge at different times. Therefore, the degree of polymer retention in FFF is directly related to the mean elevation ℓ. For well-retained polymer samples, the FFF retention time t_r is given by the theoretical equation [51]

$$\frac{t_r}{t^0} \cong \frac{1}{6(\ell/w)} = \frac{1}{6\lambda} \tag{1}$$

where t^0 is the void time (the emergence time of a nonretained species), w is the channel thickness, and λ is the dimensionless mean elevation ℓ/w. An approximate relationship between the dimensionless mean elevation and the polymer's normal

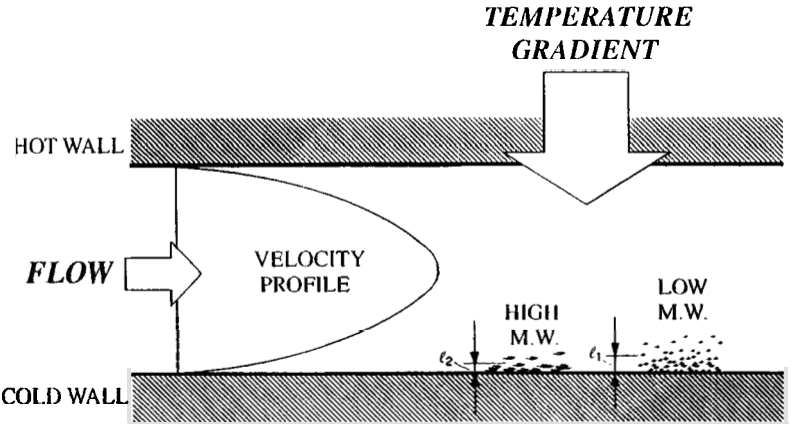

FIGURE 1. Schematic view of polymer components undergoing differential flow transport in the thermal FFF channel.

diffusion coefficient D, thermal diffusion coefficient D_T, and the temperature drop ΔT across the channel thickness is [51]

$$\lambda \Delta T = \frac{D}{D_T} \tag{2}$$

The polymer diffusion coefficient is found to obey the following scaling law [53, 54]

$$D = AM^{-b} \tag{3}$$

where M is the polymer molecular weight and A and b are constants for a given polymer-solvent system at a given temperature. For example, b approaches 0.6 for a linear flexible polymer in a good solvent [54]. Although no satisfactory theory has been developed to predict D_T, the thermal FFF results of Schimpf and Giddings for polystyrene and a few other polymer standards showed that D_T is essentially independent of the polymer molecular weight [50]. Since D_T may have a slight dependence on molecular weight for some polymer-solvent systems, we use the exponent n instead of b:

$$\lambda \Delta T = \phi M^{-n} \tag{4}$$

According to Equation 4, plotting the logarithm of $\lambda \Delta T$ against the logarithm of the polymer molecular weight M should yield a straight line whose slope is -n and intercept is $\log\phi$. This logarithmic linear relation has been confirmed by experiments on several synthetic polymers [50]. Therefore, Equation 4 provides a

basis for thermal FFF molecular weight calibration. Unlike the universal calibration parameters of SEC [30], the calibration parameters of Equation 4 are solely the properties of the polymer-solvent system and not dependent upon the characteristics of the individual separation system. By substituting Equation 4 into Equation 1, we get

$$\frac{t_r}{t^0} \cong \frac{\Delta T M^n}{6\phi} \tag{5}$$

This tells us that the retention in thermal FFF is controlled by the imposed temperature drop and the molecular weight of the polymer sample. The higher the molecular weight, the longer the retention time. However, a rigorous relationship between t_r and M requires a more sophisticated calibration procedure.

The thermal FFF calibration procedure will not be described in detail here. It should be noted that the flow velocity profile in the thermal FFF channel differs slightly from the well-known parabolic profile because the viscosity varies across the channel thickness with the temperature variation. This complicates the relationship between the mean elevation and the retention time t_r. More rigorous equations for retention as a function of dimensionless mean elevation have been derived by Gunderson, et al. [46] and amended more recently by Asten, et al. [52]. In these treatments the velocity profile is calculated using a temperature-dependent solvent viscosity. The reciprocal viscosity, called the fluidity, is approximated by a cubic polynomial expression in absolute temperature. The viscosity data for DMSO used in this work were obtained from Gaylord Chemical Corp. [55] The fluidity of DMSO can be described by the following equation

$$\frac{1}{\eta} = 1035 - 9.128\ T + 0.02536\ T^2 - 0.00001973\ T^3 \tag{6}$$

where η is the viscosity in poises and T is absolute temperature. This equation shows that there is a large variation of viscosity across the FFF channel. For example, the viscosity of the carrier near the cold wall held at 30 °C is about 2 centipoise whereas it is only about 0.74 centipoise near the hot wall at a temperature of 100 °C.

EXPERIMENTAL

Apparatus and procedures

The thermal FFF apparatus used in this work was of conventional design [47, 50]. The surfaces of the copper bars enclosing the channel were chrome plated and

finely polished. Two 1.5-kilowatt cartridge heaters were inserted into the hot bar. Passages were drilled in the cold bar to allow tap water to run through as a coolant. The temperature drop between the bars was maintained through the control of the power duty cycle of the inserted heaters with a single-board computer. A 127-μm thick Kapton polyimide spacer was clamped tightly between the bars by 20 evenly spaced bolts. The center of the spacer was cut out to leave a ribbon-like flow channel measuring 2 cm in breadth and 35.6 cm in tip-to-tip length.

In previous work, the channel inlet and outlet consisted of holes drilled directly into one of the copper bars [47, 50]. However, in the course of this study, we observed corrosion of the bared copper by DMSO. To avoid the exposure of copper to the solvent, we modified the inlet and outlet by inserting 1/16" (0.159 cm)-OD Teflon tubes with an inner diameter of 305 μm into the hot bar all the way to its surface. The tubes were mounted with Swagelog fittings.

A Kontron (London, England) Model 414 LC pump was used to deliver chromatography-grade DMSO solvent to a Rheodyne (Cotati, California) sample injector, which was connected to the inlet of the thermal FFF channel. The injection loop was 20 μL. The dead volume between the channel and the injector was 25 μL. This requires a minimum time delay of 13.5 s after injection to allow the sample to reach the channel at a flow rate of 0.2 mL/min. After arrival of the sample at the head of the channel, the solvent flow was switched to bypass the channel for 30 s to allow the polymer components to relax into their equilibrium exponential distributions under stop-flow conditions. Channel flow was then re-started to carry out the run. A detector was connected to the channel outlet using a 305 μm-ID Teflon tube. Both a Wyatt (Santa Barbara, California) Dawn F laser photometer and a Varex (Burtonsville, Maryland) Model A evaporative light scattering detector (ELSD) were used in this study.

Because the pump was not designed for working steadily for the flow rates under 0.1 mL/min, we used a circulatory loop, consisting of a 6-meter-long 254 μm-id stainless steel tube, an solvent filter, and a back pressure regulator, to split the carrier flow from the pump into two streams and send one of them back to the solvent container and the other to the injector. In this way, we were able to adjust the flow rate in the FFF channel to well below 0.1 mL/min while allowing the pump to run at a higher flow rate setting. The flow rate was always calibrated with a well-sealed burette and a stopwatch.

Materials

Model polysaccharides including pullulans, dextrans, and Ficolls were examined in this work. The characteristics and original source of each of these samples are shown in Table 1. Samples of corn starch, cationic corn starch, and isolated amylose and amylopectin, provided by H. D. Scobell of A. E. Staley Manufacturing Co. (Decatur, Illinois), were also subjected to the thermal FFF. A sample of potato starch purchased from Sigma Chemical (St. Louis, Missouri) was also studied. Most of these samples were supplied in the form of amorphous white powder. Some starch samples, however, were also supplied in solutions.

Chromatography-grade DMSO, identical to that used as the carrier, was employed as the solvent for preparing solutions of these polysaccharide samples. Reagent-grade lithium nitrate ($LiNO_3$) was used to enhance the thermal FFF retention of the cationic starch in DMSO.

Sample preparation

To prepare polysaccharide solutions from the solid powder form, samples were first weighed and then put in vials. DMSO was then added to the vials. While the samples of pullulan, dextran, and Ficoll were all readily dissolved in DMSO at room temperature, the starch samples required a much higher temperature (in the range 100 ° to 160 °C) to dissolve. A silicone oil bath and a Haake (Berlin, Germany) Model FE-2 water bath were thus used to heat the vials containing the mixture of the starch powder and DMSO. In addition, nitrogen was bubbled into the vials through an immersed 1/16"(0.15875 cm)-OD Teflon tube to help remove O_2 and mix the starch powder with DMSO. All the vials were mounted on a custom-built tilted rotor that rotated slowly until the polymers were completely dissolved. Once the solutions were made, they were all cooled to room temperature. The final concentration of the solutions for injection to the FFF channel was adjusted to 0.1 % (w/v). This concentration is below the overlap concentrations [54] of all these polysaccharides.

RESULTS AND DISCUSSION

Model polysaccharides (pullulans, dextrans, and Ficolls)

Figure 2 shows sets of thermal FFF fractograms (detector response versus time) of different molecular weight samples (see Table 1) of pullulans, dextrans,

TABLE 1. Model Polysaccharide Samples

Sample	Nominal M	M_w/M_n	[η], dl/g	Original Source
pullulan #1	100,000[a]	1.10	-	Polymer Laboratories, Ltd.
pullulan #2	186,000[a]	1.13	-	Polymer Laboratories, Ltd.
pullulan #3	853,000[a]	1.14	-	Polymer Laboratories, Ltd.
dextran #1	150,000[b]	-	0.350	Pharmacia Fine Chemicals
dextran #2	506,000[b]	-	0.530	Pharmacia Fine Chemicals
dextran #3	2,000,000[c]	-	0.700	Pharmacia Fine Chemicals
Ficoll #1	132,000	-	0.013	KABI Pharmacia Ophthalmics
Ficoll #2	321,000	-	0.172	KABI Pharmacia Ophthalmics
Ficoll #3	461,000	-	0.194	KABI Pharmacia Ophthalmics
Ficoll #4	741,000	-	0.237	KABI Pharmacia Ophthalmics
Ficoll #5	1,130,000	-	0.282	KABI Pharmacia Ophthalmics
Ficoll A	-	-	-	KABI Pharmacia Ophthalmics*

[a]From ultracentrifugal sedimentation equilibrium

[b]From light scattering

[c]From intrinsic viscosity

*Lot label 400-QC-113347

and Ficolls. For these particular FFF runs, the temperature drop ΔT of the system was set at 27.8 °C and the carrier flow rate was adjusted to 0.2 mL/min. The observation of differential retention beyond the void time t^0 confirms the applicability of thermal FFF to these three classes of polysaccharides. The relatively broad peaks (which are broader than those generated by SEC) are a result of the high selectivity of thermal FFF (much higher than SEC) and do not imply inferior resolution [51]. Rather, these broad peaks represent the broad molecular weight distributions of the polymers (see later).

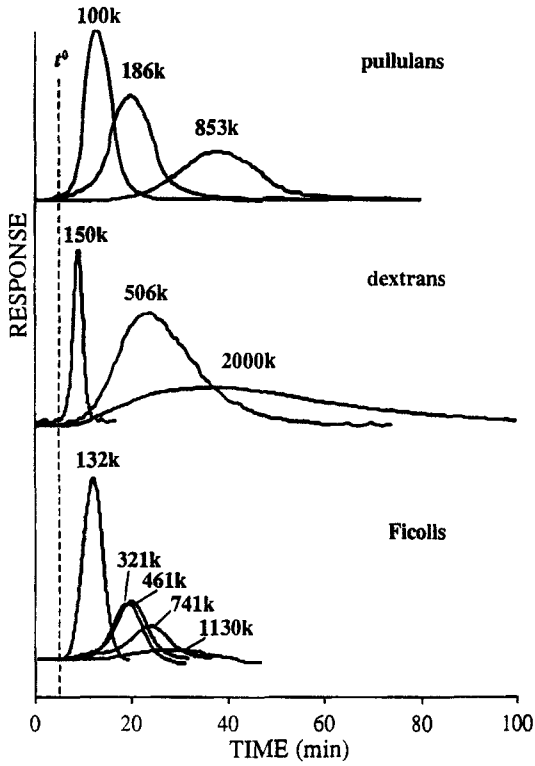

FIGURE 2. Thermal FFF fractograms of various model polysaccharides in DMSO. Conditions: sample concentration, 0.1% (w/v); flow rate, 0.2 mL/min; temperature drop, 27.8 °C; cold wall temperature, 30.0°C; detector, Varex ELSD Model A, used with 40 psi. helium carrier gas.

As described earlier, thermal FFF retention is dependent upon both the thermal diffusion coefficient D_T and the normal diffusion coefficient D. From Figure 2, it is clear that these polysaccharides, especially the pullulans, are well retained which demonstrates that the thermal diffusion effect for these polysaccharides in DMSO is quite strong, or, in other words, that the D_T values are quite high in DMSO. Kirkland and Yau reported a very weak retention (thermal diffusion) effect for the same type of polysaccharides in an aqueous carrier [48]. Thus we conclude that an organic solvent is a better choice for the carrier in the

separation of polysaccharides using thermal FFF. As mentioned above, we lack theoretical guidelines about the dependence of D_T on solvent characteristics and must rely on such empirical evidence.

According to the scaling law for D (see Equation 3), the diffusion coefficient is a function of both molecular weight M and conformation. A branched polymer assumes smaller geometric chain dimensions and hence greater values of the diffusion coefficient than a linear polymer [54]. The fact that dextrans have partially branched structures provides a tentative explanation for why dextrans are less retained than pullulans in thermal FFF for a given molecular weight (see Figure 2). Furthermore, among these three categories of model polysaccharides, highly branched Ficolls are the least retained in thermal FFF for a given molecular weight. These observations support the assumption that the diffusion coefficient D and not D_T is the parameter that dominates the changes in thermal FFF retention of these model polysaccharides. Further work is needed for verifications.

It is necessary in most FFF work to check the fractograms and retention data for any possibility of sample overloading. Once an FFF channel is overloaded with a polymer sample, the eluted polymer peak will change in position and shape with the sample amount and will thus no longer reflect properties of the polymer components such as the molecular weight. For this reason, FFF runs were carried out for several different injected sample amounts. Figure 3 shows such an "overloading" test for the sample dextran #2. For this particular sample and for the conditions used, the onset of sample overloading occurs at about 20-40 μg. The results reported below are all free from overloading effects.

In order to derive the MWD from the FFF retention data, several steps must be taken. First, the polynomial expression for DMSO fluidity shown in Equation 6 is used for determining the accurate velocity profile in the thermal FFF channel. Following this, the treatment developed by Gunderson et al. [46, 52] has been used to calculate the dimensionless mean elevation λ from measured t_rs. Thermal FFF molecular weight calibration plots are then established for each of the three kinds of polysaccharides according to Equation 4. Figure 4 shows a logarithmic plot of $\lambda\Delta T$ as a function of the molecular weight M for pullulans, dextrans, and Ficolls. Data for this plot were acquired at a fixed cold wall temperature but at several different temperature drops (27.8 °C, 37.0 °C, 44.2 °C, and 53.4 °C). The calibration parameters, n and logϕ, obtained from the least square fit are tabulated in Table 2.

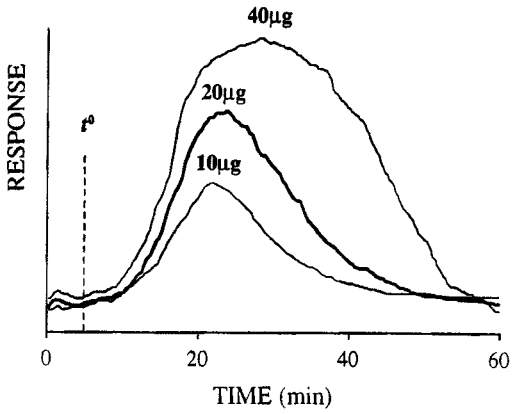

FIGURE 3. Investigation of overloading for dextran #2. Conditions: same as Figure 2.

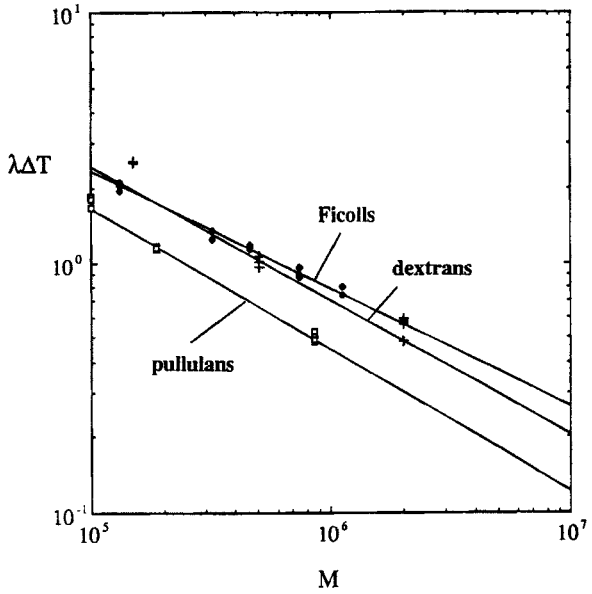

FIGURE 4. Thermal FFF molecular weight calibration plots: calculated λΔT values as a logarithmic function of the molecular weight M for pullulans, dextrans, and Ficolls at a fixed cold wall temperature of 30 °C but at several temperature drops (27.8 °C, 37.0 °C, 44.2 °C, and 53.4 °C).

TABLE 2. Molecular Weight Calibration Parameters*

Sample	Slope, -n	Intercept, logϕ
pullulans	-0.568	3.057
dextrans	-0.539	3.079
Ficolls	-0.475	2.746

*Refer to Equation 4

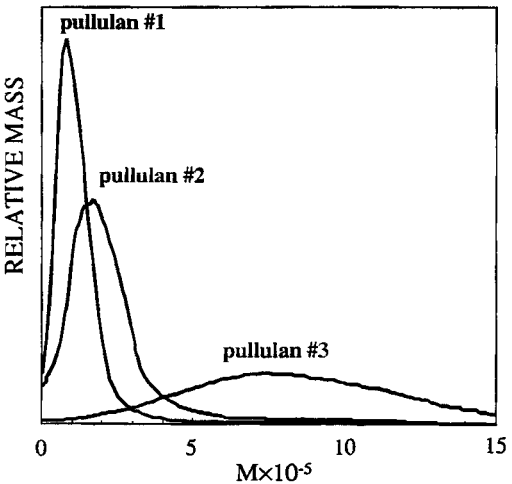

FIGURE 5. MWD curves calculated from the thermal FFF fractograms for pullulans #1, #2, and #3.

Using these calibration parameters, we have calculated the MWDs from the thermal FFF fractograms. Figures 5 to 9 show the MWD curves, the relative mass as a function of molecular weight, calculated from the fractograms using a computer program developed at our research center (the FFFRC).

Starch polymers

The cationic corn starch was well retained and fractionated in DMSO by thermal FFF, as shown in Figure 10. The amylose content is estimated from the

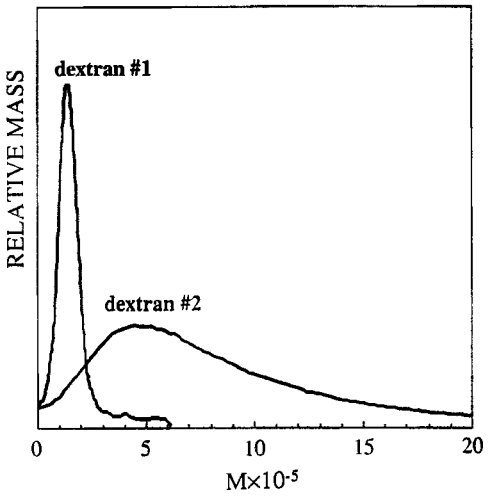

FIGURE 6. MWD curves calculated from the thermal FFF fractograms for dextrans #1 and #2.

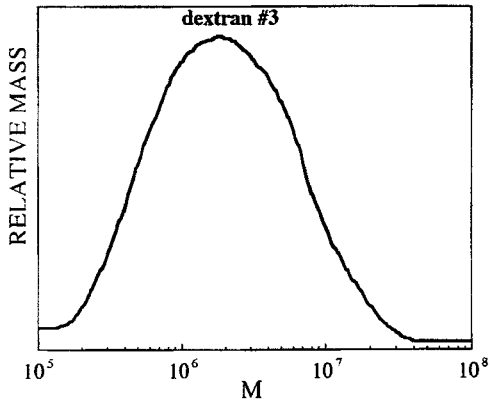

FIGURE 7. MWD curve obtained for dextran #3.

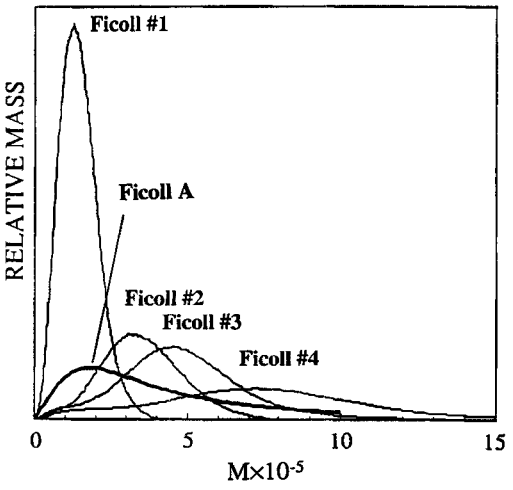

FIGURE 8. MWD curves obtained from the thermal FFF fractograms for Ficolls #1, #2, #3, #4, and A.

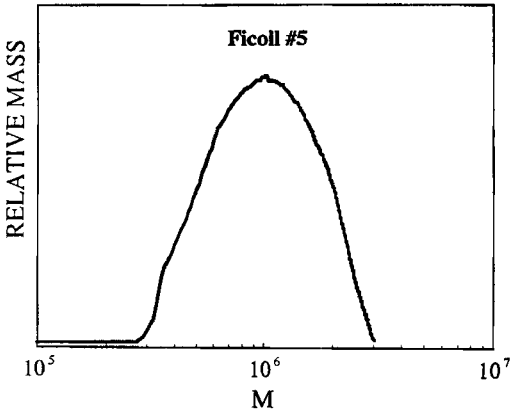

FIGURE 9. MWD curve calculated for Ficoll #5.

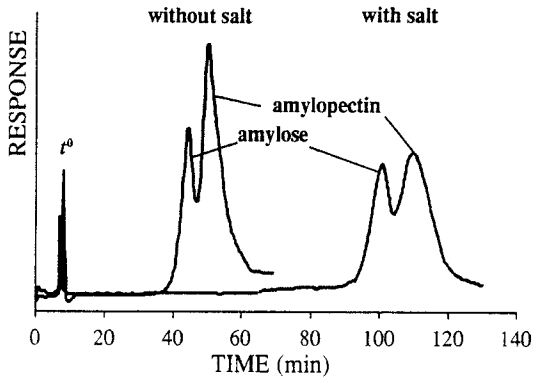

FIGURE 10. Separation of a cationic corn starch in DMSO (left) by thermal FFF. Conditions: sample concentration, 0.28 % (w/v); channel flow rate, 0.1 mL/min; temperature drop, 70 °C; detector, Wyatt Dawn F laser photometer, signal displayed at 90 °. At the right, separation in DMSO with addition of 1.0×10^{-4}M $LiNO_3$.

FFF fractogram peak areas to be about 30%, which is consistent with information from the supplier.

It was found that at an ionic strength of 10^{-4} M ($LiNO_3$ dissolved in DMSO), the thermal FFF retention was significantly enhanced, approximately doubled, as compared with that in pure DMSO (see Figure 10). Below this ionic strength, the enhancement of retention was also observed but to a lesser extent. At an even higher salt concentration, such as 10^{-2} M, we observed the reversible adsorption of cationic starch in the channel; the cationic starch peak did not elute out of the channel, even when run for a few hours, until the field (the temperature gradient) was removed. The explanation of the salt effect is not entirely clear due in part to the lack of knowledge on how D_T varies with the ionic strength. Three effects are expected to play a role. One is the unknown variation in D_T. Another is that the salt ions will reduce the thickness of the electrokinetic double layer on the accumulation wall, which tends to exclude the polymer molecules. Therefore, the addition of a salt will tend to lower the mean elevation and increase the retention time at a given external field strength (temperature gradient). The other change due to the addition of a salt is the well-known polyelectrolyte screening effect [56]. Here the added ions act as a screen to reduce the repulsion between the charged

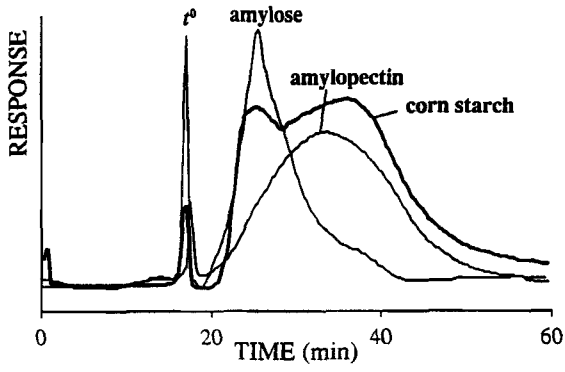

FIGURE 11. Separation of underivatized corn starch in DMSO by thermal FFF. Superimposed are the thermal FFF fractograms of corn starch and both an amylose and an amylopectin fraction isolated from corn starch. Conditions: sample concentration, 0.28 % (w/v); channel flow rate, 0.05 mL/min; temperature drop, 65 °C; detector, Wyatt Dawn F laser photometer, 90 ° angle.

chain segments. The geometric dimensions of the polyelectrolyte chain are thus reduced and the diffusion coefficient increased as salt is added. This will lead to a higher mean elevation and a shorter retention time. The salt effects shown in Figure 10 suggest that the former effect ("wall exclusion") dominates, assuming there is no large change in D_T. Such salt effects require further investigation, probably using better understood model polyelectrolytes.

Both corn and potato starches show relatively weak retention in DMSO. No observable salt effect was found on the retention of these starch samples. The partial separation of corn starch was achieved in DMSO as shown in Figure 11. The amylose and amylopectin components of the study can be identified with the first and second peaks, respectively, because isolated amylose and amylopectin samples have the same retention characteristics. The superimposed fractograms of Figure 11 illustrate this.

The thermal diffusion of starch in DMSO appears to be relatively weak. In order to achieve higher resolution in thermal FFF, a higher ΔT will be required. Another possibility includes the use of alternate solvents.

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