Separation of Amylose and Amylopectin in Corn Starch Using Dual-programmed Flow Field-Flow Fractionation

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Starch, a major storage polysaccharide in plants, is used in a wide range of industries including confectionery, beverage or liquid flavor emulsions, pharmaceuticals, cosmetic products, inks, etc.[1,2] Starch is a mixture of two macromolecular α-glucans, linear amylose and branched amylopectin.[3]

Starch applications require dissolution (or dispersion) in aqueous media under non-degradation conditions. Functional properties of starch are influenced by the average molecular weights (M) and molecular weight distributions (MWD) of the amylose and amylopectin, as was demonstrated in gels,[4] extrusion[5] and pastes.[6] Accurate determination of M and MWD of the amylose and amylopectin requires separation of the two components. Both amylose and amylopectin are usually of very high molecular weights, and are difficult to separate.

Chromatography-related techniques have been widely used for starch analysis, including size-exclusion chromatography (SEC),[7-12] high-performance anion-exchange chromatography (HPAEC).[13-15] Often chromatographic analysis of starch is not satisfactory due to degradation and/or adsorption of the sample components during elution through packed columns.[16-18] Recently, SEC coupled to multi-angle light scattering (MALS) has been demonstrated to be a useful tool for analysis of polymer systems, where SEC provides separation and MALS the molecular weight.[19-23] SEC-MALS has been used for analysis of wheat amylopectin.[17] The M and the radius of gyration (r_g) measured by SEC-MALS were significantly lower than those obtained by the batch mode of MALS due to degradation of the amylopectin during the passage through the packed column.[23,24] It was also found that some of large amylopectin molecules are adsorbed. Based on the recovery test, a significant portion (17.7-63.7%) of the sample was not recovered, resulting in reduction in measured M.[25] It has been reported that, although the starch sample has been completely dissolved in water, only 86% of the sample has been recovered through SEC columns.[16]

Field-flow fractionation (FFF) is a family of techniques that are useful for separation of wide range macromolecules and colloidal particles.[26,27] FFF possesses several merits over SEC for separation of ultrahigh molecular weight polymeric samples. Unlike in SEC, there is no packing material, minimizing degradation or adsorption of large molecules. Also there is no so-called "total exclusion limit" in FFF, and the applicable range in molecular weight is usually extended further to much higher molecular weights than in SEC. In addition, the fractionation range, resolution, and analysis time can be controlled rather easily by adjusting the flow rate and the field strength. Granular starch has been analyzed using sedimentation FFF.[28] Also derivatized potato amylopectins have been successfully analyzed by on-line coupling of flow FFF and multi angle light scattering (MALS).[29] Yet there has not been reports on separation of amylose and amylopectin in aqueous solution of a starch by FFF. Among the members of FFF family, flow FFF (FlFFF) has been widely used for separation of water-soluble polymers and biological macromolecules.[30-34] The aim of this work is to investigate the capability of FlFFF for separation of amylose and amylopectin in corn starches.

In FlFFF, the retention time t_r of a component is related with its diffusion coefficient D by[30,35-38]

$$D = \frac{w^2 V_c}{6 t_r V} \tag{1}$$

, where w is the channel thickness, V_c and V are the flow rates of the cross-flow and the channel-flow, respectively. Using eqn (1), D can be measured for each t_r over the envelope of the elution curve. This relationship between D and t_r was recognized as an effective way for determining D for proteins, viruses, and latex beads.[38-41] D can be expressed as a function of molecular weight by[42]

$$D = \frac{RT}{6\pi\eta N_0}\left(\frac{10\pi N_o}{3K}\right)^{1/3}(M_v)^{-(1+\alpha)/3} \tag{2}$$

, where R is the universal gas constant, N_o the Avogadro's number, M_v the viscosity-average M, and K (in dL/g) and α the Mark-Houwink constants. Retention time t_r is also related with the hydrodynamic diameter, d_H by[30,38]

$$t_r = \frac{\pi w^2 \eta V_c}{2kTV} d_H \tag{3}$$

, where η is the viscosity of the carrier liquid, k the

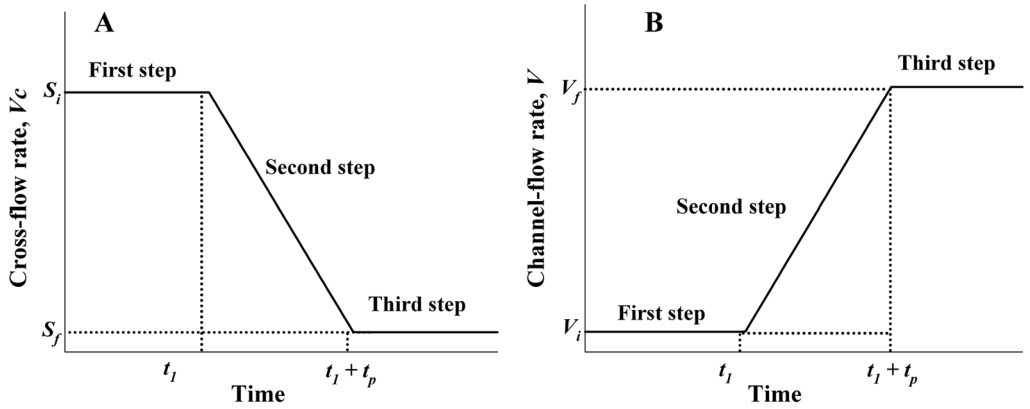

Figure 1. Profiles of cross-flow rate, V_c (A) and channel-flow rate, V (B) in a linear dual-programmed FlFFF.

Boltzmann constant, T the absolute temperature. It can be seen from Eq. (1-3) that FlFFF provides separation of components based on either molecular weight (M_v) or hydrodynamic diameter (d_H).

In FFF, the field (and/or the flow rate)-programming can be used to adjust the resolution and the analysis time, where the field (and/or the flow rate) is varied during a run. Typical programmed FFF begins with a high level of field strength suitable for retention of the smallest components, and then the field strength is gradually reduced to allow elution of the components having larger diameters.[43,44] The quantitative expressions applicable to non-programmed (isocratic) FFF can be extended to programmed FFF. In this study, a dual-programming was used, where V_c was reduced linearly while V was increased linearly as shown in Figure 1. A linear reduction of V_c (Figure 1-A) is expressed by

$$V_c(t) = V_{ci}\left[\frac{t_1 + t_p - t}{t_p}\right] \quad (4)$$

over the time interval, $t_1 \le t \le (t_1 + t_p)$. V_{ci} is the initial V_c, t_1 the pre-decay time, the time duration of the first step, and t_p the duration of programming. Likewise, a linear increase of V (Figure 1-B) can be expressed by

$$V(t) = V_i\left[\frac{t_1 + t_p - t}{t_p}\right] \quad (5)$$

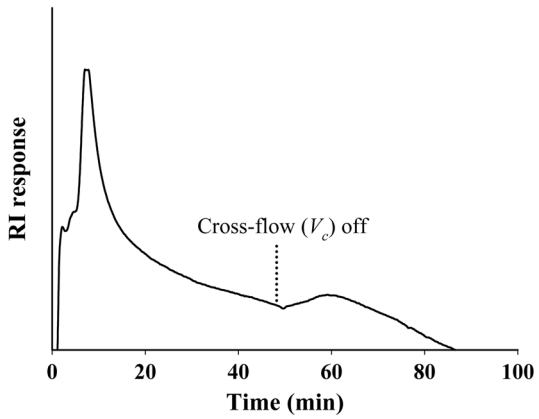

Figure 2. FlFFF fractogram of Normal corn starch obtained at V_c and V of 0.2 and 0.25 mL/min, respectively.

Figure 2 shows a FlFFF fractogram of Normal corn starch obtained at V_c = 0.2 and V = 0.25 mL/min, respectively. The first peak (corresponding to amylose fraction)[16-18,22] is eluted at around 8 min, after which the baseline keeps drifting down continuously. When V_c was turned off at around 45 min, the remainder of the sample (mainly the amylopectin fraction) was eluted, indicating the amylopectin fraction is retained too much even under this mild (low V_c) condition.

To improve resolution, a dual-programming was employed. Figure 3 shows a FlFFF fractogram of Normal corn starch obtained by a linear dual-programming with $t_1 = t_p$ = 20 min, V_{ci} = 0.763, V_{cf} = 0.058, V_i = 0.214, V_f = 0.919 mL/min, respectively. The amylose (earlier eluting one) and the amylopectin (latter) fractions are well separated. The area ratio under the elution curves of amylose/amylopectin is 36/64. Accurate conversion from the area to concentration requires area/mass calibration and correction for the change in flow rate, etc. Accurate quantitative analysis was not possible due to the lack of pure standards of amylose and amylopectin. It is interesting, however, that the area ratio determined without such correction is somewhat close to the nominal amylose/amylopectin ratio of 30/70.

Figure 4 shows a FlFFF fractogram of the High-Amylose corn starch obtained at the same conditions as in Figure 3.

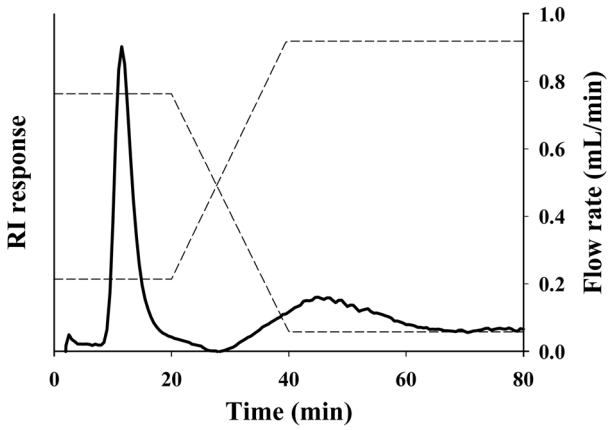

Figure 3. FlFFF fractogram of Normal corn starch obtained by a linear dual-programming. Programming parameters are: $t_1 = t_p$ = 20 min, V_{ci} = 0.763, V_{cf} = 0.058, V_i = 0.214, and V_f = 0.919 mL/min.

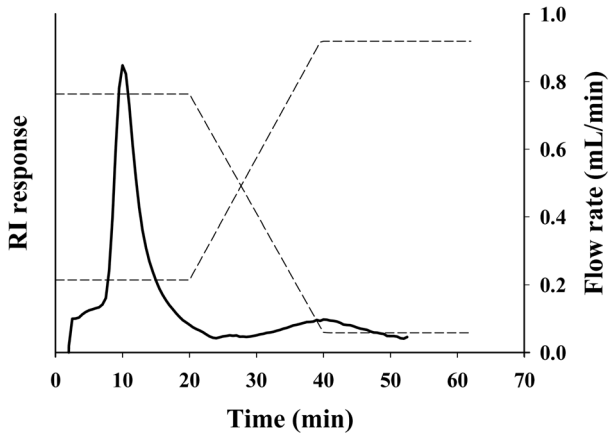

Figure 4. FlFFF fractogram of High-Amylose corn starch obtained at the same condition as in Figure 3.

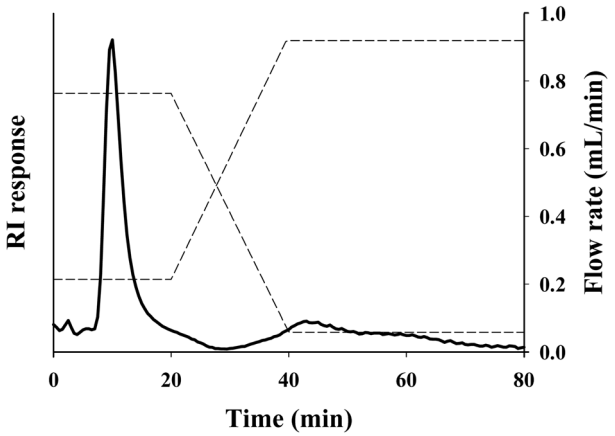

Figure 5. FlFFF fractogram of 7 days-old Normal corn starch solution obtained at the same condition as in Figure 3.

As in Figure 3, the two fractions are well separated. The area ratio of the elution curves of amylose/amylopectin is 75/25, which is again somewhat close to the nominal ratio of 70/30. As shown in both Figure 3 and 4 that the M (or the size) distributions of the amylopectin fractions are much narrower than the amylopectin fractions in both Normal and High-Amylose corn starches.

Figure 5 shows a FlFFF fractogram of the 7 days-old Normal corn starch solution obtained at the same conditions as in Figure 3. The area ratio was changed from 36/64 (Figure 3) to 52/48, which is probably due to degradation of the amylopectin fraction during storage. It seems the starch solutions prepared in this study is not quite stable, and need to be freshly prepared for each analysis.

Table 1 shows the average molecular weights (M_v) and hydrodynamic diameters (d_H) of the Normal and the High-Amylose corn starches determined by Equations (2) and (3) respectively. As expected, M_v and d_H of the amylose fractions are much lower than those of the amylopectin fractions for both samples. It is likely that the M_v and d_H values shown in Table 1 are smaller than the actual values due to the steric effect of these large molecules, which usually results in faster elution than predicted by FFF theory.

Table 1. Average molecular weights and sizes determined by FlFFF for amylose and amylopectin fraction of corn starches

Sample		Molecular weight[a] ($\times 10^6$ Da)	Hydrodynamic diameter[b] (nm)
Normal corn starch	Amylose fraction	0.144	13.4
	Amylopectin fraction	43.2	460
High-Amylose corn starch	Amylose fraction	0.112	12.0
	Amylopectin fraction	19.6	310

[a]Determined by Equation (2) with Mark-Houwink coefficients, $K = 1.32 \times 10^{-4}$ dL/g and $\alpha = 0.68$. [b]Determined by Equation (3)

In summary, FlFFF has been tested for separation of amylose and amylopectin in aqueous solution for the first time. A dual programmed FlFFF provided a good separation of the amylose and the amylopectin fractions of corn starches. If further optimized, FlFFF can become a useful tool for analysis of starches and related materials. For accurate determination of molecular weight and the hydrodynamic sizes of the amylose and amylopectin fractions, on-line coupling of a multi angle light scattering (MALS) with FlFFF is suggested.

Experimental Section

Starch solution preparation. Two types of corn starches (High-Amylose and Normal) were used in this study. The High-Amylose and Normal corn starches have the amylose/amylopectin ratio of 70/30 and 30/70, respectively. First, the starch sample (10 mg) was wetted with 100 μL ethanol and then 1 mL of 1 M NaOH was added. The mixture was heated for 2 min at 70 °C in a water bath. Then 7.9 mL of the FFF carrier liquid (water containing 0.15 M $NaNO_3$) was added, and the solution was neutralized by adding 1 mL of 1 M HCl. Finally the solution was autoclaved for 20 min at 121 °C. The final concentrations of starches were about 0.1%.

Flow field-flow fractionation (FlFFF). The accumulation wall of the FlFFF channel is an YM10 membrane (Amicon, Inc., USA) having M-cutoff of 10,000 Da. The Mylar spacer has dimensions of 29.3 cm in tip-to-tip length, 2.0 cm in breath, and 0.0194 cm in thickness. The channel volume was measured by the elution of the void peak to be 1.134 mL. The FlFFF channel was positioned vertically to avoid the influence of the gravity. Sample solution was injected using a 20 μL-loop injector (Rheodyne model 7725, Cotati, CA, USA), and eluting samples were monitored by a refractive index detector (Shodex RI-71, Showa Denko, Tokyo, Japan). Two microcomputer-controlled 6000A pumps (Waters Corp., Milford, MA, USA) were used to provide V_c and V. For dual-programming, the cross-flow pump was plumbed in a circulation mode, so that the V_c coming out of the channel goes back to the inlet of the cross-flow pump.

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