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On the determination of charge and nitrogen content in cellulose fibres modified to contain quaternary amine functionality

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ABSTRACT

Research interest in quaternization of cellulose fibres has increased considerably over the past decades. However, there is little or no consensus regarding how to characterize the material in terms of degree of substitution (DS), and the literature suggests a range of different methods focusing on charge determination as well as nitrogen content quantification. This work aims to fill the knowledge gap regarding how the different methods perform in relation to each other, and for what cellulosic systems each method has advantages, disadvantages and even potential pitfalls. FT-IR and NMR measurements are used to establish successful modification and determine the relative number of substituent groups. Another six methods are compared for the determination of the DS of cellulosic fibres and nanofibrils. The methods include Kjeldahl measurements, nitrogen determination by chemiluminescence, determination of molecular nitrogen by the Dumas method, colloidal titration, conductometric titration and polyelectrolyte adsorption. It can be concluded that most techniques investigated are reliable within certain ranges of DS and/or when using appropriate post-treatment of the quaternized material and suitable sample preparation techniques. The results from the present work hence provide recommendations to make an educated choice of method, and experimental protocol, based on the technique at hand.

1. Introduction

Striving towards a more sustainable society, there is a growing demand for reduced use of non-renewable materials from fossil-based resources, and a simultaneous push towards the development of functional and green alternatives. Using renewable and green materials from nature is an alternative to developing such materials, and the lignocellulosic feedstock provides a source that is available on a large scale (Klemm et al., 2005). Cellulosic fibres retrieved from this source might, however, need to be combined with additives or to be modified to improve, or simply change, the properties of the starting material. In papermaking, cationic starch is commonly used as a retention aid and paper strength additive (Lee et al., 2002; Prado & Matulewicz, 2014). The starch additives are prepared by quaternization, which is most commonly performed using 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) or its ring-closed counterpart 2,3-epoxypropyltrimethylammonium chloride (EPTMAC), as reagent, which yields the same functional moiety, a trimethylammonium group, on the polysaccharide chain (Choudhury, 2014; Prado & Matulewicz, 2014). Both reagents are commercially available, and the understanding of the reaction mechanism with polysaccharides is fairly well described in the literature.

The last decades of research efforts have also been focused on quaternization of lignocellulosic raw materials for different applications, but maybe most frequently to prepare cationic cellulose nanofibrils (CNFs), using the same reactions and reagents, i.e. CHPTAC and EPTMAC, as applied for the starch, and the number of scientific papers dealing with the subject has increased significantly. Using the search terms “quaternization+cellulose” in the Google Scholar database indicates an almost exponential increase in publications over the last 20 years (Fig. 1).

The starting materials presented among the literature include different types of bleached pulps (Olszewska et al., 2011; Pei et al., 2013; Willberg-Keyriläinen et al., 2019), cotton linters (Baouab et al., 2000; Hasani et al., 2009; Moral et al., 2016) and cellulose nanocrystals (CNCs) (Hasani et al., 2008; Zaman et al., 2012), but also waste

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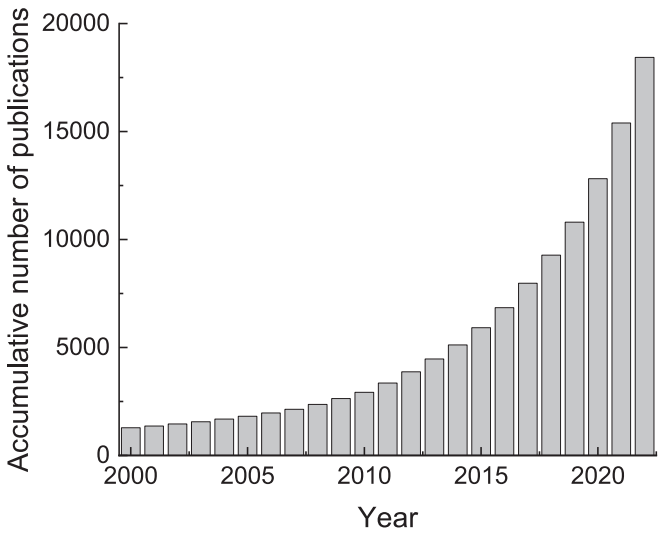
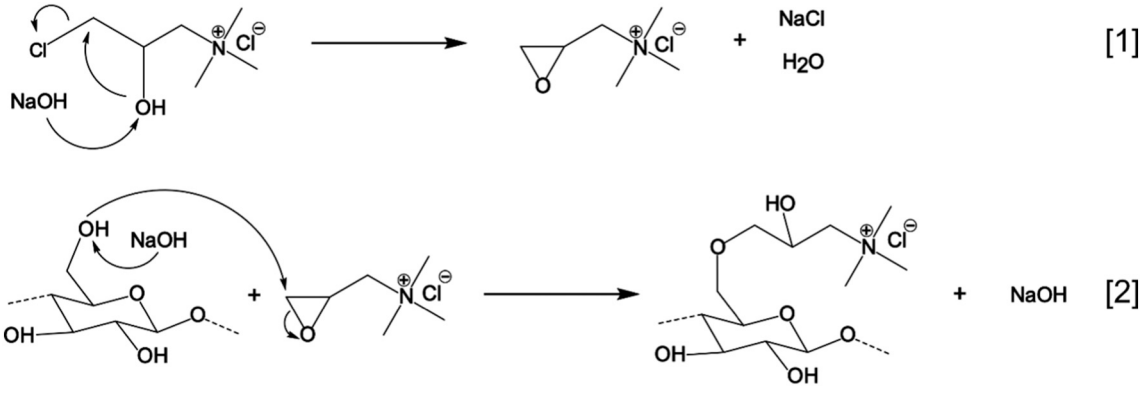


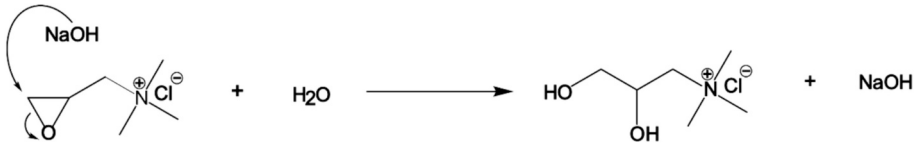
Fig. 1. Number of publications on the subject of quaternization of cellulose. Data retrieved from Google Scholar using the search-term "Quaternization+Cellulose".

materials such as wood saw dust (Baouab et al., 2001; Zghida et al., 2003), crop residues (Aguado et al., 2018) and fibre sludge (Sehaqui et al., 2015). The applications of the quaternized materials include immobilization of dyes and other anionic substances from water, which is of interest both in terms of increasing the efficiency of textile dying and removing of residual dye from waste-water (Baouab et al., 2001; Pei et al., 2013; Sehaqui et al., 2015; Zghida et al., 2003). In papermaking, the affinity to anionic substances also makes quaternized CNFs, promising candidates for further use as paper additives to improve both retention of other additives and fillers and to act as strengthening aids (Gao et al., 2016; Lu et al., 2020). An interesting example of this is the use of quaternized cellulose to enhance the adsorption of PEDOT:PSS (Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate) in the preparation of paper-based electrodes for energy storage devices (Isacsson et al., 2022). Quaternized cellulose in combination with other materials has also been studied for use in other non-conventional paper applications and dispersions of cationic CNFs and clay have been used for the production of mechanically strong composite films (Ho et al., 2012; Sethi et al., 2022). Quaternary groups are also recognized for their antimicrobial properties (Grigoras, 2021). Cellulosic materials, including fibres, CNFs and aerogels with bacterial-growth-inhibiting properties, have been developed by the build-up of polyelectrolyte multilayers based on quaternary polyvinylamine and polyacrylic acid (Henschen et al., 2016; Illergård et al., 2012). It has also been demonstrated that it is possible to achieve efficient inhibition of several types of bacteria when subjecting them to nanocomposites based on quaternized CNFs and polyvinylalcohol (Chaker & Boufi, 2015).

Quaternization of cellulose using CHPTAC or EPTMAC is described to occur through an alkali-activated epoxide-ring-opening reaction (Scheme 1), accompanied by an irreversible side reaction (Scheme 2) (Prado & Matulewicz, 2014). Since the properties of quaternized cellulose, as with any modified cellulose or cellulose derivative, will strongly depend on the number of functionalized groups, it is essential to use a reliable method for determining the DS of the quaternized material. The multifunctionality of the substituent group, i.e. being nitrogen containing, carrying a cationic charge and having an electrostatically bound counterion, provides several approaches for evaluation of the specific DS. Aguado et al. (2018) and Ho et al. (2011) use combustion-based Dumas elemental analysis to determine the nitrogen while Olszewska et al. (2011) and Zaman et al. (2012) determine the nitrogen content by chemiluminescence. Several studies also use what is referred to as classic Kjeldahl analysis to determine the nitrogen content of the quaternized samples (Rana et al., 2021; Willberg-Keyriläinen et al., 2019). Others use a determination of the counterion to the cationic charges, typically a chloride ion, through a conductometric titration for the determination of the DS, assuming a 1:1 ratio between the two (Hasani et al., 2008; Odabas et al., 2016; Pei et al., 2013). Another method to determine the charge of the modified material, and hence DS, uses a direct colloidal titration of cationic CNFs or CNCs, assuming that all charges are located at surfaces of the components and available to the titrant (Liimatainen et al., 2014; Rostami et al., 2022; Zaman et al., 2012). In addition to the above-mentioned methods, nuclear magnetic resonance spectroscopy (NMR) has been suggested as a potential method. However, the insolubility of cellulose fibres in common NMR-compatible solvents limits the use of NMR for determination of the degree of substitution (King



Scheme 1. Chemical reactions for quaternization of cellulose. [1] Conversion of CHPTAC into EPTMAC, [2] Substitution reaction for covalent linking of a quaternized group to cellulose



Scheme 2. Irreversible consumption of EPTMAC by alkaline hydrolysis

et al., 2018). de la Motte et al. (2011) investigated the possibility of using NMR for determining DS of hydrolyzed cellulosic materials, but the extraction of useful information in their study was limited due to the weak signals recorded. Only after increasing the concentration of the cationic monosaccharides by ion exchange chromatography could an estimation of DS be performed from the 1D ^{1}H NMR spectra, and these estimated values were slightly higher than the values obtained by elemental analysis (de la Motte et al., 2011; de la Motte & Westman, 2012).

From the literature it is, surprisingly, clear that there is no consensus regarding which approach or technique to use to determine the DS of quaternized cellulosic materials. To the best of the authors' knowledge, no attempts to compare the different methods have ever been presented although proper comparability is of utmost importance since the DS is expected to have a strong influence on the properties of the modified material. The present work therefore aims to fill the knowledge gap regarding the performance of commonly used methods for determining the DS of quaternized cellulosic fibres and CNFs. In the study, the five most commonly reported methods for determining the DS of quaternized cellulose are compared, these include: elemental analysis of molecular nitrogen after combustion, i.e. the Dumas method; elemental analysis by chemiluminescence emitted upon relaxation of nitrogen dioxide excited by ozone after combustion; nitrogen determination by Kjeldahl; conductometric titration of fibres; and direct colloidal titration of CNFs after fibrillation of the fibres. In addition, a sixth method based on polyelectrolyte adsorption of a small polyelectrolyte is included in the comparison. It is hypothesized that all methods included in this study can be used for the determination of the DS, but only given certain limitations and conditions. The results and conclusions presented in this work will therefore be crucial for correct comparison of results from different research groups using different techniques for determining the DS. This study might also serve as a guide when choosing an appropriate technique and methodology for characterization of quaternized cellulosic material.

2. Material and methods

2.1. Chemicals

The cationization reagent, EPTMAC, was purchased from Sigma Aldrich (by the name Glycidyltrimethylammonium chloride technical ≥90 %). Hydrochloric acid solution was prepared from 37 % ACS reagent hydrochloric acid also from Sigma Aldrich. Sodium hydroxide (pellets, purity >98.5, AnalaR NORMAPUR® Reag. Ph. Eur.), silver nitrate (*ReagentPlus*®, ≥99.0 %), dextran (from *Leuconostoc mesenteroides*, average molar mass approximately 2000 kDa), sodium bicarbonate (powder, BioReagent) and poly(acrylic acid sodium salt) (PAA, average $M_w \sim 5100$ Da) were acquired from VWR Chemicals and Sigma Aldrich, respectively. These were used without further purification to prepare the solutions used for the experiments.

2.2. Cellulose fibres

All modifications were performed using a fully bleached softwood Kraft pulp from Scots pine and Norwegian spruce, kindly provided by Stora Enso AB, Skoghall mill, Karlstad, Sweden. A thorough characterization of the starting material, including sugar analysis and fibre dimensions, is presented in Supplementary Data, Tables S1 and S2. Before any use, the pulp was washed to ensure sodium as counterion to anionic groups present and to remove dissolved and colloidal substances that might be presented in the fibres. Washings were performed batch-wise with 50 g dry pulp by suspending the never-dried pulp to a concentration of 20 g/L; the pH was adjusted to pH 2.0 by the addition of 1.0 M HCl, and thereafter left for 30 min. The suspension was then washed with deionized water until the conductivity of the filtrate dropped below 5 μS/cm. A second washing step was then performed where the still-wet fibres were resuspended to 20 g/L in a 0.001 M sodium bicarbonate solution; pH was set to pH 9.0 by the addition 1.0 M NaOH. After 30 min, the suspension was again filtrated and washed with deionized water until the conductivity dropped below 5 μS/cm. The washed pulp was collected in air-tight zip-lock bags and stored in the fridge (8 °C) until further use; dry content was measured in triplicate. The measurements were performed by overnight drying of a representative pulp sample in pre-weighted aluminum cups in a 105 °C oven, noting the weight of the material before and after drying. The dry content was calculated according to Eq. (1).

$$Dry\ content = \frac{m_{dry}}{m_{wet}} \quad (1)$$

where m_{dry} and m_{wet} are the masses of the dry and wet sample, respectively, after subtracting the mass of the aluminum cup.

2.3. Fibre modification

A wet sample containing 30 g of dry fibres was then transferred to a polyethene plastic bag and soaked in sodium hydroxide solution. This alkali pre-treatment was performed for 30 min at room temperature before EPTMAC was slowly poured into the reaction mixture. The reaction mixture was kneaded (by hand) for 5 min to ensure proper mixing before immersing the sealed bag into a 60 °C water bath. The concentrations of the reaction mixtures were set such that the final (i.e. after the addition of EPTMAC to the fibres) dry content of fibres was 10 wt% and the NaOH concentration was 4 wt%. As a guide, calculations and doses have been summarized in Supplementary Data, Table S3. The amount of reagent added was adjusted between the reaction batches to obtain materials with a range of degrees of modification. More specifically, EPTMAC was added to the reaction mixtures at the mole ratios of 0.5, 1, 2 and 4 with respect to the number of anhydroglucose units present in the fibres, assuming pure cellulose in the sample. The water content of the EPTMAC reagent corresponding to 25 wt% was adjusted for when dosing the reagent and when calculating the dry content of the reaction mixture (i.e. the cellulose dry content and alkali concentration were somewhat higher than 10 wt% and 4 wt%, respectively, before mixing). The reaction was allowed to proceed for 6 h before the material was transferred to a Büchner funnel. After removal of the alkali solution by vacuum filtration, the modified pulp was immersed in 150 mL of 1.0 M HCl before washing with deionized water. Washing was performed until the conductivity of the filtrate dropped below 5 μS/cm. The modified pulp was collected in air-tight zip-lock bags and stored in the fridge (8 °C) until further use. Dry content of the samples was measured in triplicate according to the same procedure as described for the washed pulp (Eq. (1)).

2.4. Homogenization of fibres and preparation of CNF dispersions

From each batch of modified fibres, 5 g were homogenized to CNFs. To perform this, the never-dried fibres were resuspended to a concentration of 10 g/L using deionized water and fluidized using a high-pressure microfluidizer (Microfluidics M-110EH, Microfluidics Corp., Boston, USA). The sample was subjected to one passage through a series of a 400 μm and a 200 μm chamber followed by three passages through a series of 200 μm and a 100 μm chamber to obtain a CNF gel. The pressures applied were 800 and 1600 bar, respectively, for the two chamber combinations. The dry content of the gel was obtained by overnight drying of the CNF gel at 105 °C. To minimize errors in the measurement due to moisture sorption, the samples were prepared in pre-weighted glass vials and the final dry weight was measured after cooling the samples in a desiccator. Measurements were performed in triplicate and the dry content was calculated according to Eq. (1). CNF dispersions were prepared by diluting the CNF gels to a solids content of 2 g/L followed by Ultra Turrax mixing at 12000 rpm for 10 min. Aggregates

were dispersed by sonication at 40 % amplitude for 10 min, an ice bath was used to avoid excessive heating of the sample. To assess the nano-fraction of the dispersions, they were centrifuged at 2600*g* for 1 h. No traces of a pellet could be distinguished visually, indicating that the fibres had been successfully fibrillated to CNFs. Dry content of the dispersion was determined according to the same procedure as described for the gel. Lastly, the CNFs were characterized in terms of length and width using atomic force microscopy, AFM, results are shown in Supplementary Data, Table S4 and Fig. 1S.

2.5. Analyses

2.5.1. Infrared spectroscopy

Fourier-transform infrared spectrometry (FT-IR) was performed using a PerkinElmer Spectrum 100 FT-IR equipped with a single reflection attenuated total reflection system and a MKII Golden Gate. The infrared spectra were attained from paper sheets made from the modified fibres. All samples were analysed with 32 scans, at a 1 cm^{-1} resolution, from 4000 to 600 cm^{-1}. The background was removed from the spectra together with the contributions from moisture and carbon dioxide. Spectra were normalized to the maximum peak height at 1030 cm^{-1}.

2.5.2. Nuclear magnetic resonance (NMR) spectroscopy

2.5.2.1. Solid-state cross-polarization/magic angle spinning carbon-13 (CP/MAS ^{13}C) NMR. Never-dried modified fibres and reference fibres were subjected to solvent exchange with acetone followed by drying under ambient climate. The dry samples were packed uniformly in a zirconium oxide rotor with an outer diameter of 4.0 mm. Measurements were performed at 22 °C using a Bruker Avance III AQS 400 SB operating at 9.4 T. Acquisition was performed at a MAS rate of 10 kHz and a CP pulse sequence using a 3.35 μsec proton 90° pulse, 800 μsec ramped (100–50 %) falling contact pulse and 2.5 s delay between repetitions. A SPINAL64 pulse sequence was used for ^{1}H decoupling, and a total of 16,384 transitions were recorded on each sample. Alpha-glycine was used for the Hartmann-Hahn matching procedure, as well as an external standard for calibration of the chemical shift scale relative to tetramethylsilane. The data point of maximum intensity in the alpha-glycine carbonyl signal was assigned a chemical shift of 176.03 ppm. The acquired spectra were normalized based on the maximum height of the $C_{2,3,5}$ signal at 73 ppm.

2.5.2.2. Liquid-state ^{1}H NMR. Ambiently dry fibres were dissolved at 65 °C in a Dimethyl Sulphoxide: Tetrabutylphosphonium acetate, DMSO-d_6:[P4444][OAc], (80:20 *w*/w ratio) solution according to an earlier described procedure (Fliri et al., 2023; King et al., 2018). Measurements were performed at 65 °C using a Bruker Avance III HD 400 MHz. Diffusion-edited ^{1}H measurements were acquired using a 1D bipolar-pulse pair with stimulated echo-diffusion-ordered spectroscopy pulse sequence ('ledbpgp2s1d' Bruker pulse program) with the following parameters: 3 s relaxation time, 0.5 s acquisition time, 16 dummy scans and 512 transient scans. The sweep-width was set to 20 ppm with a transmitter offset of 6.1 ppm, diffusion time of 200 ms, gradient recovery delay of 0.2 ms, eddy current delay of 5 ms, diffusion gradient pulse duration of 2.5 ms and a z-gradient strength of 80 % at >50 G/cm. To determine the relative number of substituent groups, the spectra were normalized with the area of the H2 signal followed by integration of the overlapping H10 and H5 signal. To obtain the H10 intensity the signal intensity for the H5 peak of the reference sample was subtracted in all spectra. The relative number (RN) of modified groups was calculated according to Eq. (2).

$$RN = \frac{\int H10}{9 \int H2} \quad (2)$$

where $\int H10$ is the integral of the H10 signal and $\int H2$ is the integral of the H2 signal. The numbering of protons is presented in Fig. 3.

2.5.3. Fibre saturation point

The fibre saturation point (FSP) of fully swollen fibres was determined according to the method developed by Stone and Scallan (1967). Excess water was removed from the quaternized fibres using vacuum filtration; solids contents ranged from 18 wt% to 32 wt% depending on the modified fibres propensity to swell. A sample corresponding to 0.8 g dry fibres was collected for each sample and diluted to a final concentration of 10 wt% using a 1.0 wt% dextran solution. The dispersions were mixed and stored in sealed glass vials for three days for equilibration. The liquid was extracted from the sample by squeezing followed by purification by passing the liquid through a 0.45 μm hydrophilic nylon syringe filter with a polypropylene housing (VWR International AB, Stockholm, Sweden). The dextran concentration of the liquid samples was determined using a Polartronic NH8 polarimeter (Schmidt+Haensch, Berlin, Germany). A calibration curve was constructed using a 0.5, 1.0 and 1.5 wt% dextran solution, respectively, from which the rotational angle obtained could be converted to dextran concentration of the samples. Measurements were performed in duplicate or triplicate.

2.5.4. Methods used for determination of the degree of substitution

2.5.4.1. Chemiluminescence following combustion. The content of nitrogen was determined using an ANTEK 7000 Model 737 (Antek Instruments, Houston, USA). Analyses were performed at 1050 °C, and the oxygen gas flows were set to 450, 35 and 25 mL/min for mild pyrolysis, excitation (via ozone) and carrier gas flow, respectively. Argon gas flow was set to 130 mL/min. A calibration curve was constructed using a 15 mg/g EPTMAC solution in the range of 0.8–4.0 μmol nitrogen (see Supplementary Data, Fig. S2). The samples were prepared by first drying a small amount of the quaternized fibres in a desiccator at ambient temperature, then an aliquot with a weight of 3 ± 0.5 mg was placed in a quartz boat and injected into the instrument. Analysis was performed in triplicate.

2.5.4.2. Dumas combustion method. Elemental analysis was also performed using a Flash2000 Organic Elemental Analyzer. Analyses were performed at 900 °C, the helium carrier gas flow was set to 140 mL/min and the oxygen gas flow to 250 mL/min. A calibration curve was constructed using 2,5-bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene (BBOT) standard in the range of 0.8–7.8 μmol nitrogen (see Supplementary Data, Fig. S3). The samples were prepared by drying a small amount of the quaternized fibres at 50–65 °C for 24 h followed by grinding of the sample. Aliquots in the mass range of 4.8 ± 0.5 mg were placed in tin crucibles and injected into the instrument. Analysis was performed in triplicate.

2.5.4.3. Kjeldahl method. Kjeldahl nitrogen was analysed according to the SMA 187.02 standard. In short, 150–300 mg of dried fibres was digested with a Gerherdt Kjeldatherm (Gerhardt analytical systems, Königswinter, Germany) using a mixture of sulfuric acid and potassium sulfate in the presence of a copper(II) catalyst. The ammonium ions formed were extracted by steam distillation using a Gerhardt Vapodest followed by absorption in a boric acid solution forming ammonium borate. The amount of nitrogen present in the solution was determined by potentiometric titration (Metrohm Titrando 888) of the ammonium borate with hydrochloric acid until the equivalence point was reached. Each analysis was performed in duplicate or triplicate.

2.5.4.4. Conductometric titration. Conductometric titration was performed to quantify the amount of chloride counterions present in the quaternized fibre sample. This measurement was performed for both

fibres and CNFs. The fibre sample was prepared by resuspension of never-dried modified fibres to a concentration of 5 g/L and a sample volume of 100 mL. The samples were titrated with 0.01 M silver nitrate by adding 1000 μL of the titrant every minute. The CNF sample was prepared by diluting the CNF dispersion to a concentration of 0.50 g/L and a volume of 100 mL. The samples were titrated according to a similar procedure using a 0.001 M silver nitrate solution. The conductivity of the sample was measured using an Orion Star A212 Benchtop Conductivity Meter (Thermo Scientific, Waltham, MA, USA), and the conductivity was read between each addition of silver nitrate. The amount of chloride ions present was determined from the intersect between the plateau value observed as the chloride ions were consumed and the slope of linear increase in conductivity once excess silver nitrate was being added. The mass of the fibres was determined by filtration of the titrated suspension, using a pre-weighted filter paper. The filter cake was dried overnight at 105 °C before measuring the dry weight; the weight of precipitated silver nitrate was subtracted to reach the initial mass of fibres. For the CNF samples, an exact dry mass of 0.05 g was used in each measurement.

2.5.4.5. Colloidal titration. The charge density of CNFs was determined using a ParticleMetrix Stabino system (ParticleMetrix GmbH, Munich, Germany) (Wachernig, 2009). The quaternized CNFs were titrated using a potassium polyvinylsulfate (KPVS) solution until the potential dropped below 0 mV. The charge density was back calculated using Eq. (3).

$$Q_{cat} = \frac{CQ_{an} \times V_{an} \times 1000}{V_{cat} \times C_{cat}} \tag{3}$$

where CQ_{an} is the charge concentration of the KPVS solution, in this case 0.3003 μeq/mL, and V_{an} and V_{cat} are the volumes consumed for the KPVS and CNF solution, respectively. C_{cat} is the concentration of the CNF-dispersion given in g/L. Four replicates were performed for each sample.

2.5.4.6. Polyelectrolyte adsorption. The total charge of the fibres can be measured by stochiometric adsorption of an oppositely charged polyelectrolyte (Wågberg et al., 1989), assuming that the polyelectrolyte can access all charges. To achieve this, a low-molecular-weight polyelectrolyte, polyacrylic acid (PAA) with a molecular weight of 5.1 kDa, was used together with a controlled pH of pH 10.0 to ensure full dissociation of the carboxylic groups. The adsorption experiments were performed using 5 g/L fibre suspensions with a volume of at least 50 mL and in a series of at least six samples containing an increasing amount of polyelectrolyte. Adsorption was performed for 30 min followed by vacuum filtration. The filter cake was dried overnight at 105 °C to determine the dry mass of the fibres, while the filtrate was collected for polyelectrolyte titration to determine the amount of non-adsorbed PAA.

The concentration of residual PAA in the sample was determined by polyelectrolyte titration with polydiallyldimethylammonium chloride (pDADMAC) using an equipment setup similar to that described by Horn (1978). In brief, the sample was diluted to 100 mL, followed by direct titration with a 0.2978 μeq/mL pDADMAC solution in the presence of an orto toluidine blue colour indicator. The filtrate was titrated using a Titrino polyelectrolyte titrator, and the colour change was monitored using a photoelectric measuring head. The endpoint for titration was evaluated using the Metrohm Tinet 2.4 software; a minimum of three measurements were performed on each sample. Results from the measurements were used to construct an adsorption isotherm (adsorption of polyelectrolyte as a function of equilibrium concentration of polyelectrolyte). The fibre charge was determined by extrapolating the plateau value of the isotherm to zero concentration of polyelctrolyte, as described earlier (Wågberg et al., 1989).

2.6. Calculations

Depending on the chosen method, the numbers of substituent groups are reflected as either charge or nitrogen content. These numbers are recalculated to a common unit in terms of degree of substitution, DS, according to Eq. (4).

$$DS = \frac{M_G \times N\%}{M_N \times 100 - (M_S + M_{Cl} - M_{LG}) \times N\%} = \frac{M_G \times Q}{10^6 - (M_S + M_{Cl} - M_{LG}) \times Q} \tag{4}$$

where

DS = Degree of substitution [mol_{subs}/mol_{AGU}]

N% = Nitrogen concentration [%]or [m(N)/m(sample)*100]

Q = Charge [$\mu eq/g$]

M_G corresponds to the molar mass of the anhydroglucose unit, which equals 162.1 g/mol, and M_S corresponds to the molar mass of the substituent, counterion excluded, which, for this particular modification, is 116.0 g/mol. For all methods except the conductometric titration method the counterion is expected to be a chloride ion, after conductometric titration the counterion is instead expected to be a nitrate ion. M_{Cl} corresponds to the weight of the counterion, which is 35.5 and 62.0 g/mol for the chloride ion and nitrate ion, respectively. Lastly, M_{LG} corresponds to the leaving group, in this case the removal of a proton upon the covalent linkage of the substituent.

3. Results and discussion

In the present work, cellulosic fibres were modified using EPTMAC, resulting in the covalent attachment of quaternized groups with the characteristics of being nitrogen containing and carrying a net cationic charge. A successful introduction of cationic groups to the cellulose was first established using FTIR and solid-state NMR; however, due to peak overlap, no quantitative determinations could be achieved. The FTIR and NMR spectra are shown in Fig. 2. In the FTIR spectra of the quaternized pulp, the emergence of a new peak can be distinguished at 1480 cm^{-1}, assigned to the C—N stretching vibration of the trimethylammonium group (Odabas et al., 2016; Pei et al., 2013; Rana et al., 2021; Zaman et al., 2012). Apart from this, the most salient features of the pulp fibre spectra remain unchanged after modification, indicating that the cellulose structure is maintained after modification. Characteristic peaks from the functional group introduced, including ether bonds in the region between 1030 cm^{-1} and 1163 cm^{-1}, could not be distinguished as they are also present in the cellulose structure (Zaman et al., 2012). The ^{13}C spectra of the modified pulp shows a distinct peak around 55 ppm, corresponding to the signal from the methyl moiety of the quaternary groups. The signals arising from the other carbons on the substituent, expected at 65–75 ppm, could not be distinguished due to the overlap with the characteristic signals from the cellulose backbone (Courtenay et al., 2018; Song et al., 2008; Udoetok et al., 2016; Wu et al., 2013).

Liquid-state ^1H NMR measurements were performed on the quaternized cellulose after dissolving the material in an ionic liquid, NMR-compatible, solvent. The spectra are presented in Fig. 3. From the reference material, all the characteristic signals, arising from H1–6 in the cellulose structure, could be appointed indicating that the cellulose structure had not been altered during dissolution (de la Motte et al., 2011; King et al., 2018). Increased modification with increased reagent dose could be established by the increased intensity of the signal at 3.27 ppm attributed to the nine methyl protons of the substituent group

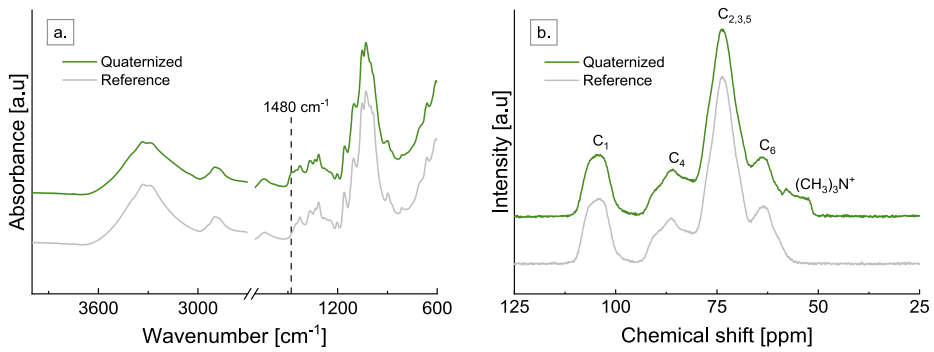


Fig. 2. a. FTIR spectra and b. ^{13}C NMR spectra of the reference pulp and quaternized pulp. The quaternized pulp corresponds the 4 EPTMAC:AGU sample with a degree of substitution of around 0.2.

which overlaps the H5 signal of the cellulose backbone (Courtenay et al., 2018; de la Motte et al., 2011). Also, the emergence of signals from H7–9 would be expected but remained unidentified due to overlap of the signals. The relative number (RN) of substituent groups was calculated using Eq. (2), the results are presented in Fig. 3. An increment of the relative number of substituents proportional to the dosing of reagent could be established, however, the data cannot be considered quantitative due to the specific pulse sequence used. The data acquisition was procured using specific pulse sequence enabling diffusion edition to attenuate the signals for the solvents. This was necessary to improve the spectral quality and enable selective integration of the relevant peaks but it will naturally also affect the other proton signals in the spectra. Furthermore, using the particular pulse sequence, some proportion of fast-relaxing species are lost due to relaxation before acquisition which has the consequence of enhancing the signal intensity of species with longer relaxation time (King et al., 2018). As the relaxation time of the methyl protons on the more flexible substituents groups is more extended than the relaxation time of the protons on the cellulose backbone increased resolution and intensity is expected for the, H10, methyl signal (Courtenay et al., 2018; King et al., 2018).

A quantitative determination of the DS could be achieved by analysing either the nitrogen content or charge of the material. The results from six different techniques, targeting either of these functionalities, are summarized and presented in Fig. 4, where the data has been recalculated to DS and charge using Eq. (4). The absence of data points for the reference material is due to the undetectable levels of nitrogen or charge, respectively.

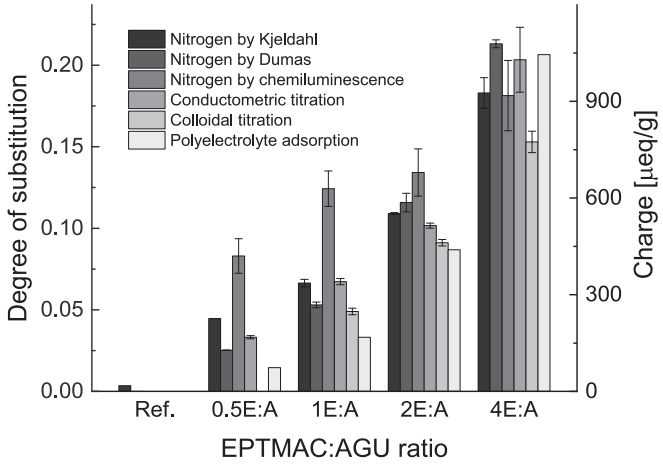


Fig. 4. Degree of substitution of quaternized cellulose determined with nitrogen and charge-based methods. The designation 0.5–4E:A corresponds to increasing dose of reagent resulting in an increase of the degree of substitution, Ref. corresponds to the non-modified pulp material.

All methods manage to capture the trend of increasing DS with increased dosing of the reagent and in general the different techniques are in fairly good agreement with each other, regardless of whether the target is the nitrogen or the charge of the material. Statistical evaluations (F- and *t*-tests, respectively) of the different data sets have been

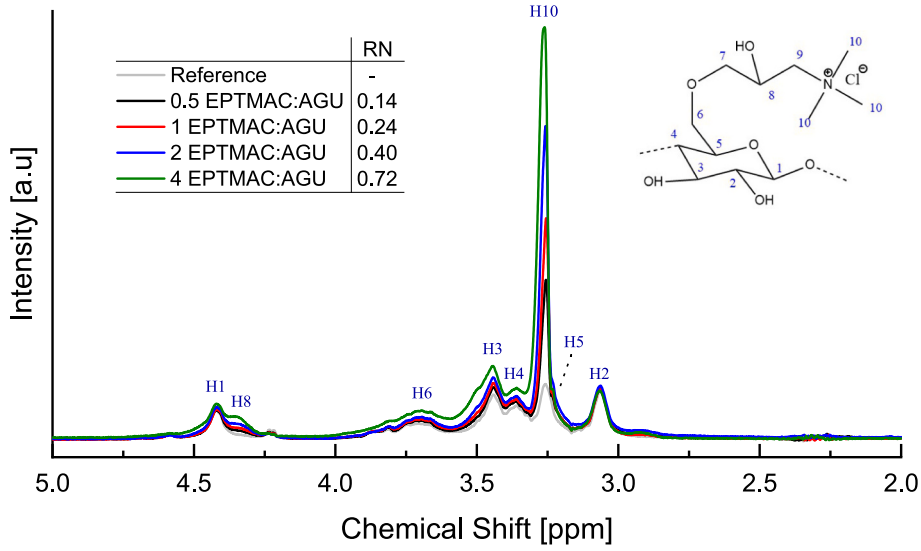


Fig. 3. Liquid-state ^{1}H NMR spectra of quaternized cellulose. The designation 0.5–4 EPTMAC:AGU corresponds to increasing dose of reagent resulting in an increase of the relative number of substituent groups (RN).

performed and are presented in Supplementary Data, Tables S5–12. As indicated by the averages and standard deviations presented in Fig. 4 the methods provide more scattered results at low DS and the results significantly differ from each other. However, as the DS is increased, most methods provide results that are not significantly different. It is apparent that nitrogen analysis by chemiluminescence tend to overestimate the DS, while the polyelectrolyte adsorption technique exhibits the opposite trend by underestimating the DS when the quaternization is modest. Apart from this, less pronounced differences can be distinguished between the different methods, and a chemical and technical background behind the techniques is crucial to fully understand the outcome. Below, each method is analysed in depth, providing their individual advantages and disadvantages.

3.1. Nitrogen-based methods

Nitrogen determination by Kjeldahl, chemiluminescence and the Dumas method all rely on different techniques for elemental quantification. While nitrogen determination by chemiluminescence and Dumas are dry-combustion techniques which typically detect all nitrogen present in the sample, Kjeldahl is a wet chemical method relaying on digestion, distillation and titration for the determination of organic nitrogen (AppliChem., 2017). The samples used in this study are expected to exclusively contain organic nitrogen and there is no statistical support for systematic underestimation of the DS when using the Kjeldahl method. However, at high degrees of modification a slight underestimation could be distinguished compared to the other methods, which could be attributed to incomplete digestion of the sample (Bradstreet, 1954). Comparing the dry-combustion methods with the other methods evaluated in the study, the chemiluminescence method clearly deviate from the other methods, overestimating the DS at low degrees of modification. The same trend is not found for the Dumas method, even though the methods have many similarities. In both techniques the sample is first converted into nitrogen oxides, but while the nitrogen oxides are reduced to molecular nitrogen in the Dumas method, the chemiluminescence method instead relies on the detection of photons emitted during the relaxation of excited nitrogen dioxide formed upon reaction of nitrogen oxide with ozone (Jones et al., 1982). Earlier studies using the same chemiluminescence instrument, an ANTEK 7000 N, found that the calibration curve start to deviate from linearity at comparably low nitrogen amounts of 0.72 nmol and a detector response of around $4{\cdot}10^5$ (Frankovich & Jones, 1998), which is much lower than the concentrations used, and counts recorded, in this study.

The construction of a reliable and linear calibration curve is crucial for both the nitrogen determination by chemiluminescence and Dumas method since the response retrieved for the samples is related to the calibration curve for the nitrogen quantification. In this study, calibration curves were prepared from EPTMAC solution (15 mg/g) and BBOT powder for the nitrogen analysis by chemiluminescence and the Dumas method, respectively, the curves are shown in Figs. S2 and S3 (Supplementary Data). While the Dumas technique yields a calibration curve with a R-square value close to 1 (0.9996) in the region of interest, the corresponding calibration curve constructed for the chemiluminescence technique exhibits a somewhat lower R-square value of 0.98, which indicates a deviation of linearity. Normalization of the response from the chemiluminescence detector with the amount of nitrogen added demonstrates a decay in sensitivity with increasing nitrogen content, indicating saturation of the detector or a limitation in the conversion of the sample into excited nitrogen dioxide. The Antek 7000 N is, indeed, built primarily for analysis of minute amounts of nitrogen in liquids and gaseous samples (Ewing, 1997), and might not be the best choice for analysing the material in question, i.e. a solid material with fairly high nitrogen content, which is also reflected in the results obtained. A smaller sample size could be considered to reduce this problem, however, this raises concerns regarding sample variability and accuracy in weighing of the sample (Mc Gill & Figueiredo, 1993).

3.2. Charge-based methods

Conductometric titration and colloidal titration was evaluated for the determination of the charge of the material. The methods are in fairly good agreement with each other and the nitrogen determination by Kjeldahl and the Dumas method. Both conductometric titration and colloidal titration relay on counter ion exchange of the charged groups and the accessibility silver ions and polyelectrolytes, respectively, is essential for the measurements. Since the comparably large polyelectrolyte is not expected to penetrate into the fibre wall, colloidal titration was only performed on CNF material. Furthermore, CNFs were only prepared from the more highly modified fibres due to difficulties with homogenization of fibres carrying little or no charge. A trend of slightly lower DS-values can be distinguished for the colloidal titration method. This could be due to the polyelectrolyte not being able to access all charges which would transpire if the homogenization or post-treatment was insufficient leaving larger CNF aggregates behind. However, no signs of aggregates could be found after centrifugation of the dispersions. In other words, the procedure of CNF gel and dispersion preparation, but also determination of dry contents, can influence the quality of the measurement.

Previously, conductometric titration has also primarily been performed on nanoscale cellulose fractions including CNCs and CNFs, where most charges can be expected on the surfaces (Hasani et al., 2008; Pei et al., 2013). The accessibility of the comparably bulkier reagent during modification, together with a good agreement with the nitrogen-based methods, also at low DS, does, however, imply that accessibility during titration is not a pronounced problem. To demonstrate this, conductometric titration was also performed using CNF dispersions prepared from the same fibres (Fig. 5) and the results show slightly lower values for the CNF dispersions compared to the fibre suspensions. As the same trend was established for the colloidal titration method one might argue that deviations in the dry-content measurements could be a reasonable explanation for the slight underestimation of the DS when CNF dispersions are analysed. However, the conductometric titration method also relies on the assumption of a 1:1 ratio between quaternized groups and chloride counter-ions and a partial counter ion exchange during homogenization and CNF dispersion preparation would affect the results in a similar way. It should be emphasised that the post-treatment of the fibres after modification is crucial to ensure a 1:1 ratio between quaternized groups and chloride counter-ions, therefore the reaction

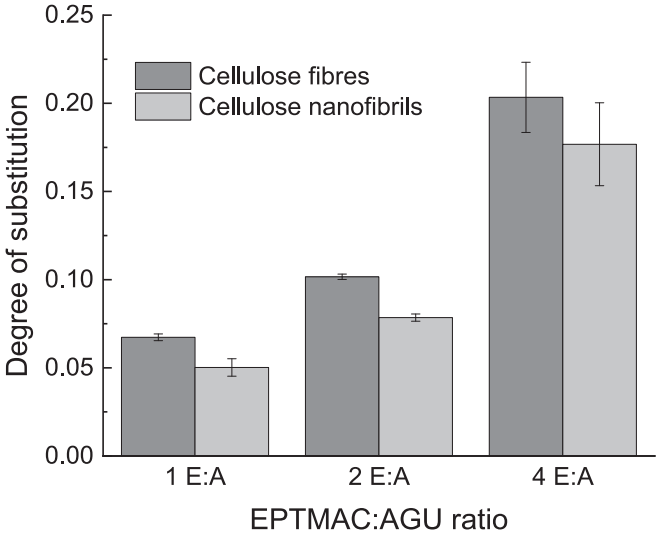


Fig. 5. Degree of substitution of quaternized fibre suspensions and quaternized cellulose nanofibril dispersions determined by conductometric titration. Increasing EPTMAC:AGU ratio indicates increasing dose of reagent and consequently a higher degree of substitution.

mixtures were neutralized with hydrochloric acid after modification followed by through washing. To demonstrate this, one experiment was performed using sulfuric acid for neutralization after the quaternization reaction, and this indeed gave a drastic underestimation of the DS (i.e. 0.005 compared to 0.079 for the 2E:A sample). On the same note, a deliberately insufficient washing resulted in an overestimation of the DS.

In general, there are not many studies present that combine charge and nitrogen-based methods for characterization of quaternized lignocellulosic material. However, Zaman et al. (2012) combined colloidal titration for charge density determination of CNCs and nitrogen analysis by chemiluminescence for determination of the DS of the material. Recalculation of the charge values reported to DS using Eq. (4) shows that both methods are in good agreement with each other for CNCs samples, especially when medium-charged CNCs with a DS of around 0.1 are used. Gao et al. (2016) also used colloidal titration for determination of the surface charge density of partially fibrillated quaternized fibres, while determining the degree of modification using the Dumas method. In this case the colloidal titration method provides a DS of 0.003 which is much lower compared to a DS of 0.154 provided by elemental analysis. This can be explained by the inaccessibility of the charges inside the fibre wall by the polyelectrolyte, which further demonstrates the importance of using well-dispersed nanofibrillated material to make a good estimation of the total charge and DS of the material. A drawback with the polyelectrolyte titration method is hence that nanofibrillated samples have to be used, adding requirements for equipment availability and an extra process step.

3.3. Polyelectrolyte adsorption method

Inspired by the colloidal titration method to determine the charge of polyelectrolyte-like CNFs and the polyelectrolyte adsorption method (Wågberg et al., 1989; Winter et al., 1986) for determining surface charge of the fibres, a novel method for determination of charge, and hence DS, was developed. When determining surface charge by polyelectrolyte adsorption, a polyelectrolyte so large that it cannot penetrate the fibre wall is used. However, with a polyelectrolyte which is really small in relation to the pores of the fibre wall, the technique can in theory also be used to assess the charge/DS of the fibres. Earlier studies on fibres carrying an anionic charge have concluded that lowering the molar mass of the polyelectrolyte increases the adsorption since the availability of the fibre wall increases, and the same is expected for an increased swelling of fibres since this will increase the pore sizes in the fibre wall and hence the accessibility of polyelectrolytes (Sjöström, 1989; Tanaka et al., 1990; Wågberg & Hägglund, 2001; Zhang et al., 2016). With the combination of a high swelling of quaternized cellulose and the use of a small-enough polyelectrolyte, it was hypothesized that the accessibility could be enhanced to such an extent that the quaternized groups inside the fibre wall could also be reached. A traditional methodology for determining fibre surface charge was used for this purpose, but instead of directly titrating the fibre charge, a series of experiments was conducted according to the methodology described by Wågberg et al. (1989). From these adsorption measurements an adsorption isotherm could be plotted, and from this isotherm it is possible to determine the charge. It was shown earlier that the amount of polyelectrolyte present in the solution will have an influence on the amount polyelectrolyte adsorbed to the available fibre surface. This is mainly due to an expansion of the polyelectrolyte layer on the fibre with increasing interaction between the adsorbed polyelectrolytes causing the charge stoichiometry between the accessible surface and the amount of adsorbed polyelectrolyte to deviate from unity. The purpose of using an adsorption isotherm instead of direct titration of the fibres is to compensate for a non-equivalent stoichiometry between amount of accessible charges on the fibre and the adsorbed polyelectrolyte (Horvath, 2006; Wågberg et al., 1989). The adsorption isotherms constructed for the quaternized fibre samples are presented in Fig. 6, and

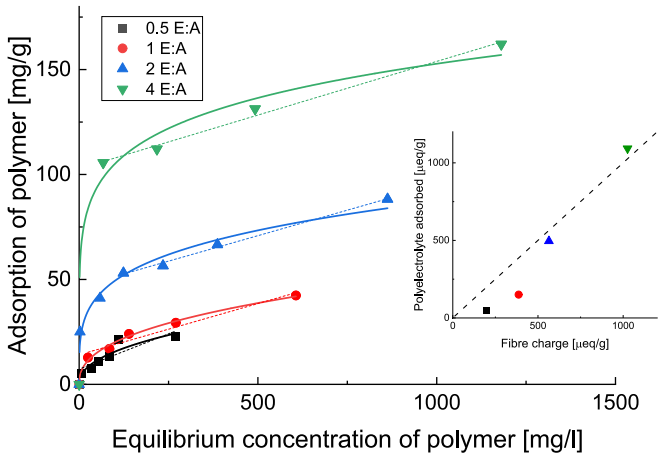


Fig. 6. Adsorption isotherms for the adsorption of polyacrylic acid (molecular weight 5.1 kDa) on quaternized cellulosic fibres; solid lines serve as a guide for the eye. The inset graph shows the amount of polyelectrolyte adsorbed as a function of total fibre charge determined by conductometric titration.

the stoichiometric adsorption of PAA is determined by extrapolating to zero equilibrium concentration.

As presented in Fig. 4, the polyelectrolyte adsorption method provides an underestimation of the DS values at the lower degrees of modification. However, at a higher degree of modification, and hence a higher total charge of the fibres, the adsorption of PAA increased significantly, and for the 2E:A and 4E:A samples, the DS predicted by polyelectrolyte adsorption can be considered to be within the same range as for the other methods. As an example, the polyelectrolyte adsorption method yielded a DS of 0.21 for the 4E:A sample, while the conductometric titration method and nitrogen determination by Dumas method gave numbers of 0.20 and 0.21, respectively. The behavior of increased adsorption with increased charge of the fibres is illustrated in the inset graph presented in Fig. 6. From this, it is clear that the charge values obtained from polyelectrolyte adsorption significantly deviates from those determined by the conductometric titration when the fibre charge is low. However, when the charge is increased, the values determined by the two methods agree. The increased adsorption also indicates an improved accessibility of polyelectrolyte to diffuse into the fibre wall, which is a result of a charge-induced swelling of the fibres arising from the build-up of an osmotic pressure inside the fibre wall

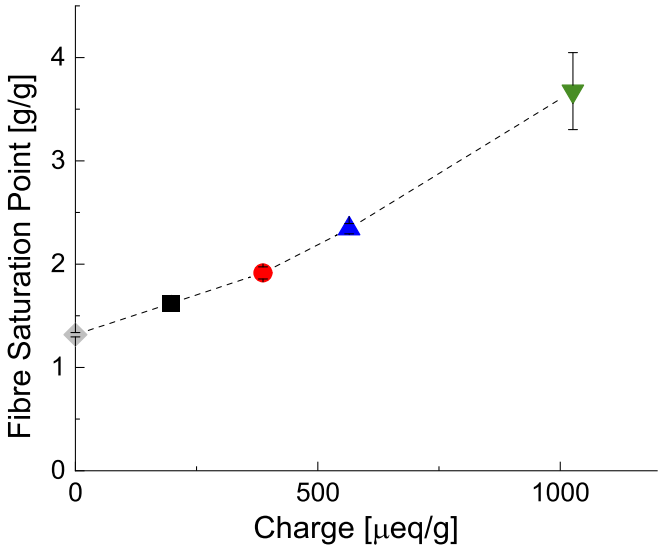


Fig. 7. Fibre saturation point as a function of fibre charge determined by conductometric titration.

Table 1
Summary of the main advantages and disadvantages of the investigated methods for determination of the degree of substitution (DS) of quaternized cellulose.

Method	Cellulose material	DS range	Advantage(s)	Disadvantage(s)
Kjeldahl	Macro-, nanofibres	Medium to high	• Direct determination of nitrogen content by titration • Only organic nitrogen included in measurement	• Several subsequent reaction steps included, labour intensive
Elemental analysis by combustion	Macro-, nanofibres	All	• Easy to operate • Fast	• Small sample volumes (risk of inhomogeneity effects)
Elemental analysis by chemiluminescence	Macro-, nanofibres	Low	• Easy to operate • Fast	• Very small sample volumes required (accuracy of sample weight and risk of inhomogeneity effects) • Detector saturation at higher nitrogen contents
Conductometric titration	Macro-, nanofibres	All	• Easy to operate • Relatively fast • Cheap	• Assumes 1:1 ratio between charged group and counterion, post-treatment of modified fibres essential. Risk of chloride contamination or counterion exchange
Polyelectrolyte titration	Nanofibres	Medium to high (>0.03)	• Relatively fast measurements	• Only applicable for CNFs, tedious and complex sample preparation • Assumes 1:1 ratio between CNF charge and charge of polyelectrolyte
Polyelectrolyte adsorption	Macrofibres	High (>0.1)	• Possible to determine DS of fibres "as they are", barely any sample preparation required	• Relatively high DS required • Time consuming measurements

(Sjöstedt et al., 2015). An enhanced swelling of the fibre wall is indeed corroborated by the FSP measurements shown in Fig. 7, showing a threefold increase in swelling for highly charged fibres compared to the non-modified starting fibres.

3.4. Summary and recommendations

Table 1 summarises the different methods evaluated in this study. Each method holds its own advantages and disadvantages which must be considered when choosing a suitable method for determining the DS of quaternized cellulosic materials.

4. Conclusions

A successful introduction of cationic groups to the cellulose could be established using FTIR and solid-state ^{13}C NMR and a relative increase in the presence of alkyltrimethylammonium groups with increasing degree of modification could be demonstrated using liquid-state ^{1}H NMR spectroscopy. Beyond this, six different methods were evaluated for determining the DS of quaternized cellulose. The results show that any of the methods can be used as long as the conditions are correct which confirms the hypothesis set for the investigation. Nitrogen analysis by chemiluminescence (using an Antek N7000) is not suitable for measurement of the relatively high amount of nitrogen present in the fibres studied, mostly due to the uncertainty introduced during weighing if the samples are very small and detector saturation which occur when too-high amounts of nitrogen is present. The Dumas combustion method and the Kjeldahl method perform well for nitrogen analysis, both for relatively low and high nitrogen contents, despite the drawbacks of requiring low sample volumes, with a risk of inhomogeneous sampling and labour intensive efforts, respectively. Conductometric titration with silver nitrate also worked well for both low-DS and higher-DS material, but a correct pre-treatment of the quaternized material is essential to meet the requirement of a 1:1 ratio between substituent group and counterion chloride. If the fibres can be mechanically fibrillated to CNFs, polyelectrolyte titration is a valid choice for measuring their surface charge and thereby provides a representative DS value. However, the measurements can lead to an underestimation of the DS due to limited access to all charges. Limited accessibility is also a drawback shared by the polyelectrolyte adsorption method. It can, however, be concluded that the polyelectrolyte adsorption values are in good agreement with other methods at a higher DS due to increased accessibility with enhanced swelling of the fibre (herein supported by FSP measurements); hence, it is recommended that this method is used only for quaternizations exceeding a DS of 0.1. All in all, post-treatment of the modified material, the macroscopic dimensions of the material, i.e. whether cellulose fibres, nanofibrils or crystals are used, and the estimated DS should be considered when choosing a suitable technique for determining the DS. This study serves as a guide when making a qualified choice of method for determining DS (i.e. charge or nitrogen content) and when evaluating and comparing the results in relation to other studies.

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CRediT authorship contribution statement

Johanna Sjölund: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Gunnar Westman:** Writing – review & editing, Supervision, Conceptualization. **Lars Wågberg:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Per A. Larsson:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2024.122734.

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