Carbohydrate Polymers 106 (2014) 166–171

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Characterisation of cationic potato starch by asymmetrical flow field-flow fractionation. Influence of ionic strength and degree of substitution

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ARTICLE INFO

Article history:
Received 26 September 2013
Received in revised form 3 January 2014
Accepted 2 February 2014
Available online 18 February 2014

Keywords:
Molar mass distribution
Cationic starch
Size separation
Field-flow fractionation
Multi-angle light scattering

ABSTRACT

The properties of a paper sheet depend on the absorption together with the physico-chemical properties of additives used in the paper processing. The effect of ionic strength and degree of substitution of cationic potato starch on the elution pattern of asymmetrical flow field-flow fractionation was analysed. The effect of starch derivatisation, in either dry or wet phase, was also investigated. Average molar mass showed no difference between the starches obtained from the two derivatisation processes. Apparent densities showed that dry cationic starch had higher density than wet cationic starch for a hydrodynamic radius between 50 and 100 nm. Elution times of native and three cationic starches increased when the ionic strength increased from 50 to 100 mM. No differences in the molar mass among cationic starches with different degree of substitution suggested no degradation due to a derivatisation process. Large sample loads can be used at 100 mM without overloading.

1. Introduction

Starch is a very useful raw material with a wide field of applications from gelling systems of foods to manufacturing of paper and adhesives (Swinkels, 1985). The role of starch in paper manufacture is to improve the strength of paper, the retention of fines and fillers, or a combination of both (Roberts, 1996). Cationic starch is preferred because the positive charge that has been introduced onto the starch molecule chain tends to form an electrostatic bond with negative charge sites on the cellulosic fibres. This in turn results in better retention of the starch in the paper web. By using cationised starches, the addition rate of starch in paper manufacture can be raised substantially without increasing the amount of soluble starch wasted in circulating water, that is, without increasing the chemical and biological oxygen demand of circulating and waste waters.

Several methods have been developed for the cationisation of starches. The cationising chemical can be selected from a group comprising both tertiary and quaternary reagents that are capable of reacting with the OH groups of starch. Among the available methods (Vihervaara, Bruun, Backman, Paakkanen, 1990; Kweon, Hoover, Sosulski, Bhirud, 1997), the most commonly used is the so-called wet method. In this method, starch is mixed with water to form an aqueous suspension with a concentration of approximately 40%. The cationising chemical is added and pH is adjusted to within pH 11–12. At these conditions and a temperature of 40–45 °C, starch will be cationised in a reaction completed in approximately 12–16 h. Methods requiring no suspending medium at all have also been developed. These are called dry cationisation methods. According to such a method, the cationising chemical is blended with a mixture of pulverised starch and a basic catalyst such as NaOH or $Ca(OH)_2$ at a temperature between 5 and 40 °C in the presence of a finely divided silica for times between 10 s to 25 min.

The surface chemistry of a paper is very important on the properties of the final paper sheet. The aqueous suspension of fibres and fillers and also the added chemicals need to be retained efficiently during the sheet formation. This retention process is controlled to a large extent by the surface characteristics of the individual components and by the molecular and colloidal interactions, which take place in the aqueous phase. In addition the effective functioning of

Abbreviations: AsFlFFF, Asymmetrical flow field-flow fractionation; CP, Cationic potato starch; CPAP, Cationic potato amylopectin; DS, Degree of substitution; FFF, Field flow fractionation; F_c, Cross flow; F_{out}, outlet flow; MALS, Multi-angle light scattering; NP, Native potato starch; PAP, Potato amylopectin; RI, Refractive index; SEC, Size exclusion chromatography.

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0144-8617/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2014.02.002

many of the chemicals which are added depends upon their absorption, molar mass, charge density, conformation and orientation at the fibre (Roberts, 1996).

The need of techniques to measure the properties of natural polymers was early recognised due to the often very complex nature of these molecules. Most often in the industry, the average molar mass is the property that is primarily asked for since it may indicate the expected performance of the polymer in a specific application. Today, viscometry, ultracentrifugation, light scattering as well as mass spectrometry belong to the more important techniques for the determination of the average molar mass. However, it is not often enough to know just the average molar mass but also the molar mass distribution. The development of size exclusion chromatography (SEC) facilitated fractionation of polymers which rendered possible the determination of molar mass distributions. However, charged polymers may interact with the stationary phase resulting in adsorption or high molar mass polymers run the risk of be degraded by shear forces found in high resolution SEC columns (Cave, Seabrook, Gidley, Gilbert, 2009; Modig, 2005). To overcome such effects a unique analytical methodology called asymmetrical flow field-flow fractionation (AsFlFFF) is especially suited to analytical separation of bio-colloidal particles that are difficult to solve by other methods (Giddings, 1993; Litzén & Wahlund, 1989). The separation in all field flow fractionation (FFF) methods is obtained along a thin open flow channel. A laminar flow of a liquid carrier with a parabolic flow profile makes the sample components to elute at different retention times. The retention is result of a transversal field that concentrates the sample components near one of the walls of the channel (accumulation wall). This wall is made up of an ultra filtration membrane across which the cross flow permeates. The transversal field is counteracted by diffusion, which results in those differently sized sample components differing in their position above the accumulation wall. Due to parabolic velocity profile of the channel flow, differently positioned components are transported at different speed. The method is generally suited to size separations of colloidal particles between 2 and 500 nm. AsFlFFF may be coupled with MALS and RI detectors resulting in a rapid system for molar mass determination (Roessner & Kulicke, 1994; Wittgren & Wahlund, 1997). This enables determination of molar mass and distributions from rather small macromolecules ($\sim 10^3$ Da) up to very large ones ($>10^9$ Da) and moreover also generates data on the molecular conformation and shape (Wittgren, Borgström, Piculell, & Wahlund, 1998).

Previous study has shown the applicability of AsFlFFF for the analysis of cationic potato amylopectin (Lee, Nilsson, Nilsson, & Wahlund, 2003; Shirzad-Semsar, Scholz, Kulicke, 2007; Krentz et al., 2006). Modig, Nilsson and Wahlund, (2006) considered the influence of ionic strength on the elution pattern of cationic starch analysed by using AsFlFFF. However, the effect of changes on the degree of substitution was not considered. Radosta et al. (2004) showed the properties of cationic starch prepared by different derivatisation processes by SEC. As was seen before, SEC in combination with RI detector together with a calibration procedure was used for the determination of high molar mass molecules. However, the results were very dependent of the choice of appropriate references substances for the calibration procedure. Moreover, standards are unavailable for branched polymers as amylopectin (Gidley et al., 2010). Improvements in the molar mass determination were done by adding a light scattering detector to the SEC-RI system. The new system had no need of reference substances. AsFlFFF coupled to MALS and RI detectors permit to perform analyses in shorter times and without the problems that SEC may present and were previously described.

In the present paper the molar mass, molar mass distribution and apparent density of dry and wet cationic starches were investigated. Moreover, the effect of ionic strength and degree of substitution of cationic potato starch on the elution pattern was studied by asymmetrical flow field flow fractionation and multi-angle light scattering were studied (MALS).

2. Materials and methods

2.1. Materials

All the starch samples were obtained from Lyckeby Stärkelsen AB (Kristianstad, Sweden).

The characteristics of the starch samples are summarised in Table 1.

Sample and chemical solutions utilised during the analyses were prepared with filtered (0.2 μm pore-size regenerated cellulose filter, order number 18407, Sartorius AG, Goettingen, Germany) de-ionised (Millipore Corp., Bedford, MA, USA) water.

2.1.1. Equipment

The AsyFlFFF instrument was an Eclipse F Separation system (Wyatt Technology, Santa Barbara, CA, USA) which is an asymmetrical flow FFF instrument (Andersson, Wittgren & Wahlund, 2006). It was connected to a Dawn DSP laser photometer (Wyatt Technology), which is a MALS detector, and an Optilab DSP interferometric refractometer (Wyatt Technology), which is a refractive index (RI) detector. Both used 632.8 nm An Agilent 1100 series isocratic pump (Agilent Technologies, Waldbronn, Germany) with an in-line vacuum degasser and an Agilent 1100 series autosampler delivered the carrier flow and handled sample injection onto the AsyFlFFF channel. A Teflon filter-holder with a 20 nm pore size aluminium oxide filter (Anodisc 25, Whatman International, Maidstone, UK) was placed between the pump and the channel to ensure that a particle free carrier entered the channel. A PEEK pre-column filter

Table 1
Degree of substitution and derivatisation process of starch samples.

Starch sample	Degree of substitution	Derivatisation process	Method of analysis
Native potato (NP)	-	Wet-JC[a]	2.2.2, 2.2.3
	-	Wet-FD[b]	2.2.2, 2.2.3
Cationic potato (CP)	0.05	Wet	2.2.1
	-	Dry	2.2.1
Cationic potato (CP)	0.065	Wet-JC[a]	2.2.2, 2.2.3
		Wet-FD[b]	2.2.2, 2.2.3
Cationic potato (CP)	0.10	Wet-JC[a]	2.2.3
Cationic potato (CP)	0.18	Wet-JC[a]	2.2.3
Potato amylopectin (PAP)	0.065	Wet-JC[a]	2.2.2
		Wet-FD[b]	2.2.2
Cationic potato amylopectin (CPAP)	0.065	Wet-JC[a]	2.2.2
		Wet-FD[b]	2.2.2

[a] JC: jet-cooked.
[b] FD: jet-cooked + freeze drying + re-dissolution at 100 °C.

with a 2 μm PEEK frit (Upchurch Scientific, Oak Harbor, WA, USA) was placed between the channel and the MALS detector to remove large-sized particulate impurities. The ultrafiltration membrane forming the accumulation wall of the AsyFlFFF channel was made of regenerated cellulose with a cut-off of 10 kDa (C010F, Nadir Filtration, Wuppertal, Germany).

2.2. Methods

2.2.1. FFF analysis. Cationic starch with derivatisation in dry or wet phase

Commercial cationic starches with a degree of substitution (DS) of 0.05 with derivatisation in either wet or dry phase were utilised. The samples were dissolved in a tubular reactor at 120° C (jet cooking) and freeze-dried before the analyses. The freeze-dried starch samples were re-dissolved in 50 mM $NaNO_3$ solution (0.1% starch concentration) by heating at elevated pressure in a ROTH Hochdruck-Laborautoklav Modell II (Carl Roth GmbH & Co KG, Karlsruhe, Germany) laboratory autoclave with a programmable temperature control unit (Leeman, Santacruz, & Wahlund, 2009). The starch dispersion was heated from room temperature (approx. 25 °C) to 150 °C in approximately 15 min, then kept at 150 °C for 40 min (pressure around 17 bar), and after that, cooled in a water bath to approximately 90 °C and immediately analysed. Flow FFF analyses were performed by injecting 10 μg of the prepared starch solution. A 50 mM $NaNO_3$ with 0.02% NaN_3 solution was used as carrier. The sample was eluted by using an outlet flow (F_{out}) of 1.0 mL min^{-1} and a cross flow (F_c) of 1.0 mL min^{-1} that decreased exponentially with a half time of 4 min.

2.2.2. FFF analysis. Influence of freeze-drying

Native potato, together with potato amylopectin, cationic potato amylopectin and cationic potato starch with DS of 0.065 with derivatisation in wet phase were dissolved in a tubular reactor at approximately 120 °C. After dissolving, one part of the samples was straight forward analysed in a flow FFF system (not freeze dried samples) whereas another part of the dissolved samples was freeze-dried before the analysis (freeze dried samples). The freeze dried samples were re-dissolved in 50 mM $NaNO_3$ solution at 100 °C for 15 and 40 min in an autoclave as previously described. Starch samples of 6 μg and 0.1% concentration were injected. The carrier solution as previously described together with a constant F_c = 0.1 mL min^{-1} and F_{out} = 0.5 mL min^{-1} were employed.

2.2.3. FFF analysis. Influence of ionic strength and degree of substitution

Native potato starch and three cationic potato starches with DS of 0.065, 0.10 and 0.18 with derivatisation in wet phase were investigated. Starch solutions of 0.1% concentration were dissolved in the proper carrier and cooked in an autoclave as previously described. Carriers of 50 and 100 mM $NaNO_3$ with 0.02% NaN_3 were used. Flow conditions were F_{out} = 1.0 mL min^{-1} and a cross flow of 1.0 mL min^{-1} that decreased exponentially with half time of 4 min. Three different sample loads were employed; being 5, 10 and 20 μg of the dissolved starch.

2.2.4. Data evaluation

A computer software Astra (Wyatt Technology, Santa Barbara, CA) was used for calculation of the molar mass and the z-average root-mean-square radius ($r_{rms,z}$). The calculations were performed according to Wyatt (1993). The molar mass and the $r_{rms,z}$ were obtained by linear fitting to data in Debye plots according to the Berry method (Berry, 1966), together with guidelines described by Andersson (2003). The plots were obtained by using at least scattering intensities from 5 different angles. A refractive index increment, dn/dc, for starch of 0.146 g mL^{-1} was taken from the literature (Paschall & Foster, 1952) and was used for all starch samples. The second virial coefficient, the A_2 term, (equation 1) was unknown and set to zero, i.e. assuming it to be negligible. Since the concentration of the sample in the MALS cell was very low (<10^{-5} g mL^{-1}) this is a valid assumption. The recovery of starch from the AsFlFFF was calculated by integration of the RI-signal and combination with the loaded mass of starch, with values between 92 and 96%.

$$\frac{R_\theta}{Kc} = M_w P(\theta) - 2A_2 M_w 2P2(\theta)c + \ldots \quad (1)$$

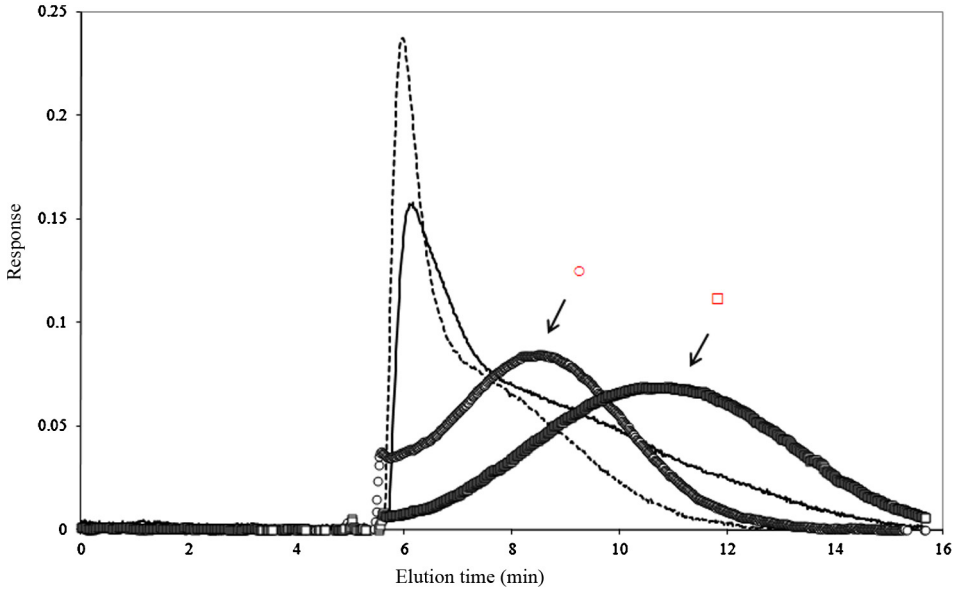

Fig. 1. MALS and RI signals of cationic starches derivatised by wet or dry phase. — RI dry cationic starch, ○ MALS dry cationic starch, – RI wet cationic starch, □ MALS wet cationic starch. A 50 mM $NaNO_3$ carrier. F_c = 1.0 mL min^{-1} that decreased exponentially with a half time of 4 min, F_{out} = 1.0 mL min^{-1}.

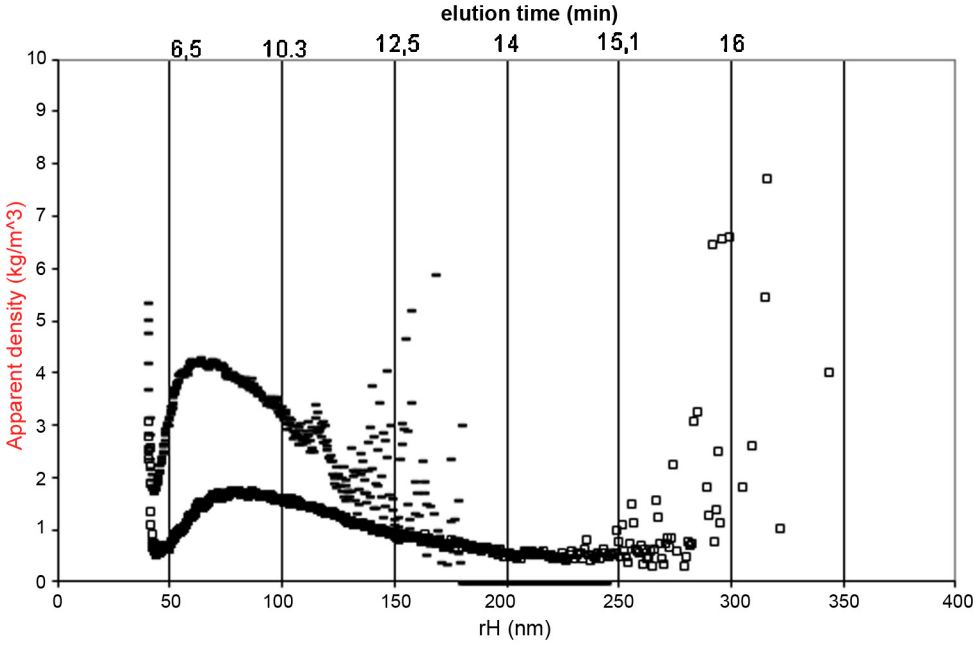

Fig. 2. Apparent density of cationic starches derivatised in wet and dry phase. –Dry cationic starch, □ wet cationic starch. A 50 mM $NaNO_3$ carrier. F_c = 1.0 mL min^{-1} that decreased exponentially with a half time of 4 min, F_{out} = 1.0 mL min^{-1}.

The size parameter, hydrodynamic radius r_H, can be determined from the retention time together with the Stokes–Einstein equation (Eq. (2))

$$r_H = \frac{kTV^\circ t_r}{\pi\eta t^\circ F_c w^2} \quad (2)$$

where r_H is the hydrodynamic radius (assuming spherical sphere), k the Boltzmann constant, T the temperature in Kelvin, V° the void volume, η the viscosity of the carrier liquid, t° the void time, F_c the crossflow rate and w the channel thickness.

Assuming homogenous distribution of mass and a spherical shape, the apparent density can be obtained from (Eq. (3)).

$$\partial = \frac{Mq}{V(r_{rms})} \quad (3)$$

where M is the molar mass, V the volume and q is a scaling constant relating the physical radius of a sphere and r_{rms}.

3. Results and discussion

3.1. Cationic starch derivatisation in dry or wet phase

RI fractograms from wet and dry cationic starches showed two populations not completely resolved. Dry cationic starch had a low molar mass population eluting between 5.5 and 7 min and a high molar mass between 7 and 11 min (Fig. 1).

Wet cationic starch showed shifted elution times for both populations, being 5.5–7.5 min and 7.5–14 min respectively. Average molar mass showed no difference between wet and dry cationic starches, with values of 2.2×10^6 and 2.3×10^6 g mol^{-1} respectively. These values are different than the $23–37 \times 10^6$ g mol^{-1} molar mass obtained for dry cationic starch by Radosta et al. (2004). Differences may be due to the different technique and/or sample preparation utilised during the analysis. MALS fractograms showed that wet cationic starch had material of high molar mass in a higher amount than dry cationic starch above approximately 10 min elution time. Results of the molar mass distribution for both starches showed scattered values before 6 min elution time making the data in that region not reliable (Fig. S-1). Wet cationic starch had a material of low molar mass, between 1×10^5 and 3×10^5 g mol^{-1}, which was not present in dry cationic starch. Wet cationic starch had also a fraction between 1×10^7 and 1.7×10^7 g mol^{-1} that was not present in dry cationic starch. The broader molar mass range of wet cationic starch was also observed by the higher polydispersity index, 5.4, compared to dry cationic starch with a value of 2.6. Results of the cumulative molar mass showed that for any weight fraction below 0.8, dry cationic starch had material of higher molar mass than wet cationic starch (data not shown).

Considering the results above 8 min elution time (Fig. S-2), where both starches showed no scattered values of rms radius, wet cationic starch had a fraction of slighter higher rms than dry cationic starch, being 42 nm and 37 nm respectively. Results of the apparent densities calculated based on r_H showed that dry cationic starch had higher density than wet cationic starch for a hydrodynamic radius (r_H) between 50 and 100 nm (Fig. 2). Differences on the starch density may influence the level of retention of starch

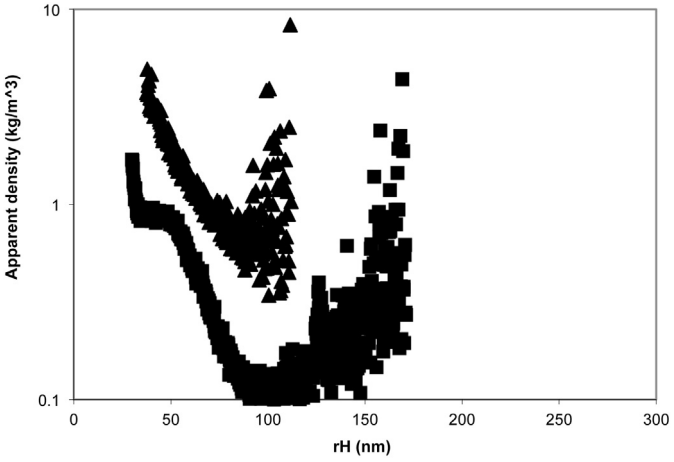

Fig. 3. Apparent density of cationic potato starch. ▲jet-cooked, ■ jet-cooked plus freeze-drying together with re-dissolving at 100 °C for 15 min. A 50 mM $NaNO_3$ carrier. F_c = 1.0 mL min^{-1}, F_{out} = 1.0 mL min^{-1}.

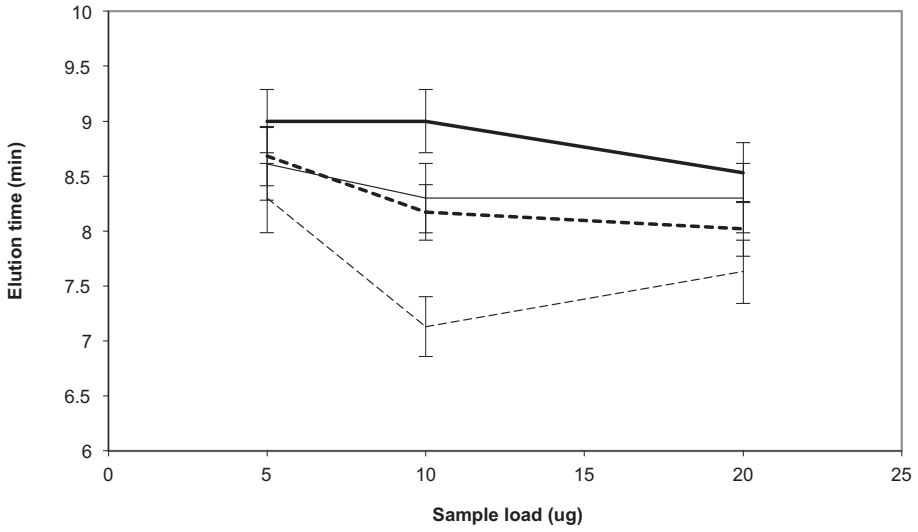

Fig. 4. Elution time vs. sample load of cationic potato starch with degree of substitution (DS) of 0.065 and 0.10. Continuous line 100 mM and dotted line 50 mM $NaNO_3$. — DS 0.065, —DS 0.10,— DS 0.065, — DS 0.10. F_c = 1.0 mL min^{-1} that decreased exponentially with a half time of 4 min, F_{out} = 1.0 mL min^{-1}.

on the paper sheet. Scattered values of density of dry cationic starch were observed for r_H above 120 nm making difficult to conclude if there were differences in that region. The variation of density as function of size suggested that density is strongly dependent on the size of the molecule, as was expected. The difference in elution behaviour between dry and wet cationic starches may be explained by the difference in density.

3.2. Influence of freeze-drying

Differences on the apparent densities were observed on NP (data not shown) and CP starches (Fig. 3) due to freeze-drying. Both freeze dried starches had apparent densities which were lower than the corresponding not freeze-dried samples. These results suggest the formation of a less compact structure during freeze drying.

When the jet-cooked samples were freeze-dried and re-dissolved at either 100 °C for 15 min or 150 °C for 40 min, different starch degradation was obtained. Increase of the heating treatment during re-dissolution from 100 °C for 15 min to 150 °C for 40 min led to a reduction of molar mass in the order of ten times from 3.8×10^7 to 3.2×10^6 g mol^{-1} for PAP starch. The higher heating treatment produces also a reduction in an apparent density from 35 to 1.7 g cm^{-3} for PAP starch.

3.3. Ionic strength and degree of substitution

RI fractograms (Fig. S-3) showed that the elution times of native and three cationic starches increased when the ionic strength increased from 50 mM to 100 mM as was shown previously by Modig et al. (2006).

Peak asymmetry (defined as the ratio, at 10% of the peak height, of the distance between the peak maximum and the backside of the curve and the front side of the curve) was kept constant between 5 and 10 μg sample load for both ionic strengths (50 and 100 mM) and decreased for further higher loads (data not shown). Results of the elution times (time at peak maximum) of the three cationic starches at both ionic strengths (50 mM and 100 mM) showed that the increase of sample load led to shorter elution times (Fig. 4). Similar results were obtained by Modig et al. (2006). The larger quantity of material can cause exclusion of some particles from the volume immediately adjacent to the accumulation wall which is already occupied by a portion of a sample. This volume exclusion effect forces molecules to be located into regions distant from the wall making the sample to elute earlier (Cölfen & Antonietti, 2000). Therefore, too high mass load (i.e. overloading) may thus provide experimental conditions where separation according to size is non-functional during a certain fraction of the elution period.

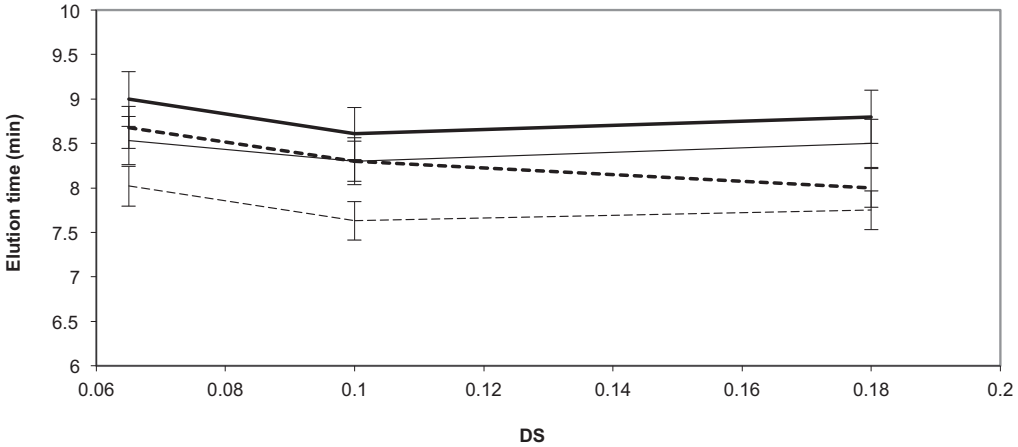

Fig. 5. Elution time vs. degree of substitution (DS) of cationic potato starch with DS of 0.065, 0.10 and 0.18. Sample loads of 5 and 20 μg. Continuous line 100 mM and dotted line 50 mM $NaNO_3$. - - - 5 μg and 100 mM, - - - 5 μg and 50 mM, – 20 μg and 100 mM, - - - 20 μg and 50 mM. F_c = 1.0 mL min^{-1} that decreased exponentially with a half time of 4 min, F_{out} = 1.0 mL min^{-1}.

No differences in the molar mass range were observed among cationic starches with different DS, which suggest no degradation due to a different degree of derivatisation process (results not shown).

Results of elution time vs. DS (Fig. 5) showed that a higher DS led to shorter elution times for the two sample loads of 5 and 20 μg. However no variation on the elution time or a very slight one was observed when the DS increased from 0.10 to 0.18. For a constant DS, smaller differences in elution time between 5 and 20 μg sample load were observed for 100 mM compared to 50 mM carrier (Fig. 5). According to Barman and Moon (2000), if elution time does not change with sample load, then overloading is absent. The smaller changes in elution time at higher ionic strength suggested that larger sample loads could be used at 100 mM without overloading problems.

Peak asymmetry slightly increased when sample load increased from 5 to 10 μg and decreased afterwards for higher mass load (data not shown). At the lowest ionic strength, the changes on peak asymmetry were much pronounced than at the higher ionic strength. The less overloading effect may be explained by charge shielding, causing reduction of repulsive intermolecular interactions.

4. Conclusions

The dry cationisation processing of starch leads to a formation of a more dense starch compared to cationic starch obtained from the wet cationisation. Differences on the starch density may influence the level of retention of starch on the paper sheet. No differences on the average molar mass between the starches obtained from the two derivatisation processes were found. However wet cationic starch had material of high molar mass in a higher amount than dry cationic starch. Additionally, no differences were obtained for apparent densities based on hydrodynamic radius. Dissolution of a starch at 150 °C for 40 min for further analyses produces depolymerisation of the starch. Milder thermal treatments should be used. The use of moderate ionic strengths, 50 mM or 100 mM, and sample loads between 5 and 10 μg are recommended to obtain low overloading effects and high detector signals.

Acknowledgement

This work was supported by Centre for Amphiphilic Polymers from Renewable Soruces, Lund, Sweden.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.02.002.

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