Interaction of industrially relevant cationic starches with cellulose

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Abstract

Industrially relevant, commercially available cationic starches have been investigated towards their interaction capacity with cellulose thin films derived from trimethylsilyl cellulose (TMSC). The starches used in this study stem from different sources (potato, pea, corn) and featured rather low degrees of substitution ranging from 0.030 to 0.062. The interaction of those starches with cellulose thin films was studied by surface plasmon resonance spectroscopy under flow conditions using concentrations of 1.0 $mg \cdot ml^{-1}$ and a flow rate of 25 $\mu l \cdot min^{-1}$. All the investigated starches employed in this study were capable to efficiently interact with the slightly negatively charged cellulose surface leading to irreversible deposition on the surface. As complementary techniques atomic force microscopy and x-ray photoelectron spectroscopy were used to confirm the presence of the starches on the cellulose film surface. Further, dynamic light scattering and size exclusion chromatography measurements were performed to correlate adsorbed amount, particle size and molecular weight of the starches to their interaction behavior.

Keywords: cationic starch; cellulose thin films; nanocellulose; surface plasmon resonance

1. Introduction

Cationic polymers are important materials for tailoring the properties of cellulosic substrates such as fibers and pulps. These polyelectrolytes carry a large amount of positive charges on their backbone and are capable to efficiently interact with negatively charged polymers. (Dobrynin, Deshkovski, & Rubinstein, 2001) The cationic polymers very often take the role of adhesion promoters, thereby triggering or enhancing the adsorption of other functional molecules/particles. Consequently, the interaction of many cationic polymers with a variety of cellulosic substrates has been extensively studied in the past. (Geffroy, Labeau, Wong, Cabane, & Cohen Stuart, 2000; Guan, Qian, Xiao, Zheng, & He, 2008; Hasani, Cranston, Westman, & Gray, 2008; K. S. Kontturi et al., 2009; K. S. Kontturi, Tammelin, Johansson, & Stenius, 2008; Lee, Lee, & Youn, 2014; Tamilselvan Mohan et al., 2014; T. Mohan, Ristic, et al., 2013; T. Mohan, Zarth, et al., 2013; Petersen, Radosta, Vorwerg, & Kießler, 2013; Schwikal et al., 2011; Terada, Samoshina, Nylander, & Lindman, 2004a, 2004b; Yokota, Kitaoka, & Wariishi, 2007) This originates from the importance of cationic polymers in fiber manufacturing processes as well as in papermaking. For instance, cationization of cellulosic textile fibers is often employed to increase anionic dye uptake on and into the fiber matrix. (Nechwatal, Michels, Kosan, & Nicolai, 2004) Further, the deposition of cationic starches has also been proposed to induce antimicrobial action against several microorganisms. (Guan, Qian, Xiao, & Zheng, 2008) In contrast, in paper industry the addition of cationic polymers and here in particular of cationic starches is pivotal to produce papers with better retention properties and higher dry strength.(Nachtergaele, 1989) A major issue to unravel the interaction of such cationic starches with cellulosic materials is their intrinsic inhomogeneity (e.g. diameter, morphology, accessibility, composition etc.) which

makes it difficult to extract fundamental parameters governing their interaction behavior in real time. Although there are some methods available which can track changes on e.g. fiber matrices such as isothermal titration calorimetry (thermodynamics) and zeta potential (charge) measurements, an elegant way to overcome problems associated with inhomogeneity is to use materials with a well-defined morphology, chemistry and charge.(Rohm, Hirn, Ganser, Teichert, & Schennach, 2014) A particularly well established system has been introduced by Klemm in 1993 and further developed by Kontturi which is based on mostly amorphous cellulose thin films. (E. Kontturi, Thüne, & Niemantsverdriet, 2003; Schaub, Wenz, Wegner, Stein, & Klemm, 1993) These two-dimensionally constrained films can be easily prepared by depositing solutions of a precursor (trimethylsilyl cellulose, TMSC) on a flat surface and by a subsequent spin coating step smooth films are obtained. These silylated cellulose films can be simply converted to cellulose thin films by exposing them to hydrochloric acid vapors over a period of several minutes. Their defined morphology, chemical composition and the homogenous surface allow for a detailed investigation of the surface properties and their interaction with other biomolecules such as proteins, or other polysaccharides. (Niegelhell et al., 2016; Hannes; Orelma, Johansson, Filpponen, Rojas, & Laine, 2012; Strasser et al., 2016) In this context, just a few studies focus on how cationic polysaccharides interact with cellulose thin films. Examples include different types of chitosans, cationic celluloses, cationic xylans and also cationic starches. (Lee et al., 2014; Tamilselvan Mohan et al., 2014; T. Mohan, Ristic, et al., 2013; T. Mohan, Zarth, et al., 2013; Hannes Orelma, Filpponen, Johansson, Laine, & Rojas, 2011) In these studies researchers employ cationic starches with different degrees of substitution (0.2-0.75) and focus on whether and to which extent the presence of electrolytes

change the interaction capacity with cellulose and as comparison to silica. (K. S. Kontturi et al., 2009; K. S. Kontturi et al., 2008) As for other charged polymers (Geffroy et al., 2000; Hasani et al., 2008; Tammelin, Saarinen, Österberg, & Laine, 2006; Yokota et al., 2007) it was demonstrated that the degree of coiling strongly determines the adsorption of the starches onto the surfaces. (Tammelin, Merta, Johansson, & Stenius, 2004) The degree of coiling in turn strongly correlates with the amount of electrolyte in the solutions since it prevents repulsion between individually charged segments by charge screening. Without such a screening the cationic polymers adopt a flat-like, extended conformation usually leading to the formation of thin layers. (Dobrynin et al., 2001; Dobrynin & Rubinstein, 2005) However, the use of starches directly employed for papermaking processes do often have rather low degrees of substitution DS (<0.08). Therefore, the repulsion between the substituted segments is small and can become at a certain point negligible leading to coiled conformations even in the absence of electrolytes. In this study, we aim at a deeper understanding on how such lowly substituted starches interact with cellulose thin films in real time using surface plasmon resonance spectroscopy (SPR). The employed cationic starches are manufactured in large quantities and are intended for papermaking issues. They feature DSs ranging from 0.030 to 0.062.

2. Experimental

2.1. Materials

Trimethylsilyl cellulose (TMSC, Avicel, M_w = 185,000 g·mol^{-1}, M_n = 30,400 g·mol^{-1}, PDI = 6.1 determined by SEC in chloroform) with a DS_{Si} value of 2.8-2.9 was purchased from TITK (Rudolstadt, Germany). SPR gold sensor slides (CEN102AU) were purchased from Cenibra, Germany. Milli-Q water (resistivity = 18.2 MΩ^{-1}·cm^{-1})

from a Millipore water purification system (Millipore, USA) was used for contact angle determinations and SPR experiments. Chloroform (99.3%), hydrochloric acid (37%), hydrogen peroxide (30% in water) and sulfuric acid (95%) were purchased from Sigma Aldrich and used as received. Wet end starches commonly used in paper production were used without further purification. Cationic starches were cooked at appropriate temperatures (95-125°C).

2.2. Substrate Cleaning and Film Preparation.

Prior to spin coating, SPR gold sensor slides/silicon wafers were immersed in a "piranha" solution containing H_2O_2 (30 wt.%)/H_2SO_4 (1:3 v/v) for 10 min, then extensively rinsed with MilliQ water and blow dried with N_2 gas. 100 µl of a TMSC solution (0.75 wt% in chloroform) were deposited onto the substrate and then rotated for 60 s at a spinning speed of 4000 rpm and an acceleration of 2500 $rpm \cdot s^{-1}$. To convert TMSC into pure cellulose, the sensors/wafers were placed in a polystyrene petri-dish (5 cm in diameter) containing 3 ml of 10 wt.% hydrochloric acid (HCl). The dish was covered with its cap and the films were exposed to the vapors of HCl for 12 min. The regeneration of cellulose from TMSC was verified by water contact angle (**Figure S1**) and ATR-IR (**Figure S2**) measurements as reported elsewhere. (Tamilselvan Mohan et al., 2012; T. Mohan et al., 2012)

2.3. Multi Parameter Surface Plasmon Resonance Spectroscopy – MP-SPR.

MP-SPR spectroscopy was accomplished with a SPR Navi 200 from Bionavis Ltd., Tampere, Finland, equipped with two different lasers (670 and 785 nm) in both measurement channels, using gold coated glass slides as substrate (gold layer 50

150 nm, chromium adhesion layer 10 nm). All measurements were performed using a full
151 angular scan (39–78°, scan speed: 8°·s^{-1}).
152 For the adsorption experiments, solutions of cationic starches have been prepared by
153 heating dispersions with a concentration of 1 mg·ml^{-1} (bone-dry substance) over a
154 period of one hour at 95°C. The resulting clear solutions were then directly used for
155 the adsorption experiments. For the adsorption experiments, the cellulose thin films
156 were allowed to equilibrate for 45 minutes before a continuous flow of the starch
157 solutions was pumped through. The starches were allowed to adsorb for 10 minutes
158 (flow rate 25 µl·min^{-1}). Afterward, the surfaces have been rinsed with MilliQ water to
159 remove loosely bound material.
160 The adsorption of cationic starches onto the cellulose surfaces was quantified
161 according to equation 1, which considers the dependence of the angular response of
162 the surface plasmon resonance in dependence of the refractive index increment
163 (*d*n/*d*c) of the adsorbing layer.

164 $$\Gamma = \frac{\Delta\Theta \times k \times d_p}{dn/dc} \qquad (1)$$

165 For thin layers (<100 nm), $k \times d_p$ can be considered constant and can be obtained by
166 calibration of the instrument by determination of the decay wave length λ_d. For the
167 SPR Navi 200 used in this study, $k \times d_p$ values are approximately $1.09 \cdot 10^{-7}$ cm/° (at
168 670 nm) and $1.9 \cdot 10^{-7}$ cm/° (at 785 nm) in aqueous systems. Since the degree of
169 substitution of the starches is very low, *d*n/*d*c values for native starches have been
170 used (0.150 $cm^3 \cdot g^{-1}$) to determine the amount of adsorbed masses. (Theisen, Johann,
171 Deacon, & Harding, 2000)
172
173 2.4. Dynamic Light Scattering

The DLS equipment consisted of a diode laser (Coherent Verdi V5, λ= 532 nm) and a goniometer with single-mode fiber detection optics (OZ from GMP, Zürich, Switzerland). The data was acquired by an ALV/SO-SIPD/DUAL photomultiplier with pseudo-cross correlation and an ALV 7004 Digital Multiple Tau Real Time Correlator (ALV, Langen, Germany). The correlation functions were determined and stored by the ALV software package. The light scattering (LS) was measured ten times 30 s at a scattering angle of 90° at 25°C and the resulting correlation functions were averaged. The hydrodynamic radius was calculated by the optimized regulation technique software (Schnablegger & Glatter, 1991) by means of the cumulant method. (Koppel, 1972) All cationic starch solutions have been centrifuged at 4000 rpm for 30 s prior to measurements.

2.5. Atomic Force Microscopy – AFM.

Surface morphology and roughness of the N_2-dried films were obtained in tapping mode in ambient atmosphere at room temperature by a Veeco Multimode Quadrax MM scanning probe microscope (Bruker; Billerica, MA, USA) using Si-cantilevers (NCH-VS1-W from NanoWorld AG, Neuchatel, Switzerland) with a resonance frequency of 320 kHz and a force constant of 42 $N \cdot m^{-1}$. Root mean square (RMS) roughness calculation and image processing was performed with the Nanoscope software (V7.30r1sr3, Veeco).

2.6. X-ray photoelectron spectroscopy

XPS spectra were recorded using a Thermo Scientific instrument equipped with a monochromatic Al-Kα X-ray source (1486.6 eV). High resolution scans were acquired at a pass energy of 50 eV and a step size (resolution) of 0.1 eV. Wide scans were

199 acquired with a pass energy of 100 eV and a step size of 1.0 eV. Photo-electrons
200 were collected using a take-off angle of 90 º relative to the sample surface. Charge
201 compensation was performed with an argon flood gun. All analyses were performed
202 at room temperature.
203
204 2.7. Size Exclusion Chromatography (SEC).
205 Size exclusion chromatography was performed on a SEC-3010 equipped with an
206 autosampler, a pump with degasser and a refractive index detector from WGE Dr.
207 Bures (Dallgow, Germany). Two analytical columns from AppliChrom (ABOA CatPhil
208 P-350 300 mm × 8 mm) were used at a flow rate of 1 ml·min^{-1}. The temperature of
209 the column oven was set to 30 °C. 0.1 M $NaNO_3$ was used as eluent. The calibration
210 curve was made with pullulan standards purchased from PSS (Mainz, Germany) with
211 molar masses ranging from 1,080 g·mol^{-1} to 708,000 g·mol^{-1}.
212
213 2.8. Contact Angle (CA) and Surface Free Energy (SFE) Determination.
214 Static contact angle measurements were performed with a Drop Shape Analysis
215 System DSA100 (Krüss GmbH, Hamburg, Germany) with a T1E CCD video camera
216 (25 fps) and the DSA1 v 1.90 software. Measurements were done with MilliQ water
217 and di-iodomethane using a droplet size of 3 µl and a dispense rate of 400 µl·min^{-1}.
218 For these experiments ex-situ coated silicon wafers have been used since the
219 adsorption slot of the SPR slides is just a few mm^2. The ex-situ adsorption was
220 performed in a similar manner as the in situ experiments using SPR. This involved
221 the swelling of the films in water for 45 minutes (deposition of ca 500 µl MilliQ water
222 on a 1 cm × 2 cm cellulose coated silicon wafer), then the water was exchanged by
223 the cationic starch solutions (1 mg·ml^{-1}) and allowed to adsorb for 10 minutes.

Afterwards, the samples were rinsed with 500 ml of MilliQ water and dried in air overnight at ambient temperatures. All measurements were performed at least 3 times. SCA were calculated with the Young-Laplace equation and the SFE was determined with the Owen-Wendt-Rabel-Kaelble (OWRK) method. (Kaelble, 1970; Owens & Wendt, 1969; Rabel, 1971)

3. Results and discussion

For the determination of the interaction capacity with cellulose thin films, different industrially relevant cationic starches (CS) were selected which feature very low degrees of substitution (0.030-0.062). As samples four different starches from different origins as well as a blend of two starches have been chosen for this study (**Table 1**).

Table 1. List of cationic starches used in this study including information on source, dry solids content, degree of substitution (DS) and molecular weight.

Source	Label	Dry Solids Content [%]	DS[a]	M_n [g·mol^{-1}]	M_w [g·mol^{-1}]	PDI
corn	A	87.3	0.058	7.7×10^4	1.7×10^5	2.18
potato	B	83.9	0.030-0.036	4.3×10^5	1.4×10^6	3.20
corn, potato	Blend A:B 80:20	85.2	0.050-0.056	n.d.	n.d.	n.d.
pea	C	81.9	0.047	3.7×10^5	7.4×10^5	1.99
waxy corn	D	86.9	0.062	1.3×10^6	2.4×10^6	1.94

[a] According to the manufacturer. (n.d. non determined)

Since the investigated starches do not completely dissolve at room temperature in water, they were cooked at appropriate temperatures (95-125°C, 1 mg·ml^{-1} bone-dry

241 substance) over a period of 60 minutes to obtain macroscopically clear solutions
242 suitable for adsorption experiments. It should be noted here that all the starches have
243 been used at their native pH values (5.0-5.8) (**Table S1**); any adjustment of pH value
244 or addition of electrolytes was intentionally avoided for this study. These
245 macroscopically clear solutions were investigated by size exclusion chromatography
246 and dynamic light scattering after cooling to room temperature. Size exclusion
247 chromatography results are in agreement with literature, where molar masses of
248 approximately 10^5-10^6 g·mol^{-1} and 10^7-10^8 g·mol^{-1}, have been determined for
249 amylose and amylopectin respectively. (Banks & Greenwood, 1975) In general, waxy
250 corn starches feature amylose contents of maximum 1% (Pérez & Bertoft, 2010) and
251 therefore CS D displays the highest molar mass of the herein studied starches
252 concomitant with a small polydispersity index. Starches containing relatively high
253 amylose contents, such as pea starch (40-70%) and corn starch (30 %) feature lower
254 molar masses compared to starches derived from waxy corn or potato (20% amylose
255 content). (McCready, Guggolz, Silviera, & Owens, 1950; Rosin, Lajolo, & Menezes,
256 2002)
257 As depicted in **Figure 1**, all of the investigated starches form particles with a size
258 (median hydrodynamic diameter) ranging from 57 (CS D) to 100 nm (Blend, CS C).
259 However, the size distribution is at least of bimodal nature and shows larger particles
260 (micron range) as well as very small particles with a size of ca 10 nm. It is important
261 to point out that particles with larger size lead to a response with higher intensity than
262 smaller particles. Interestingly, there are not any particles in the micron range size
263 present for the starch solutions consisting mostly of amylopectin (CS D). It could be
264 concluded that the large particles present in solutions of the other compounds (CS A,
265 B, and C) are composed of starch clusters formed by aggregation of amylose and

amylopectin. (Shirazi, van de Ven, & Garnier, 2003a) Although amylopectin is the starch component with higher molecular weight, it is of branched nature and therefore the hydrodynamic radius is smaller for CS D than for the other starches containing non-branched amylose.

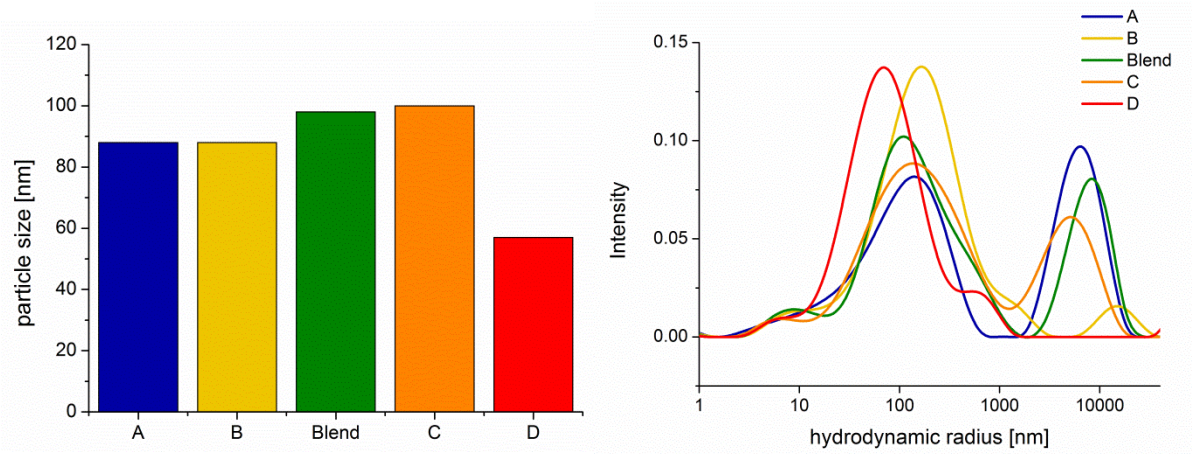

Figure 1. Left: Comparison of the median particle size of the different cationic starches in aqueous solution (c = 1 mg·ml^{-1}) determined by DLS in the 57-100 nm regime. Right: Intensity weighted size distribution of the cationic starch solutions obtained by DLS.

For adsorption experiments using surface plasmon resonance spectroscopy, the same dissolution procedure was applied as for the samples used in SEC and DLS experiments. These adsorption experiments were designed in way that after equilibration of the cellulose thin films over a period of 45 minutes in water, the cationic starches were pumped through the microfluidic channel and allowed to interact until a plateau was reached (i.e. after 10 minutes). Afterwards, the samples were rinsed with water in order to remove loosely bound materials from the cellulose thin films. The change in the surface plasmon resonance angle is then used to determine the amount of deposited cationic starches onto the surfaces according to equation (1).

As depicted in **Figure 2**, the cationic starches irreversibly adsorb onto the cellulose thin films and a final rinsing step did not remove a significant amount of material from any of the films. This can be attributed to reorientation of the adsorbed molecules as well, since SPR detects changes in refractive index which is traceable to adsorption, desorption or density variations. Furthermore, adsorption occurs spontaneously and kinetics is fast, demonstrating a high affinity of the CS to the cellulose films. However, the extent of adsorption strongly varies for all the investigated samples ranging from 0.74 ± 0.15 (CS D) to ca. 3.94 ± 0.13 $mg \cdot m^{-2}$ (CS A). An exception in terms of kinetics is the starch B sample whose adsorption profile is much slower than for all the other investigated samples. Factors governing the kinetics in the adsorption of polymers can be related to molecular weight and to the PDI, to mention just two parameters. It is known from literature that a polymer with higher PDI is prone to slower adsorption than the same polymer with a smaller PDI. Interestingly, the PDI is the highest for CS B sample which could be the reason for the rather slow adsorption on cellulose thin films. (Kronberg, Holmberg, & Lindman, 2014) This phenomenon initially proven for proteins and often recalled the Vroman effect, relates to species with a lower molar mass which are slowly replaced by those with a higher mass, thereby causing much slower kinetics.

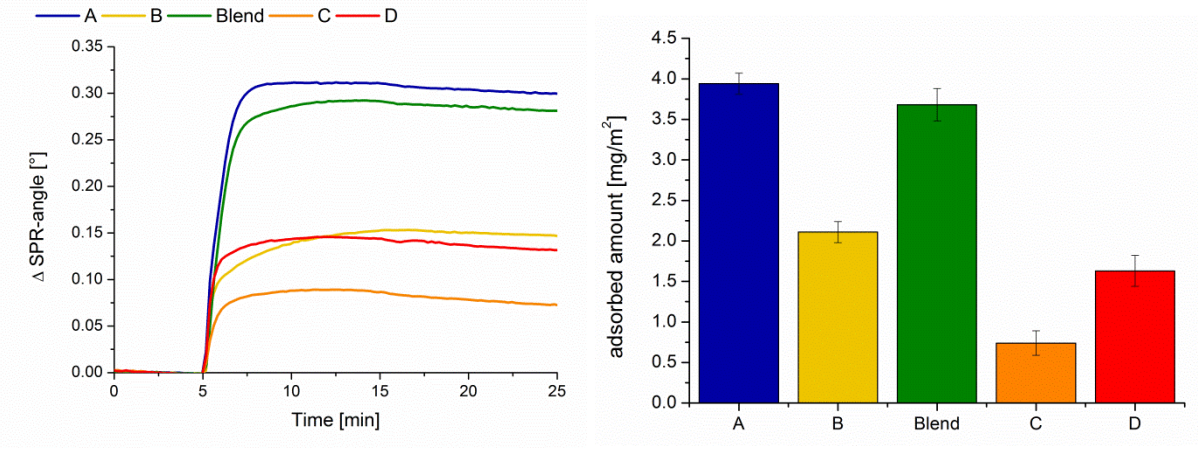

Figure 2. Comparison of the adsorption behavior of the different starches (c= 1 $mg \cdot ml^{-1}$, flow rate 25 $\mu l \cdot min^{-1}$) as a function of the SPR angle (left) and corresponding adsorbed masses (right).

A similar behavior is observed for the blend of CS A and CS B (80:20). Here, the extent of adsorbed material (3.68 ± 0.2 $mg \cdot m^{-2}$) matches the calculated value of the individual components (3.57 ± 0.13 $mg \cdot m^{-2}$) but the adsorption kinetics is rather different. In the beginning of the adsorption experiment, the blend behaves like CS A (same slope) with a very fast adsorption rate. However, after 2 minutes the slope of the curve becomes less steep i.e. the adsorption is slower and proceeds in a similar fashion as the curve of CS B. As for the CS B adsorption, the fraction with higher molar mass slowly replaces those with lower molar mass as mentioned in literature for other polymer blends having different molar masses. (Dijt, Cohen Stuart, & Fleer, 1994; Fu & Santore, 1998)

After drying in air at room temperature, the same films from SPR experiments were subjected to XPS measurements to confirm the presence of nitrogen stemming from the cationically charged groups (i.e. ammonium groups) in CS on the cellulose films. The XPS results are shown in **Table 2** and clearly show the presence of the cationic species on those films exposed to CS solutions whereas the neat cellulose films do not contain any nitrogen. However, a quantitative assessment using XPS is not always straightforward since the recorded intensities depend on several parameters e.g. the homogeneous distribution of starch particles throughout the sample. Besides the N_{1s} also the Cl_{2p} signal is observed, which relates to the chloride counterion and matches the nitrogen content. Carbon and oxygen signals arise from the backbone of starch and the underlying cellulose layer, which is plausible since even some signal

originating from the gold substrate is detectable to a minor extent. The full survey scans are available in the Supporting Materials (**Figure S1-S6**).

Table 2. Elemental composition (atom%) of the pure and cationically modified cellulose films determined by XPS.

	atom%					
	Cellulose	A	B	blend	C	D
C_{1s}	58.1	59.4	58.9	59.2	59.1	59.2
O_{1s}	38.0	36.8	38.5	37.9	39.0	38.9
N_{1s}	**<LOD**	**0.8**	**1.1**	**1.1**	**0.9**	**1.0**
Cl_{2p}	<0.5	1.0	0.9	0.9	0.8	1.0

Further, the SPR samples were subjected to atomic force microscopy. The topography images (**Figure 3**) do not exhibit large differences between the different samples before and after adsorption, albeit a decrease in surface roughness is observed after CS adsorption. This can be explained by cationic starch particles filling the pores and holes present on the cellulose thin film. Despite the similar chemical nature of adsorbens and the cellulose surface, the amplitude images provide (**Figure 4**) better insights. In those images, it can be clearly seen that larger particulates are present for CS A and the blend, whereas for the other samples rather small features are present. The size of these features is in good agreement with the results obtained by DLS, where both, CS A and the blend, feature a bimodal size distribution with some large particles. CS C displays aggregates with large particles size too, however, the amount deposited on the cellulose films is rather low to be detected by AFM.

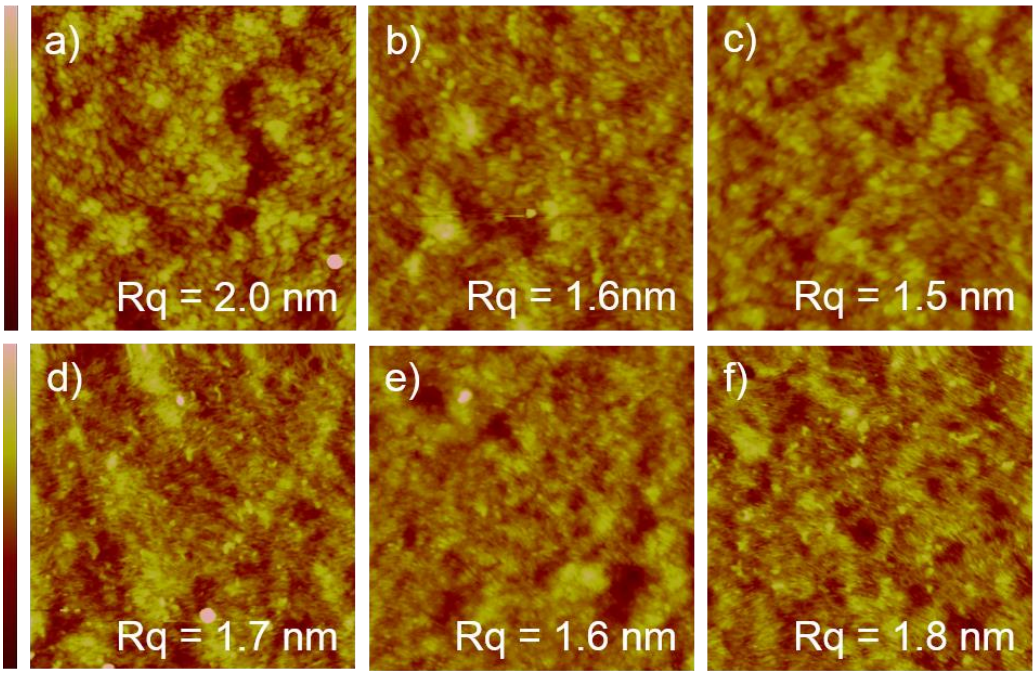

Figure 3. Comparison of AFM topography images (1x1 μm^2, z-scale 20 nm) of the different samples before (a) and after adsorption (b-f). b: CS A, c: CS B, d: blend, e: CS C, f: CS D. All samples have been measured in air at ambient atmosphere.

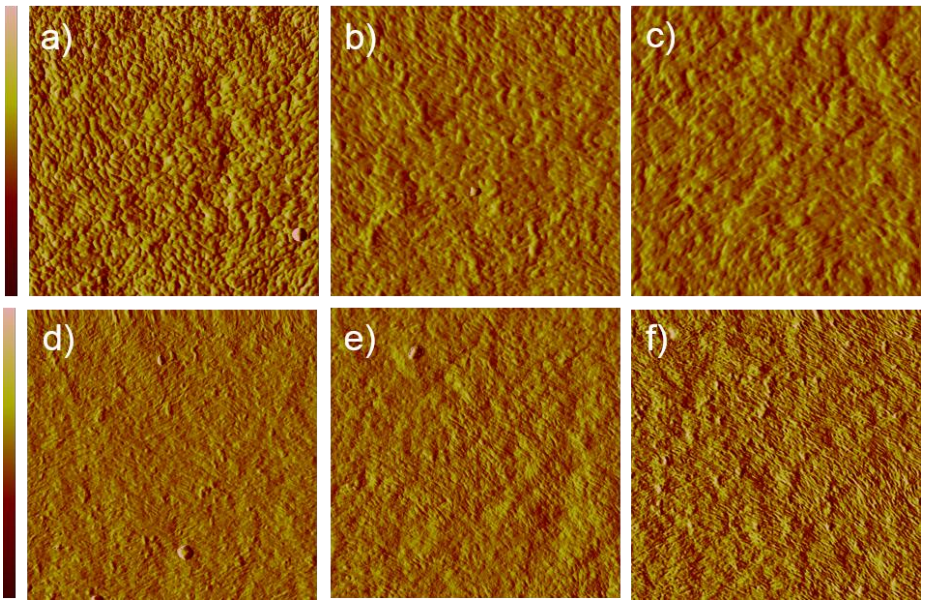

Figure 4. Comparison of AFM amplitude images (1x1 μm^2, z-scale 10mV) of the different samples before (a) and after adsorption (b-f). b: CS A, c: CS B, d: blend, e: CS C, f: CS D. All samples have been measured in air at ambient atmosphere.

The modification of a surface by a charged polymer should impact the hydrophilicity of the surface to some extent as well. The surface free energies of the films, determined by wettability studies using water and diiodomethane, increased after the deposition of the cationic starches on the surfaces (**Figure 5**). However, the increase in SFE does not significantly relate to the amount of cationic starch deposited on the surface and SFEs in the range of 70 $mJ \cdot m^{-2}$ have been determined. Only for the sample with the lowest affinity, a slightly lower SFE was observed. However, even for the CS with a rather low affinity to the surface, the SFE increased compared to cellulose (67 $mJ \cdot m^{-2}$ for CS D vs. 62 $mJ \cdot m^{-2}$ for cellulose). The increase in SFE can be mainly attributed to higher polar contributions to SFE in the case of CS compared to cellulose.

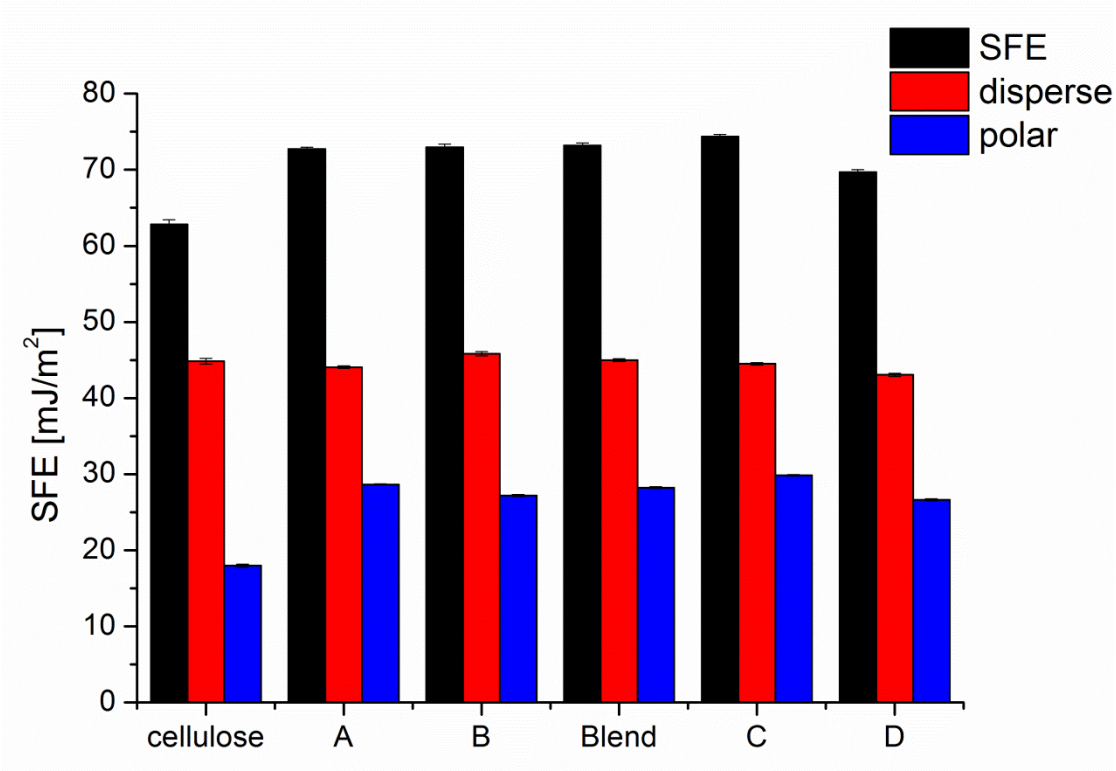

Figure 5. Comparison of the SFE of non-modified cellulose thin films and those modified with CS.

The results can be rationalized in following respects. The interaction of any type of polymer with a surface is governed by the properties of the individual components (e.g. charge density), the conformation of the adsorbens (flat vs. coiled) and the solubility of the polymer at the interface.(Dobrynin & Rubinstein, 2005) The SPR data shows significant differences in the interaction capacity of cellulose thin films with different cationic starches. These differences depend partially on the charge of the substrate and the adsorbens. However, for the industrially relevant CS, the amount of cationic groups is rather low. When considering an average length of 0.5 nm of an anhydrous glucose unit (AGU), the mean distance between the charges ranges from 8 to 15 nm for the CS with the lowest and highest DS, respectively. Considering the molecular weights of the different compounds employed in this study, the amount of charged groups that are present on the polymer backbone were calculated and are shown in **Table 3**. The calculations assume perfectly flat, non aggregated starch molecules and neither take into account the uneven distribution of the cationic groups within the starch molecules nor the difference in cationization of amylose and amylopectin within the type of CS. In general, amylose is better accessible for cationic modification than amylopectin leading to a higher DS. (Steeneken & Smith, 1991) Despite the low mean number of charges per molecule compared to cationic starches with higher degree of substitution, a relatively high interaction capacity is found for the starches applied in this study. In terms of nitrogen content determined by XPS, all the modified cellulose featured higher N contents than those reported in literature where many CS with higher DS had been used (0.2 and 0.75). (K. S. Kontturi et al., 2008)

Table 3. Mean distance between charges, mean length of starch molecules and number of cationic charges present on the polymer backbone in an extended flat conformation of different CS calculated from molecular weights and DS.

	mean distance charges [nm]	mean length molecule [nm]	mean number of charges
A	8.6	376	44
B	15.2	2810	185
Blend	9.4	3514	372
C	10.6	1715	161
D	8.1	5690	706

Results from cationic starches with the same particle size reveal the influence of the DS and the amount of cationic charges at the polymer backbone. For instance, CS A (3.94 ± 0.13 $mg \cdot m^{-2}$) and CS B (2.11 ± 0.13 $mg \cdot m^{-2}$) features the same particle size, but a different DS. Higher DS basically leads to more electrostatic attraction between the cellulosic substrate and the compound leading to more pronounced interaction and finally adsorption. Additionally, CS A and CS B have a similar content of amylose, therefore the uneven distribution of cationic charges between amylose and amylopectin can be considered negligible. This effect is observed for the blend (3.68 ± 0.2 $mg \cdot m^{-2}$) and CS C (0.74 ± 0.15 $mg \cdot m^{-2}$) as well. In this case, the amylose content differs, which may have an effect in the adsorption behavior.

CS B and CS C (**Table 3**) exhibit different adsorption capacities on the cellulose films, even though they contain a similar amount of cationic charges. Interestingly, the compound with the higher DS (CS C) adsorbs to a lesser extent, whereby the adsorbed amount of the CS that forms the smaller aggregates (CS B) is more than twice as high. This indicates that besides the DS, additional factors influence the adsorption behavior of the cationic starches. One of these factors is the solubility of the polymers which is dependent on molecular weight, the amylose/amylopectin ratio

414 and the charge. (Kronberg et al., 2014) Polymer solubility increases with the amount
415 of charges on a polyelectrolyte leading to lower adsorption. However, this is in
416 contradiction with the electrostatic interaction between adsorbens and substrate,
417 since a high DS favors electrostatic attraction to the negatively charged cellulose
418 substrate (ζ-potential ca. -18 mV at pH 5-6 (Ristić et al., 2014)). This phenomenon is
419 observed for compounds with the same particle size, such as CS A and CS B or the
420 blend and CS C, where electrostatic attraction is overruled by solubility. Besides
421 charge, molecular weight and particle size control the solubility. In principle, low
422 molecular weight polymers are better soluble than those with high molecular weight,
423 therefore large molecules tend to adsorb in larger amounts but - as already
424 discussed above - slower. The same effect applies for particle sizes. Different
425 observations have been made by Shirazi and coworkers, who investigated the
426 adsorption of cationic starches on pulp fibers. (Shirazi, van de Ven, & Garnier,
427 2003b) They proposed an initial adsorption step of starch aggregates followed by a
428 deposition of individual macromolecules onto the cellulose fiber surface.
429 The influence of particle hydrodynamic radius can be illustrated by comparing CS A
430 (88 nm), the blend (98 nm) and CS D (57 nm), all having a similar DS. Compared to
431 CS D for instance, larger amounts of CS A and the blend (compared to CS D) adsorb
432 onto the cellulose films since larger particles are less soluble and tend to adsorb to a
433 higher extent. Moreover, conformation and the resulting availability of attachment
434 sites are decisive factors as well. As seen in the above mentioned case of CS C and
435 CS B, this parameter is important and can be ascribed to the amylose content of the
436 starches. Since amylose is the low molecular polymer in starch and is better
437 accessible to the cationization procedure than amylopectin, therefore featuring a
438 higher DS, the amylose proportion of the cationic starch keeps dissolved while larger

starch clusters adsorb. CS D, the cationic starch with the highest molecular weight and amylopectin content, displays theoretically the highest amount of charges (~706) present on the polymer backbone. However, due to the conformation of amylopectin, fewer charges are accessible for interaction with the surface. (Shirazi et al., 2003b; Swerin & Wågberg, 1994) The branched structure of amylopectin permits less freedom in chain movement and does not easily rearrange compared to amylose. In addition to that, CS D forms rather small particles, thereby increasing their solubility.

Of course ionic strength is an essential parameter in the interaction of oppositely charged materials, because it impacts conformation by charge screening. Although the investigation of the influence of ionic strength on the adsorption behavior was not the main focus of this work, for the sake of comparison one sample (CS B) was selected and adsorbed at different electrolyte concentrations (1 mM, 10mM, 100 mM NaCl) in the same SPR setup as for the other samples. The results are summarized in **Figure S8-S12**. Results follow the expected trends and fit to data reported in literature. (Shirazi et al., 2003b) The particle size of CS B decreases upon addition of salt to the CS solution from 88 nm (pure water) to approximately 75 nm due to screening of the positive charges and reduction of electrostatic repulsion in the polymer. The increase of the ionic strength to 1 mM NaCl leads to a higher extent of adsorption. However, a further increase in salt concentration does not only decrease intramolecular repulsion but the surface-starch interactions are screened too, leading to even smaller adsorbed amounts of CS B than in pure water.

4. Conclusion

In summary, the adsorption behavior of industrially relevant cationic starches with low degrees of substitution (0.03-0.06) on cellulose thin films was investigated in the

absence of any electrolytes and at native pH value of the starches. It turned out that all the investigated starches irreversibly adsorb onto cellulose, whereas the extent of mass deposited on the surfaces varies from ca 0.7 to ca 4.0 $mg \cdot m^{-2}$. For CS with the same size, the adsorption capacity is mainly influenced by the DS. For those CS, a high DS leads to higher deposition on the surface. In these cases, electrostatic interactions overcome solubility of the polymers. When it comes to starches with very similar DS, the particle size dominates adsorption, since smaller particles are better soluble and therefore adsorb to a lesser extent. Another crucial factor is the conformation of the polymer and concomitant the availability of attachment sites, which is among others dependent on the amylose-amylopectin ratio of the starch. In conclusion, cationic starch adsorption behavior is primarily governed by the interplay of solubility and electrostatic interaction which are both influenced by different parameters such as particle size, molecular weight, conformation and DS. The influence of the substrate's negative charge stemming from partial oxidation i.e. carboxylic groups on the adsorption behavior of cationic compounds is an additional factor, which is certainly critical for industrial materials featuring various degrees of oxidation. Currently, we are investigating whether these results are transferable to industrially manufactured materials such as fibers and papers.

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