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Translational Diffusion Coefficients and Hydrodynamic Radii of Normal Corn Starch in Aqueous Media from Asymmetrical Flow Field-Flow Fractionation Experiments

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Abstract

Starch is a highly disperse material with broad distributions of molecular sizes and geometries. Its dissolution in aqueous media is difficult to achieve and it tends to form aggregates through both inter- and intra-molecular interactions. Asymmetrical flow field-flow fractionation (AF4) is a suitable technique for the separation of such macromolecular and colloidal systems. A major advantage of AF4 is the direct correlation of translational diffusion coefficients with retention time and experimental parameters. In this article, the hydrodynamic and diffusive mobility of normal corn starch dissolved in 0.035 M KSCN was investigated by systematically varying the cross flow rates (applied forces); the translational diffusion coeffcients for normal corn starch in aqueous medium were found to range between 9.9 x 10^{-9} cm^2/s and ~2.5 x 10^{-7} cm^2/s with varying F_{cr} rates. Diffusion coefficient ranges shifted to higher diffusion co-efficient values at higher cross flow rates (applied forces). This behaviour, which may be attributed to the increased retention of very large starch molecules/particles at high F_{cr} rates, is further confirmed by the decrease in apparent molar mass and mass recovery values.

Keywords

Starch, Mobility of Starch Populations, Asymmetrical Flow Field-Flow Fractionation, Amylopectin, Amylose, High Pressure Microwave Vessel

1. Introduction

Starch is one of nature's most complex and elegant materials. It occurs in various types of plant tissues and organs including leaves, roots, stems, grains and fruit. It is one of the most abundant carbohydrate sources in the world and is an important energy source for animals and humans [1]. Starch materials are highly versatile and have numerous applications in the food

and non-food industries [2]. Starch is deposited in eukaryotic cells in the form of granules and the morphology and size of the granules vary considerably for different botanical sources [2]. Starch consists of two polysaccharides: amylose-type (long-chained, with no or little branching) and amylopectin-type (short-chained, highly-branched) [1, 2]. Amylose is a α-glucan polymer containing 99% α(1→4) and 1% of α(1→6) glycosidic linkages. The literature values for molar masses for amylose (weight average molar mass M_w) range from ~1 x 10^5 to ~3 x 10^6 g/mol [1-3]. Amylopectin is a highly branched polysaccharide consisting of α-1,4 glucan linkages (95%) and with branching points arising from α-1,6 bonds (5%) [2]. The molar mass (weight average molar mass M_w) values reported for amylopectin-type range from 1 x 10^7 to 1 x 10^9 g/mol [4]. In starch granules, the amylose and amylopectin molecules are organised radially with their single reducing end groups towards the centre, or *hilum* [5]. The crystalline lamellae are formed from amylopectin double helices, whereas the amorphous lamellae contain the branch points of the amylopectin side chains [2].

Complete dissolution of starch in aqueous medium is very difficult to achieve and this limitation is a major obstacle in the characterisation of native starches [6-9]. Dissolution of starch is achieved by disrupting the starch granule to release the starch macromolecules, typically by heating in excess water [2, 4]. The presence of inter- and intra-molecular interactions of starch-glucan molecules in aqueous media results in the formation of supramolecules [3,8-9]. The formation, disintegration and re-formation of these supramolecular assemblies are dynamic processes inherent to the molecular characteristics of the starch materials. The aggregation of starch molecules is not easy to control, and this leads to overestimation of the molar mass and size characterisation [4]. The degree of aggregation of starch molecules in aqueous media is mainly influenced by the dissolution protocols but also depends on botanical origins, method of extraction of starch, etc [6-9]. Detection and investigation of the aggregation of these supramolecular assemblies is therefore valuable in understanding the interactions of starch molecules, which in turn would provide information regarding controlling the aggregation process.

Asymmetrical flow field-flow fractionation (AF4) is a powerful technique for the separation of macromolecules and colloids [10-11]. Aqueous solutions containing macromolecules/colloids are introduced into an AF4 channel (Fig. 1). The upper channel wall is impermeable (PMMA) and the lower channel wall comprises a semi-permeable membrane with a known molar mass cutoff. The separation of the molecules/particles is governed by the difference in their translational diffusion coefficients (D_T) [10]. In AF4 the incoming aqueous flow from the channel inlet is split into two: the axial channel flow (F_{ch}) and the perpendicular cross flow (F_{cr}) (Fig.1). Molecules/particles of different sizes with varying D_T are separated by the velocity gradient inside the channel. After injection, the molecules are forced towards the accumulation wall by cross flow (applied force). Small molecules (high D_T) travel further away from the accumulation wall in the area of faster channel flow. Conversely, the large molecules (low D_T) are positioned close to the membrane where the channel flow is slower. Therefore, separation of the macromolecules/particles occurs according to their D_T values.

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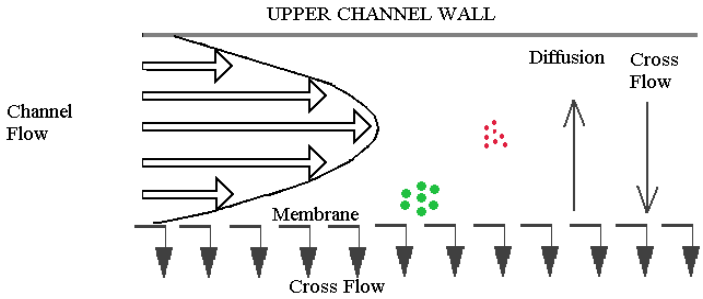

Fig. 1: Asymmetrical Flow Field-Flow Fractionation (AF4): AF4 Channel (top) & separation principle of asymmetrical flow field-flow fractionation (bottom).

AF4 coupled with multi-angle light scattering and a refractive index detector (AF4/MALS/RI) has been employed for the characterisation of a variety of starch materials by a number of research groups [3, 4, 8, 9, 11-17]. One of the main advantages of employing AF4/MALS/RI for the characterisation of starch materials is the ability to directly determine D_T and hydrodynamic radii R_h (assuming a spherical geometry) from the retention time and known AF4 parameters, as shown in eq. 1-2 [10].

$$D_T = \frac{(t^0 F_{cr} w^2)}{6 V^0} \frac{1}{t_r} \qquad \text{eq. 1}$$

$$R_h = \frac{k_B T V^0}{\pi \eta t^0 F_{cr} w^2} t_r \qquad \text{eq. 2}$$

t^0 is the void time, F_{cr} is the cross flow rate, w is the channel thickness, V^0 is the geometric void volume, t_r is the detected retention time, k_B is the Boltzmann's constant, T is the absolute temperature and η is the viscosity coefficient of the solvent.

In this article, the diffusion coefficients of normal corn starch dissolved in aqueous media by microwave heat treatment (HPMV) for 60 seconds determined using AF4/MALS/RI are discussed. F_{cr} rates were systematically varied at a constant F_{ch} rate of 0.7 mL/min to investigate the effect upon D_T and R_h of normal corn starch.

2. Materials and Methods

Pre-gelatinised normal corn starch was dispersed in 0.035 M KSCN and then heated in a high pressure microwave vessel (HPMV) for 60 s. The final concentration of 0.24 mg/mL of normal corn starch was obtained by heating the aqueous dispersion (20-30 mg of starch material in 50 mL of 1M KSCN) in a HPMV (Polycarbonate model 4782) at 90% power for 60 s in a microwave oven (800W). After cooling the HPMV in an ice bath for 1 h the stock solution was diluted with de-ionised water to yield a starch sample dissolved in 0.035M KSCN, which was then filtered through an 8 μm cellulose nitrate filter.

The AF4/MALS/RI set-up (ConSenxus, Ober-Hilbersheim, Germany) comprised: a vacuum degasser (Cambridge Scientific Instruments Ltd., Ely, UK); a 0.22 μm inline filter

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(Millipore (U.K.) Ltd., Watford, UK); a Flow Box P 2.1; a Savebox V5 linked to an AF4 channel (polysulphone membrane (M_w-cut-off of 10 kDa) and a 190 μm spacer), on-line multi-angle laser light scattering (Wyatt Dawn® EOS (λ=690 nm)) and a refractive index detector (Wyatt Optilab DSP). The calibration constant for the MALS detector was 9.04 x 10^{-6} (toluene calibration). The AUX calibration constant value for the RI detector was 2.369 x 10^{-5} determined from NaCl calibration curves. The AF4 system was controlled by WinFFF software (v. 4; ConSenxus) and the MALS and RI data were collected using ASTRA for Windows 4.90 (Wyatt Technology Corporation, Germany). The optimum focusing time (7 min) was determined from visual observation of amylopectin-azure solution at a channel flow rate (F_{ch}) of 2.5 ml/min and a cross-flow rate (F_{cr}) of 2.4 ml/min. The channel thickness of 165 μm was experimentally determined using bovine serum albumin (BSA) (Sigma Aldrich, Gillingham, UK) (literature value of D_T for BSA = 5.96 x 10^{-7} cm^2/s at 294 K).
Apparent molar mass ($M_{w,\,app}$) and radius of gyration ($R_{g,app}$) were determined using the Berry fit method (eq. 3) from the excess Rayleigh ratios (R_θ) measured at 18 different angles ranging from 26°-143° [3, 4, 17].

$$\sqrt{\frac{K\,c}{R_\theta}} = \sqrt{\frac{1}{M_w} + \frac{16\pi^2}{3\lambda^2} R_g^2 \sin^2\left(\frac{\theta}{2}\right)} \qquad \text{eq.3}$$

M_w represents the weight-average molar mass, R_θ, the excess Rayleigh ratio, K is an optical constant and λ is the laser wavelength. M_w is deduced from the intercept ($c \to 0$ and $\theta \to 0$), while R_g is obtained from the slope ($\theta \to 0$ extrapolation).

3. Results & Discussion

The elution behaviour of normal corn starch was investigated by varying the cross flow rates (F_{cr}) in regular increments ranging from 0.1 mL/min to 0.7 mL/min at a constant channel flow rate (F_{ch}) of 0.7 mL/min. The light scattering (LS) at 90° and refractive index (RI) signals for native sago and normal corn starches are shown in Fig. 2a & Fig. 2b, respectively.
Significant broadening of LS signals and a decrease in LS intensity is observed as F_{cr} rates decrease. This is attributed to the retention of very large molecules/particles at higher F_{cr} rates (increased applied force). A majority of the material elutes within the initial 10 minutes of elution, as seen from the RI signals.

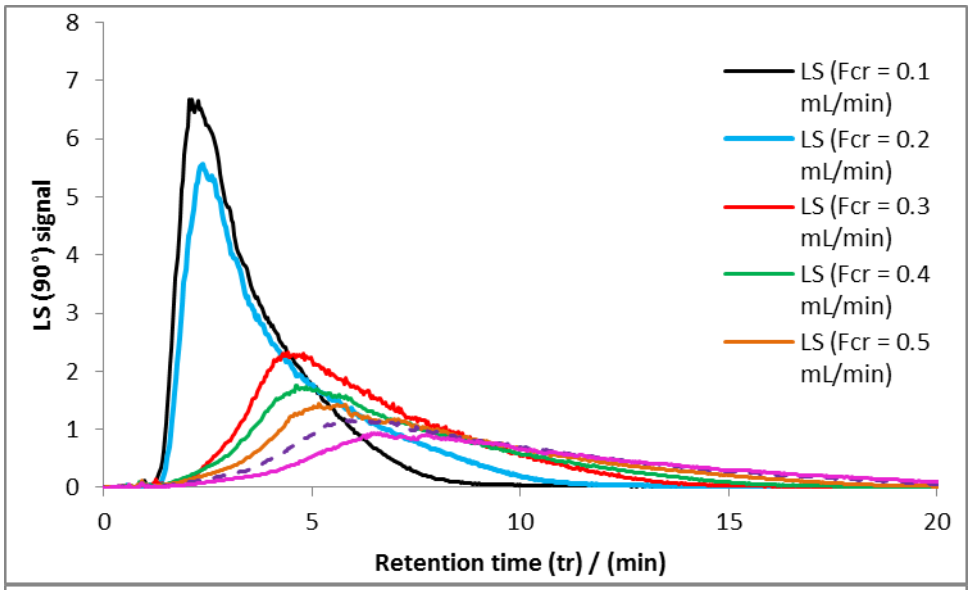

Fig. 2a: Light Scattering (LS) signals at 90° for normal corn starch obtained at a fixed channel flow (F_{ch}) rate of 0.7 mL/min with varying cross flow (F_{cr}) rates ranging from 0.1-0.7 mL/min.

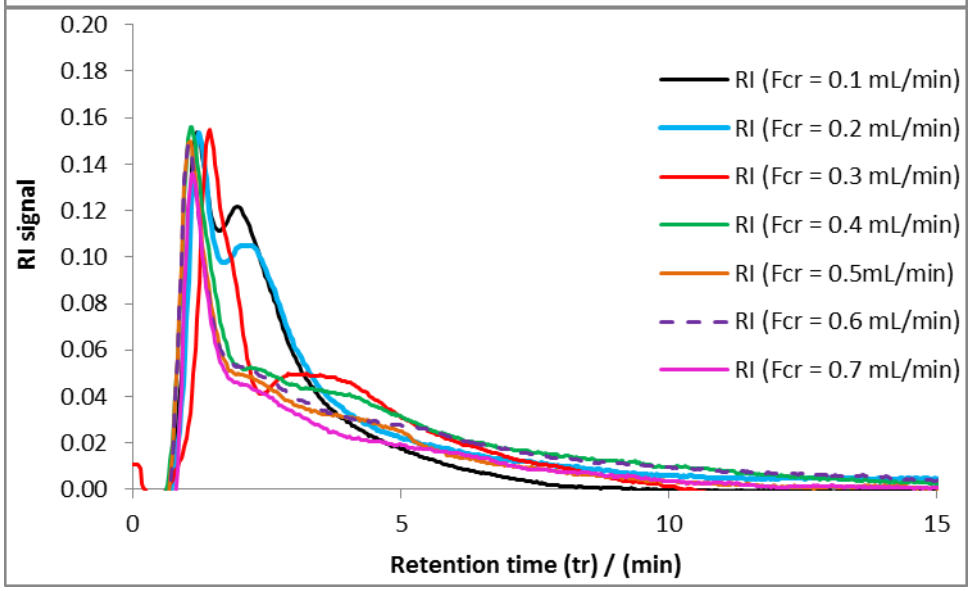

Fig. 2b: Refractive Index (RI) signals at 90° for normal corn starch obtained at a fixed channel flow (F_{ch}) rate of 0.7 mL/min with varying cross flow (F_{cr}) rates ranging from 0.1-0.7 mL/min.

Assuming a spherical geometry, the translational diffusion coefficient values (D_T) for normal corn starch were determined from the observed retention time (t_r) and known AF4 parameters employing eq. 1. The hydrodynamic radii (R_h) distributions for normal corn starches were determined using eq. 2. RI profiles plotted against D_T (Fig. 3a) and R_h (Fig. 3b), show that normal corn starch is highly heterogeneous in nature. An increase in the retention of very large molecules/particles occurs as a function of increasing cross flow rates, as D_T shifts to higher values (faster moving, small molecules/particles) (Fig. 3a and Table 1).

The decrease in the average apparent molar masses ($M_{w,app}$) (employing both RI and LS data determined using eq. 3) and mass recoveries also confirm that very large molecules/particles are retained in the AF4 channel at high F_{cr} rates (applied force), as seen from Table 1. A comparison of the $M_{w,app}$ distributions at a low F_{cr} rate of 0.2 mL/min range and a high Fcr rate of 0.5 mL/min is shown in Fig. 4a.

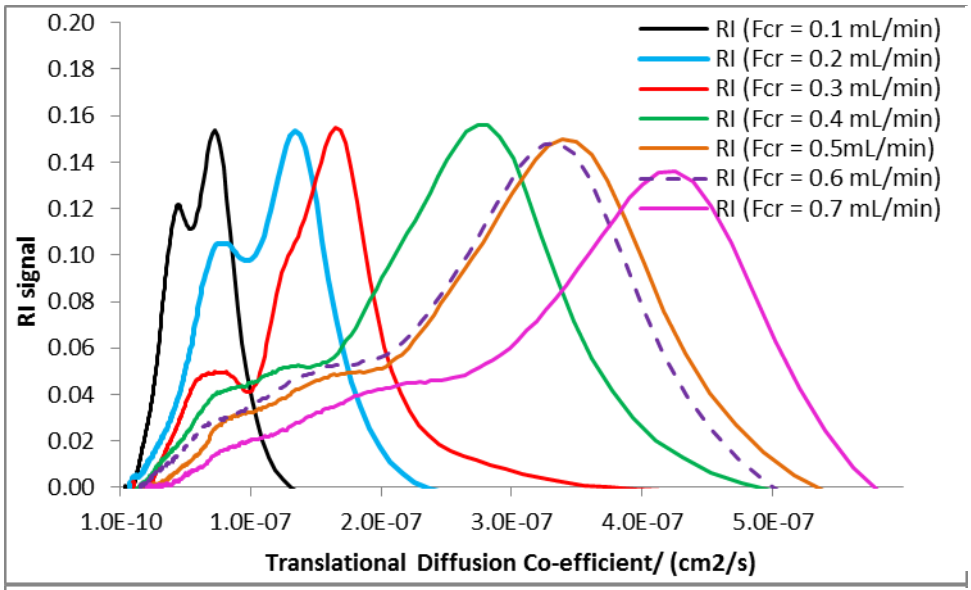

Fig. 3a: Refractive Index signal (RI) for normal corn starch obtained at a fixed channel flow (F_{ch}) rate of 0.7 mL/min with varying cross flow (F_{cr}) rates ranging from 0.1-0.7 mL/min plotted as a function of translational diffusion co-efficient.

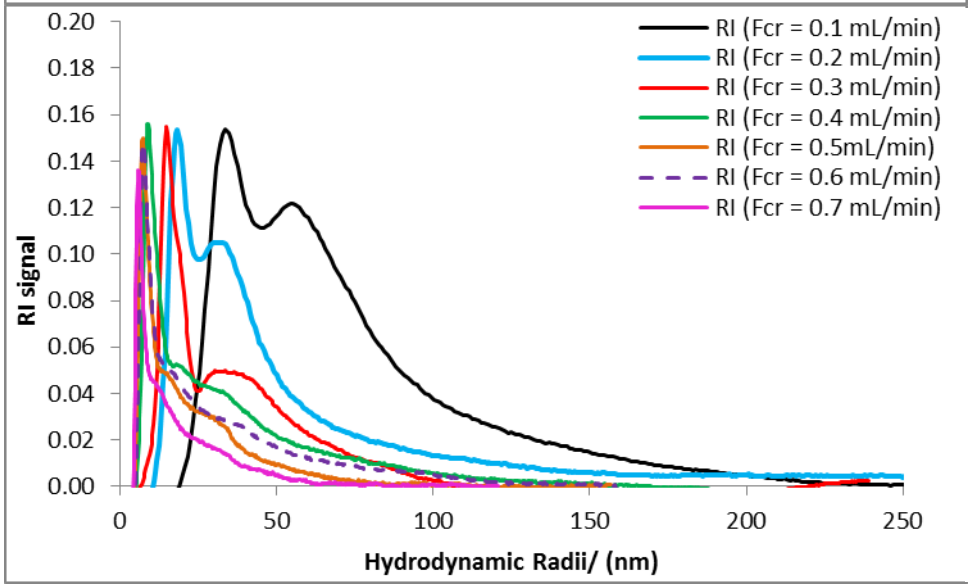

Fig. 3b: Refractive Index signal (RI) for normal corn starch obtained at a fixed channel flow (F_{ch}) rate of 0.7 mL/min with varying cross flow (F_{cr}) rates ranging from 0.1-0.7 mL/min plotted as a function of hydrodynamic radii (R_h).

The $M_{w,app}$ distribution for normal corn starch obtained at a low F_{cr} rate of 0.1 mL/min range from ~8 x 10^6 g/mol to ~120 x 10^6 g/mol and is higher than the $M_{w,app}$ distribution obtained at a high F_{cr} rate of 0.4 mL/min. The corresponding distributions of apparent radii of gyration ($R_{g,app}$) for normal corn starch (Fig. 4b) range from ~28 nm to 200 nm.

The D_T ranges observed for normal corn starch vary with applied cross flow rates (increased applied force) and this behaviour is also seen with the $M_{w,app}$ and mass recovery values. At a low F_{cr} rate of 0.1 mL/min, the presence of large aggregates is observed which are retained in the channel at higher F_{cr} rates, resulting in the shift to higher diffusion coefficients.

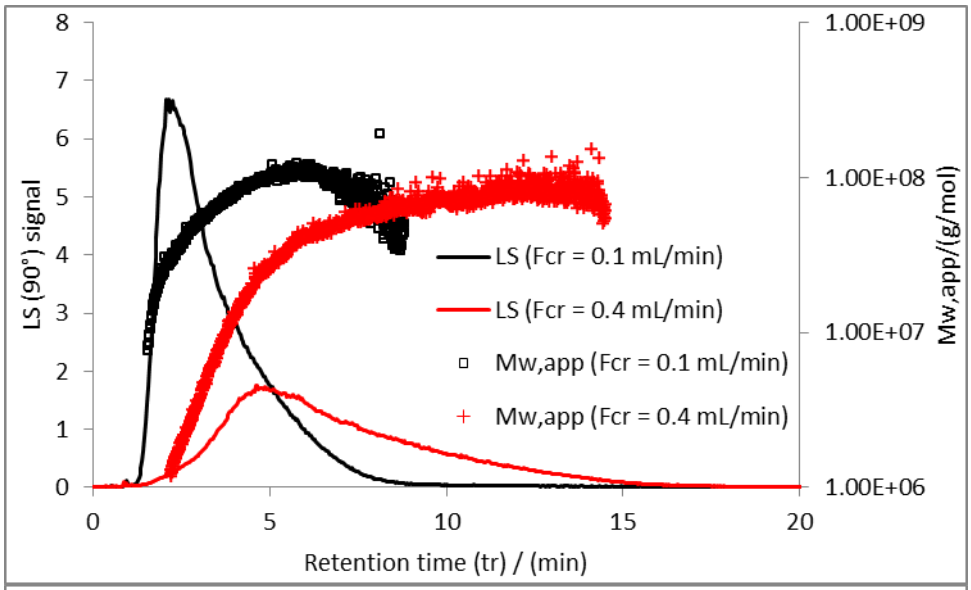

Fig. 4a: Apparent molar mass (Mw,app) for normal corn starch obtained at different cross flow (F_{cr}) rates (0.1 mL/min and 0.4 mL/min) at a fixed channel flow rate of 0.7 mL/min with superimposed light scattering profiles

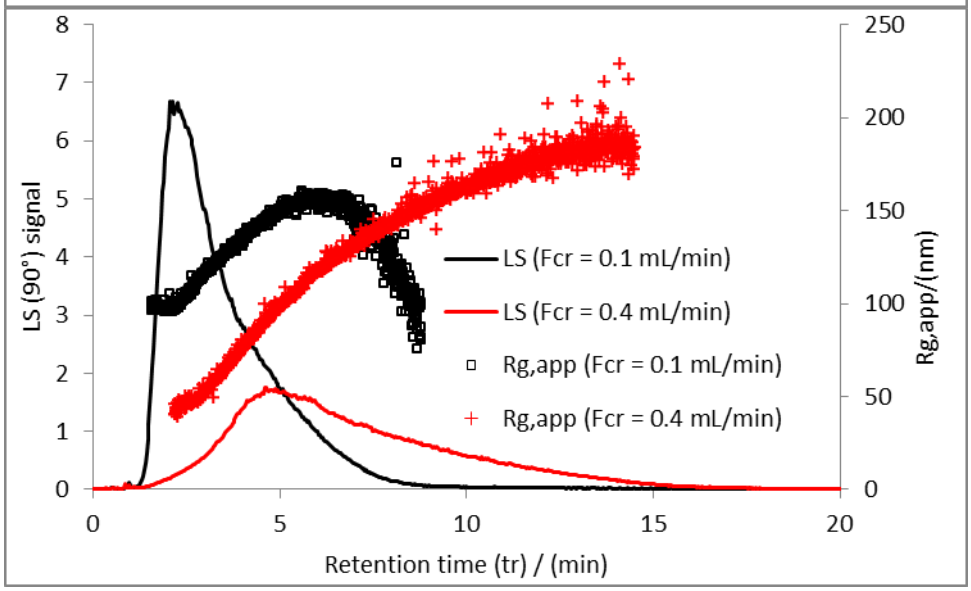

Fig. 4b: Apparent radii of gyration (R_g,app) for normal corn starch obtained at different cross flow (F_{cr}) rates (0.1 mL/min and 0.4 mL/min) at a fixed channel flow rate of 0.7 mL/min with superimposed light scattering profiles

F_{cr}/ mL/min	Light Scattering detectors included	$M_{w,app}$/ g/mol	$R_{g,app}$/ nm	D_T/ (cm^2/s)	R_h/ nm	Recovery %
0.1	69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142 °, 153°, 163°	49 x 10^6	139	5.5 x 10^{-8} - 9.9 x 10^{-9}	44 - 248	72
0.2	69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142 °, 153°, 163°	48 x 10^6	139	1.0 x 10^{-7} - 1.6 x 10^{-8}	24 - 155	61
0.3	52 °, 60°, 69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142 °, 153°, 163°	28 x 10^6	136	1.6 x 10^{-7} - 1.8 x 10^{-8}	15 - 133	59
0.4	35°, 43°, 52 °, 60°, 69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142°, 153°, 163°	32 x 10^6	143	1.4 x 10^{-7} - 2.0 x 10^{-8}	18 -125	55
0.5	35°, 43°, 52 °, 60°, 69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142°, 153°, 163°	35 x 10^6	134	2.0 x 10^{-7} - 2.5 x 10^{-8}	12 -97	52
0.6	35°, 43°, 52 °, 60°, 69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142°, 153°, 163°	23 x 10^6	126	2.1 x 10^{-7} - 2.4 x 10^{-8}	13 - 104	48
0.7	26°, 35°, 43°, 52 °, 60°, 69 °, 80°, 90°, 100°, 111°, 121 °, 132 °, 142°, 153°, 163°	27 x 10^6	119	2.5 x 10^{-7} - 2.9 x 10^{-8}	10 - 83	40

Table 1: The effect of varying cross flow (F_{cr}) rates upon the apparent molar masses ($M_{w,app}$), apparent radii of gyration ($R_{g,app}$), translational diffusion coefficient (D_T), hydrodynamic radii (R_h) and mass recoveries for normal corn starch.

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4. Conclusions

The investigation of the translational diffusion coefficients (D_T) of starch materials provides valuable information on their macromolecular nature and dissolution state. The D_T of normal corn starch solubilised in 0.035 M KSCN heated in a HPMV for 60 s were determined from AF4 measurements, assuming a spherical geometry. The effect of varying cross flow (F_{cr}) rates upon the molecular characteristics and diffusion coefficients demonstrated the presence of large molecules/particles in the normal corn solution. D_T distributions (determined from AF4) of normal corn starch and their corresponding apparent molar mass and radii of gyration distributions (determined from MALS and RI data) vary considerably as a result of varying cross flow rates (applied force). These variations in D_T and R_h in particular demonstrate that normal corn starch molecules/supramolecules are very sensitive to applied forces and variations in cross flow (F_{cr}) rates. An increased retention of normal corn starch molecules/particles was observed at high cross flow rates (applied stresses), as observed from the shifts in the diffusion coefficients of starch to higher values. The translation diffusion coefficients of highly disperse materials (heterogeneous macromolecules) such as starch which tend to form large supramolecular assemblies in aqueous media can be determined from AF4 measurements.

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