Minimally invasive monitoring of cellulose degradation by desorption electrospray ionization and laser ablation electrospray ionization mass spectrometry†

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Minimally invasive desorption electrospray ionization-mass spectrometry (DESI-MS) and laser ablation electrospray ionization-MS (LAESI-MS) were used to look for soluble cellulose degradation products produced by accelerated aging in unsized cotton paper. Soluble extracts from papers aged 144 to 26 856 hours were first analyzed in solution using traditional electrospray ionization-MS (ESI-MS). Results were compared to those from direct analysis of condensed phase degradation products extracted from the absorbent paper substrate using DESI-MS and LAESI-MS. ESI-MS results showed evidence of oligosaccharide degradation products ranging from cellobiose to cellononaose; using DESI-MS and LAESI-MS, products from cellobiose to cellodecaose and glucose to cellooctaose, respectively, were observed. As degradation proceeded, increased quantities of both low and high molecular weight oligosaccharides were observed. The analytical approaches developed in the control study were applied for the detection of degradation products in two naturally-aged books dating from the 19th century, both made from cotton and linen. Oligosaccharides ranging from glucose to cellopentaose were observed.

Introduction

Determining the condition and degradation rate of cellulose in aging paper has been of great concern in applications where the long term physical properties of the paper are important.[1–6] These performance properties are compromised by chain breaking of the cellulose molecule in the amorphous phase, with heterogeneous hydrolysis being the most common degradation reaction in most circumstances.[7,8] Tracking the loss of average molecular weight has long been used to study paper degradation, but such destructive testing is impractical for papers in active use. Even though it has been shown that a single fiber taken from a sheet of paper can be sufficient for molecular weight determination,[9] such efforts necessarily raise issues about how representative such small samples are and whether results from individual fiber analyses can be compared over time in order to monitor changes in average molecular weight.

Other strategies to track paper degradation have aimed to follow cellulose breakdown by measuring the production of very low molecular weight breakdown products such as glucose[10,11] and furfural.[12] Recently, an entire series of soluble cellulose oligomers were observed in degrading paper using ESI-MS.[5] These oligomers represent products of multiple hydrolytic reactions in a cellulose chain, which thus free the oligomer from the amorphous phase of the cellulose. Experiments and computer simulations both demonstrated that low molecular weight oligomers (e.g., glucose and cellobiose) are most abundant early in the degradation, with higher molecular weight oligomers becoming detectable at later stages of aging.[5] These oligomers are particularly well suited for tracking cellulose breakdown for they are stable and likely to originate from primary hydrolytic reactions which are the important degradation chemistry. However, in this earlier study, the soluble oligomers were prepared for mass spectrometric analysis using an invasive solvent extraction method that resulted in the destruction of the sample.[5]

Recent advances in surface analysis mass spectrometry have permitted samples to be investigated under ambient pressure conditions, allowing minimally invasive analysis of soluble compounds with almost no sample preparation. Two such techniques are desorption electrospray ionization mass spectrometry (DESI-MS) and laser ablation electrospray ionization-mass spectrometry (LAESI-MS). DESI-MS works by extracting the analyte from a matrix with a spray of ionized solvent. It directs the desorbed ions contained in secondary droplets into a mass spectrometer for analysis.[13–15] By optimizing the experimental parameters, the detection limit of DESI-MS has been reduced to the attomole level.[16] LAESI-MS works by using a mid-infrared (mid-IR) laser to excite the water content of the

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sample, which results in a microscopic ablation, producing a plume of ions and neutral species. A stream of electrically charged solvent droplets from an ESI source passes through the plume and ionizes the neutral species which are then introduced into a mass spectrometer.[17,18] These atmospheric-pressure mass spectrometric techniques have found wide use in examining pharmaceutical, and biological samples.[19–21] DESI-MS has also been employed to examine inks deposited on paper.[22,23] Neither technique has heretofore been used to study polymer degradation *via* the detection of soluble oligomer scission products located within the substrate itself. Their potential for analyzing paper with minimal sample preparation and negligible destruction to the sample itself therefore makes them natural candidates for the determination of paper condition and deterioration rates.

This paper reports the results of a study examining whether the minimally invasive DESI-MS and LAESI-MS techniques can be used to track the low molecular weight hydrolytic breakdown products of cotton cellulose generated during artificially aging in a humid oven and therefore embedded in an absorbent substrate. The results are compared to a destructive sampling technique that is known to be capable of tracking these products. Also, the two techniques are used to see if similar oligomers can be found in two rag papers from books that have degraded through natural aging processes. If so, then the two atmospheric pressure techniques may be used to non-destructively probe *in situ* the condition of papers used in art objects or in electrical applications.

Experimental

Aging of paper

Several samples were prepared as controls to look at the effect of absorbent paper on the detection of oligosaccharides using mass spectrometry. For DESI-MS, two aqueous solutions, one containing 1000 pmol μL^{-1}, the other 100 pmol μL^{-1}, of D-(+)-glucose (Sigma-Aldrich), D-(+)-cellobiose (Fluka) and D-(+)-cellotetraose (Sigma-Aldrich), respectively, were deposited onto separate pieces of pure, unaged cotton cellulose paper (Whatman no. 42 quantitative paper [W42]) and the water was allowed to evaporate. For the 1000 pmol μL^{-1} sample, it was estimated that the abundance of each oligosaccharide analyzed to generate the mass spectrum was approximately 23 000 pmol cm^{-2} as 3 mL of the solution were deposited on 128 cm^2 of paper. For the 100 pmol μL^{-1} sample, the amount of each oligosaccharide present in the paper was approximately 2300 pmol cm^{-2}. For LAESI-MS, a solution of 1000 pmol μL^{-1} D-(+)-cellobiose was deposited onto a piece of unaged W42 and the water was allowed to evaporate to prepare the sample for analysis. Inhomogeneous oligomer concentrations are expected across the paper due to inhomogeneous evaporation, but this was not investigated.

Plain sheets of W42 were also used to study the effect of thermal aging on the generation of oligosaccharide degradation products. The batch of W42 paper used in this study was originally purchased in 1997 from the manufacturer (Whatman) and therefore may have undergone some degradation in the interim. A cold water extract of the paper exhibited a pH of 5.0 according to the ASTM D778-97 standard test method. The W42 samples were hydrolyzed in a controlled humidity Blue M oven at 90 °C and 50% relative humidity (RH). For this oven treatment, stacks of 40 sheets measuring 20 cm × 20 cm were placed between two 23 cm × 23 cm glass plates. The stack configuration retained some but not all of the volatile degradation products generated during the oven aging that might otherwise have been completely retained had the sheets been sealed in a vial.[24] Three individual sheets were removed from the stack at various time points ranging from 144 h to 26 856 h of oven-aging. Samples removed from these aged sheets for analysis were always taken from a section at least 2.5 cm from the edge of the paper.

Finally, two naturally aged books made from cotton and linen rags were also examined in this study. They were part of a collection of books donated to the Art Conservation Research Center in 2005. The pH of both books was measured using the same ASTM standard method mentioned above. One book, published in 1833 and entitled A history of the United States of America from the discovery of the continent by Christopher Columbus to the present time was published in Hartford, CT by D. F. Sumner & Co (pH 5.5). The second book, published in 1857 and entitled Scenes de le vie de province; illusions perdues was published in Paris, France by Librairie Nouvelle (pH 4.2). The history of storage and handling of these books is unknown. An Olympus BX61 microscope operated with Slidebook 5.0 software was used to determine the type of fiber used to make the pages of the books.

Electrospray ionization and desorption electrospray ionization

For the ESI-MS experiments of the oven-aged W42 samples, unknown quantities of solvent soluble degradation products were extracted from 0.094 g swatches of oven aged W42 paper by placing the weighed portion into the top portion of a tipped Petri dish to which 10 mL of water were added. The water was allowed to evaporate leaving the solvent soluble residues in the bottom of the Petri dish. The extraction procedure was repeated using first methanol and then dimethyl acetamide (DMAc). Following the final extraction and evaporation, the residue in the bottom of the Petri dish was scraped out with a razor blade, weighed, transferred to a vial, and dissolved in water to yield a nominal concentration of approximately 500 pmol μL^{-1}. Though it was known that several oligosaccharides might be in the residue, to prepare a 500 pmol μL^{-1} nominal concentration solution it was assumed that the molar mass of the residue was equivalent to glucose, 180 g mol^{-1}.

Direct analysis of cellulose degradation products in oven- and naturally-aged samples without solvent extraction was achieved using DESI-MS. The DESI source was built in house and was modeled after a sprayer design by Takats *et al.*[25] The O.D. and I.D. of the fused silica capillaries (Polymicro Technologies) were 360 μm and 250 μm for the outer gas flow capillary and 197 μm and 98 μm for the inner solvent flow capillary. The inner capillary protruded from the outer capillary by 0.1–0.2 mm. The DESI sprayer was clamped to a micromanipulator (World Precision Instruments) mounted on a magnetic stand so that the spray incidence angle (α) and the x, y, z positions could be adjusted. Adjustment of the micromanipulator also allowed for control of the spray incidence angle. A second micromanipulator and magnetic stand were used to control the sample holder. A paper

sample was mounted on a 2.5 cm × 5 cm Teflon® sheet held by a second micromanipulator on a magnetic stand. To prevent movement during analysis, paper samples were attached to the Teflon with double sided tape (3M). During analysis, the DESI sprayer was placed 1–2 mm away from the sample (tip to surface distance), at approximately 65° incident angle measured from the surface, and a collection angle of 0°.[14]

For both ESI-MS and DESI-MS, cellulose degradation products were detected by a ThermoFisher LCQ quadrupole field ion trap mass spectrometer operated with version 1.3 Xcalibur software. Data were collected in positive ion mode. Initially, full scans (150–2000 m/z) were collected to observe all possible cellulose degradation products that fell within the detection range of the instrument. Following an initial experiment, to improve the signal to noise ratio, the mass range was limited to only include the mass ranges where oligosaccharides were observed.

For ESI-MS spectra, an average of at least 35 scans of 20 microscans each were collected (e.g., 700 microscans in total). A microscan represents one complete mass analysis scan and many microscans are typically signal averaged before display or storage. The electrospray voltage was 4.5 kV, the heated capillary temperature was 250 °C, the capillary voltage was 22 V, the tube lens potential was 16 V, the solvent mixture was 90 : 10 : 0.1% methanol : water : acetic acid and the flow rate was 5 μL min⁻¹. The HPLC grade methanol (Fisher Scientific), distilled water (in house, pH 5.5), and the glacial acetic acid (Acros Organics) were used in the experiments.

Using DESI-MS, the spray voltage was 4.5 kV, the heated capillary temperature was 200 °C, the heated capillary voltage was 45 V, the tube lens potential was 120 V, the solvent mixture was 20 : 80 methanol : water and the flow rate was 3 μL min⁻¹. The sample was manually maneuvered under the DESI source sprayer at ∼1 mm min⁻¹ to generate a mass spectrum with the highest signal intensity. Linear scanning of the sample helped reduce any effects of solvent washing.[26] Nitrogen was used as the nebulizer gas and was supplied at a pressure of 150–160 psi behind the sprayer. In the DESI-MS experiments, the signal response of the data from the LCQ instrument was 10 to 100 times lower than for normal ESI some of which can be attributed to the amount of paper sampled. Mass spectra shown here were the average of at least 35 scans with each scan consisting of 10 microscans.

Laser ablation electrospray ionization

Direct analysis of cellulose degradation products formed during thermal and natural aging was also completed using LAESI-MS. Before analysis, samples were pre-wetted with 5–20 μL of 1 mM aqueous potassium bromide (KBr) (J. T. Baker, NJ) solution. The water promoted the absorption of mid-IR energy, while the potassium ion facilitated the production of ions in LAESI-MS. Laser ablation of the paper samples was performed using a diode pumped Nd:YAG laser-driven optical parametric oscillator (Opotek, CA) running at 2940 nm wavelength, 100 Hz repetition rate, and 5 ns pulse width. The laser beam was aligned by gold-coated mirrors (PF10-03-M01, Thorlabs, Newton, NJ) and focused by a 75 mm focal length plano-convex zinc selenide lens (Infrared Optical Products, NY). The ablated material in the

plume was ionized using a home-built electrospray setup operating in cone-jet mode[27] that supplied the solvent using a low-noise syringe pump (Physio 22, Harvard Apparatus, MA).

Ions produced by LAESI were detected with a Waters Q-TOF Premier orthogonal acceleration time-of-flight mass spectrometer with a modified interface.[17] The mass spectra were collected from the ablation of a single spot using ∼1000 laser shots in 10 seconds. Each mass spectrum was the average of 10 scans. The spray voltage was 2.7–2.9 kV, and a mixture of 50 : 50 : 0.1% methanol : water : acetic acid (Sigma Aldrich, MO, Acros Scientific, Belgium, and Fluka, Germany, respectively) was supplied with a flow rate of 200 nL min⁻¹. All the ions were detected in positive mode. The observed oligosaccharides were primarily singly charged potassium adducts and, occasionally, singly charged sodium adducts. The sampling inlet of the mass spectrometer was heated to 80 °C.
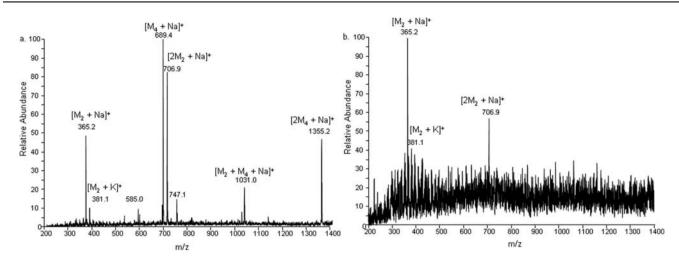
Results and discussion

Control experiments

A control study was performed to determine the nature of the cellulose degradation products, i.e., oligosaccharides, in a mass spectrum when sampled directly from an absorbent paper substrate. This study was also conducted to see if it would be possible to estimate the relative amounts of oligosaccharides in a sample based upon their peak intensities relative to the base peak. Tests were performed on papers having high concentrations of oligosaccharides and low concentration, representing very degraded and slightly degraded papers, respectively.

Fig. 1 shows the DESI mass spectra of two solutions of glucose, cellobiose and cellotetraose from a piece of unaged W42 paper. Fig. 1a shows the DESI-MS mass spectrum of the unaged W42 containing 23 000 pmol cm⁻² of each oligosaccharide. Fig. 1b is the DESI mass spectrum of the unaged W42 paper with 2300 pmol cm⁻² of each oligosaccharide.

In Fig. 1a, representing oligomer concentrations typical of very degraded papers, sodiated peaks of cellobiose (m/z 365.3) and cellotetraose (m/z 689.4) were seen. The polymer unit or mer is identified by the letter M and followed by a subscribed number indicating the degree of polymerization of the adduct (e.g., "2" represents cellobiose [degree of polymerization $n = 2$]). Also, a potassiated adduct of cellobiose was seen at m/z 381.1. Trace amounts of sodium and potassium must have been present in the water used to analyze samples.

Fig. 1a also shows peaks at m/z 706.9, 1031.0, and 1355.2. These peaks represent sodium bound dimers of cellobiose–cellobiose ($[2M_2 + Na]^+$), cellobiose–cellotetraose ($[M_2 + M_4 + Na]^+$), and cellotetraose–cellotetraose ($[2M_4 + Na]^+$), respectively. Sodium bound dimers are often observed in ESI mass spectra and their presence in a mass spectrum is molecule, concentration, and instrument dependent.[28]

A sodiated glucose peak at m/z 203.0 was not observed in any of the ESI or DESI mass spectra. It was noted, however, that after the sample was analyzed and the DESI sprayer was moved to clean the heated capillary, a sweet odor was detected. Since the oxidation of glucose can occur at temperatures as low as 160 °C,[29] it is suspected that the glucose was degraded on the surface of the heated capillary during analysis. Lowering the
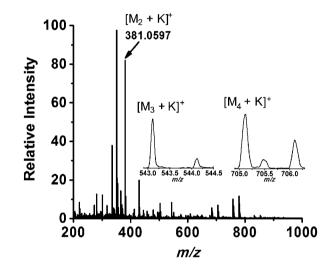
Fig. 1 DESI-MS of (a) 23 000 pmol cm^{-2} and (b) 2300 pmol cm^{-2} each of glucose, cellobiose and cellotetraose deposited and dried on unaged W42. (M is the polymer unit; Na—sodium adduct; K—potassium adduct; 2 and 4—cellobiose and cellotetraose, respectively).

temperature of the heated capillary to 150 °C, the lowest setting for the instrument, the glucose ion was still not detected by the mass spectrometer.

Though the concentrations of the cellobiose and cellotetraose were the same, it was not possible to change the instrumental parameters such that the abundances of the two sodiated adducts in the spectra were the same. However, by adding up the relative abundances of all the peaks associated with cellobiose and cellotetraose, respectively, the total relative abundance for cellobiose was 242 units (48 for the sodiated cellobiose peak + 10 for the potassiated peak + 164 for the cellobiose–cellobiose dimer peak + 20 for the cellobiose–cellotetraose dimer peak) and for cellotetraose, 212 units (100 for the cellotetraose peak + 20 for the cellobiose–cellotetraose dimer peak + 92 for the cellotetraose–cellotetraose dimer peak). These values came within ~12% of one another in representing equivalent amounts of each oligosaccharide. This indicated that it may be possible to use DESI-MS spectra to estimate the relative quantity of various oligosaccharides present in degraded paper samples.

Fig. 1b, representing oligomer concentrations typical of slightly degraded papers, shows that at lower oligosaccharide concentrations, the sodiated peak of cellobiose (m/z 365.2), the sodium bound dimer of cellobiose–cellobiose (m/z 706.9), and the potassiated cellobiose peak (m/z 381.1) were still observed. For unknown reasons, cellotetraose and glucose were not detected. It appears that, at these low concentrations, the DESI-MS spectra distort the relative abundances of the oligosaccharides present in the sample. For slightly degraded papers, which will have very low abundances of oligosaccharides, and essentially no higher molecular weight degradation products, accurately determining the relative abundances of the products is not possible. However, using this instrumental set up and analyzing an absorbent sample manually doped with sample, it was possible to determine the amounts of oligosaccharide relative to one another if the concentrations were at or above 23 000 pmol cm^{-2}.

Fig. 2 shows the LAESI-MS mass spectrum of unaged W42 doped with 23 000 pmol cm^{-2} cellobiose. Though it was not the base peak, a potassium adduct of cellobiose was easily observed at m/z 381.1. Cellotriose (m/z 543.1) and cellotetraose (m/z 705.1)

Fig. 2 LAESI-MS of W42 doped with 23 000 pmol cm^{-2} cellobiose (K—potassium adduct; 2, 3, 4—cellobiose, cellotriose, and cellotetraose, respectively).

may have been present in the unaged W42 paper. LAESI-MS analysis did not show dimers in the mass spectra.

Using both DESI-MS and LAESI-MS, it was possible to detect oligosaccharides from an absorbent substrate, however, the two techniques gave slightly different mass spectra. DESI-MS spectra did not show glucose ions in spite of the fact that it was present in the sample. Also, at high concentrations of 23 000 pmol μL^{-1}, DESI produced an abundance of sodium bound dimers, generating more complex spectra relative to the LAESI-MS spectrum. Interpretation of data without dimer formation is less complex, so using LAESI-MS or operating the DESI-MS LCQ in a low energy tube-lens CAD mode or with a higher heated capillary temperature might reduce the abundance of these dimers. At concentrations less than 23 mM one would expect to see less dimerization. As seen in Fig. 2, LAESI also produced many abundant peaks that are unassigned; this adds to the complexity of analyzing those spectra. More work needs to be completed to confirm whether DESI-MS and LAESI-MS may

be used to quantitatively determine the relative amounts of oligosaccharides present in the paper sample.
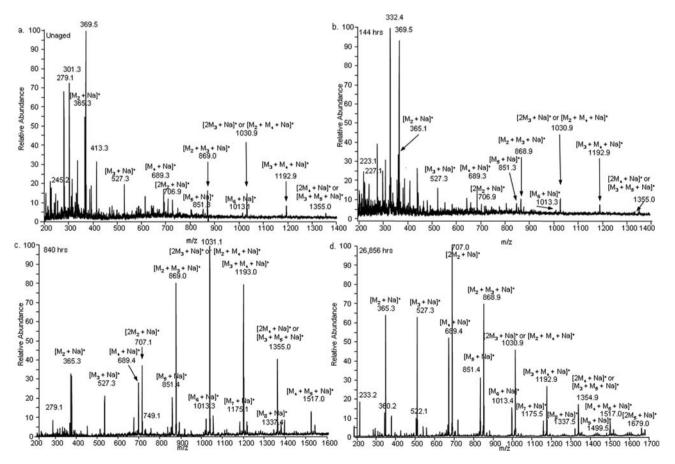
Artificially aged papers

To probe the effectiveness of DESI-MS and LAESI-MS in tracking cellulose degradation, W42 paper was artificially aged in a humid oven to induce hydrolysis. Hydrolysis caused low molecular weight oligosaccharides to be formed and the progress of oligosaccharide formation in cotton W42 paper was monitored at four time points: unaged W42, and W42 aged for 144 h, 840 h and 26 856 h. Though the concentration of oligomers analyzed using ESI-MS may have been higher due to the amount of paper used to make the extractions, results from DESI-MS and LAESI-MS were compared to the outcome of ESI-MS analysis to determine if the minimally invasive techniques showed similar trends. If so, the minimally invasive techniques might be used *in lieu* of ESI-MS.

Fig. 3 shows the mass spectra from ESI-MS analysis of the oven-aged W42 samples at the four different time points. The spectrum of unaged W42, Fig. 3a, shows the following sodium adducts of the oligosaccharides: cellobiose (*m/z* 365.3), cellotriose (*m/z* 527.3), cellotetraose (*m/z* 689.3), cellopentaose (*m/z* 851.3), and cellohexaose (*m/z* 1013.1). In addition, sodium bound dimers of cellobiose–cellobiose (*m/z* 706.9), cellobiose–cellotriose (*m/z* 869.0), cellobiose–cellotetraose or cellotriose–cellotriose

(*m/z* 1030.9), cellotriose–cellotetraose (*m/z* 1192.9), and cellotetraose–cellotetraose or cellotriose–cellopentaose (*m/z* 1355.0) were observed. Fig. 3b, the W42 aged 144 h, shows the same oligosaccharides and sodium bound dimers that were observed in the unaged W42 sample. At 840 h of aging (Fig. 3c), it was observed that the cellulose had undergone further degradation. Oligomer degradation products ranging from cellobiose (*m/z* 365.3) through celloheptaose (*m/z* 1175.1) and cellooctaose (*m/z* 1337.4) were seen. Also, higher abundances of the sodium bound dimers were seen, ranging from cellobiose–cellobiose (*m/z* 707.1) to cellotetraose–cellopentaose (*m/z* 1517.0).

Fig. 3d, W42 aged 26 856 h, shows that the cellobiose (*m/z* 365.3) and cellotriose (*m/z* 527.3) peaks had increased in intensity relative to the 840 h sample due to further degradation of the cellulose. Also, two newly developed peaks, sodiated cellononaose (*m/z* 1499.5) and a sodium bound dimer of cellopentaose–cellopentaose (*m/z* 1679.0) were seen. Table S1 in the ESI† summarizes the findings from the ESI-MS experiments.

Fig. 4 shows the DESI-MS results from analysis of the unaged W42 and three oven-aged W42 samples. Using DESI-MS, the sodiated oligosaccharides observed in both the unaged (Fig. 4a) and the 144 h (Fig. 4b) sample were cellobiose (*m/z* 365.2), cellotriose (*m/z* 527.2), cellotetraose (*m/z* 689.3), and cellopentaose (*m/z* 851.4). Potassiated cellobiose (*m/z* 381.2) was seen at both time points while potassiated cellotriose (*m/z* 543.1) was only observed in the unaged sample. The sodium bound

Fig. 3 ESI-MS of (a) unaged W42 and W42 oven-aged at 90 °C and 50% RH for (b) 144, (c) 840 and (d) 26 856 hours (Na—sodium adduct; 2, 3, 4, 5, 6, 7, 8, 9—cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, celloheptaose cellooctaose and cellononaose, respectively).
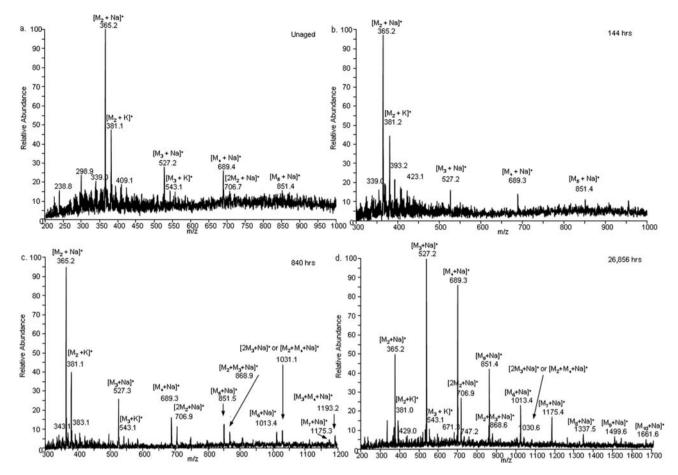
Fig. 4 DESI-MS of (a) unaged W42 and W42 oven-aged at 90 °C and 50% RH for (b) 144, (c) 840 and (d) 26 856 hours (Na—sodium adduct; K—potassium adduct; 2, 3, 4, 5, 6, 7, 8, 9, 10—cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, celloheptaose, cellooctaose, cellononaose and cellodecaose, respectively).

cellobiose–cellobiose dimer (m/z 706.7) was only seen in the unaged sample. This similarity between the unaged and 144 h aged spectra was also observed in the spectra at the same time points using ESI-MS, however, fewer sodium bound dimers were seen in the DESI-MS than as a result of the ESI-MS process. Cellohexaose was not observed in the DESI-MS possibly due to the low signal-to-noise ratio of both spectra.

Fig. 4c, the DESI mass spectrum after 840 h of aging, shows the sodiated species of cellobiose (m/z 365.3), cellotriose (m/z 527.3), cellotetraose (m/z 689.3), cellopentaose (m/z 851.5), cellohexaose (m/z 1013.4), and celloheptaose (m/z 1175.3). In addition, the sodium bound dimers of cellobiose–cellobiose (m/z 706.9), cellobiose–cellotriose (m/z 868.9), cellotriose–cellotriose or cellobiose–cellotetraose (m/z 1031.1) and cellotriose–cellotetraose (m/z 1193.2) were observed. Potassium adducts of cellobiose (m/z 381.1) and cellotriose (m/z 543.1) were also observed. The emergence of higher molecular weight degradation products as well as the increased intensity of all the oligosaccharide peaks relative to the base peak indicated that a greater degree of degradation had occurred compared to the less aged samples. The DESI-MS results at 840 h of aging were similar to ESI-MS at the same aging time in that higher molecular weight oligomers, more dimers, and greater amounts of both were observed. Compared to ESI-MS, the highest molecular weight oligomer,

cellooctaose, and the cellotetraose–cellotetraose and cellotetraose–cellopentaose dimers were not observed in the DESI-MS most likely due to the lower concentration of these oligomers found in the gas phase.

At 26 856 h, Fig. 4d shows that as aging proceeded, similar oligosaccharides as seen in the 840 h sample were observed. In addition, the following higher molecular weight oligosaccharides were also seen: cellooctaose (m/z 1337.5), cellononaose (m/z 1499.6) and cellodecaose (m/z 1661.6). At this time point increased quantities of oligosaccharides were detected compared to less degraded samples; this was especially true at the low mass-to-charge ratio end of the spectrum. These results showed the presence of the same sodiated oligomers as observed in the ESI-MS results at the same time, indicating that it should be possible to track the ongoing degradation process using DESI-MS. The ESI mass spectra showed the presence of more dimers and this is likely due to the higher concentration of oligomers in the gas phase. Table S2 in the ESI† summarizes the findings from the DESI-MS experiments.

Slightly different mass spectra were generated when the unaged W42 and three oven-aged W42 samples were analyzed using LAESI-MS (Fig. 5). Unaged W42 (Fig. 5a), only showed a potassiated cellobiose peak at m/z 381.1. When aged to 144 h (Fig. 5b), potassiated glucose (m/z 219.0), cellobiose (m/z 381.1),
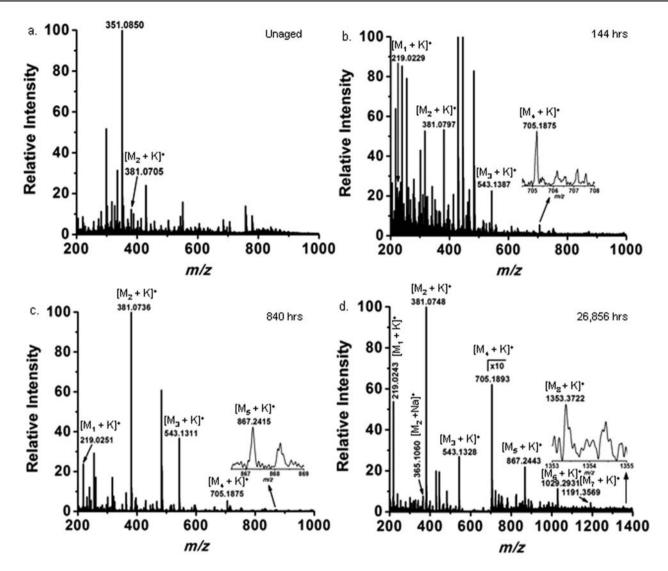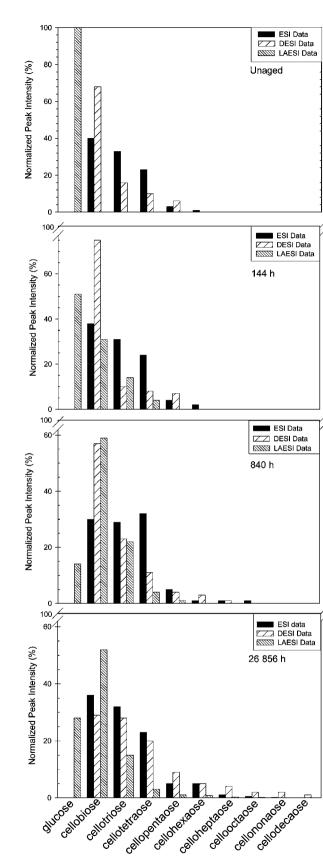
Fig. 5 LAESI-MS of (a) unaged W42 and W42 oven-aged at 90 °C and 50% RH for (b) 144, (c) 840 and (d) 26 856 hours (Na—sodium adduct; K—potassium adduct; 1, 2, 3, 4, 5, 6, 7, 8—glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, celloheptaose and cellooctaose, respectively).

cellotriose (m/z 543.1) and cellotetraose (m/z 705.2) were observed.

As aging progressed, LAESI-MS of the 840 h oven-aged sample showed potassiated oligosaccharides of glucose (m/z 219.0), cellobiose (m/z 381.1), cellotriose (m/z 543.1), cellotetraose (m/z 705.2) and cellopentaose (m/z 867.2) as well as increased quantities of the lower molecular weight cellulose oligosaccharides relative to the noise (Fig. 5c). At 26 856 h of aging (Fig. 5d), in addition to seeing the peaks observed at 840 h, cellohexaose (m/z 1029.2) and celloheptaose (m/z 1191.4), and cellooctaose (m/z 1353.4) were also observed. Cellononaose and cellodecaose were not observed in the LAESI mass spectrum, as were seen with DESI-MS and ESI-MS. Regardless, the results from LAESI-MS showed that as degradation proceeded, more degradation products were being generated, hence, LAESI-MS can also be used to monitor cellulose degradation with time. Table S3 in the ESI† summarizes the findings from the LAESI-MS experiments.

Due to the likely differences in concentrations of oligosaccharides analyzed by each technique as well as the fact that two different mass spectrometers were used to generate the data, it was not possible to determine which technique was the most sensitive. However, it was of interest to see if the DESI technique (average sample area ~1.25 to 2 cm^2) or LAESI technique (average sample size 5 × 10^{-4} cm^2) could be used *in lieu* of the destructive sampling method used to prepare samples for ESI (average sample area 121 cm^2). In order to do this, Fig. 6 compares normalized histograms of the changes in the total relative intensities of the degradation products with time. Though the concentration of any of the oligosaccharides is unknown, calculation of the total intensities at each time point was carried out in the same manner discussed for Fig. 1a to compare whether or not DESI and LAESI results were similar to ESI results. Normalized peak intensities were determined by dividing the total intensities of one oligosaccharide (including all adducts and dimers) by the total of all the peak intensities of the

Fig. 6 Normalized total peak intensities of oligosaccharides determined by ESI-MS, DESI-MS, and LAESI-MS.

oligosaccharides observed in a given sample. This analysis was done for each technique.

A comparison of DESI and LAESI with ESI shows similar patterns of the oligosaccharide mass distribution and changes to the distribution with aging. At short aging times, all three techniques showed lower relative abundances of higher molecular weight oligosaccharides. As degradation proceeded, all techniques showed increasing quantities of high molecular weight oligosaccharides. It was proposed previously that smaller oligosaccharides are generated first during degradation because they can be produced during any secondary chain break reaction while larger oligosaccharides accumulate later.[5]

Both DESI-MS and LAESI-MS adequately tracked changes to the cellulose molecule with time, however the DESI-MS was able to detect higher molecular weight degradation products compared to LAESI-MS. This may have been due to the larger amount of paper analyzed to generate a spectrum or to other instrumental parameters. The LAESI-MS technique showed the presence of glucose in samples while the DESI-MS system used here did not. Ideally, both high molecular weight and low molecular weight products would be equally detectable. Regardless, both techniques were similar to ESI-MS in that they both tracked changes to cellulose as degradation proceeded. Hence, the DESI-MS and LAESI-MS techniques, where smaller quantities of material of unknown analyte concentration are analyzed, are adequate substitutes for the time consuming and sample destroying technique described earlier.[5]

A clear advantage then of LAESI vis-à-vis excising a 1 g piece of paper for ESI-MS analysis is the size scale of sample necessary to generate data. While our experiments used a laser spot size of about 250 μm, recently, by using an optical fiber to deliver the mid-IR light, LAESI-MS has shown the ability to obtain mass spectra from ablation spots 30 μm in diameter.[30] This advantage, analyzing micron sized areas *in situ*, offers the analyst control over the specific location to be analyzed. Further, the small sample area will permit one to examine and compare degradation products formed in foxed[31] or stained portions of paper[32] to those generated in surrounding, clean areas. However, a disadvantage of LAESI-MS analysis was also found: during the laser ablation step, a microscopic hole sometimes formed where the laser ablation took place. Hole formation occurred in more aged papers but not in less aged samples and was related to the inherent low tensile strength of the paper fiber web.[33] Since holes for these experiments were on the scale of 200 μm, the scale of which is close to the limit of detection for the human eye, it will be important to select a spot for analysis that would minimally impact the appearance of any sample as a whole. However, on the occasions that a hole was formed during the LAESI-MS analysis, *i.e.* when 1000 laser pulses were used, this result indicated that the entire depth of the paper was analyzed and any potential bias toward surface-only analysis was avoided.

During analysis, sample spots probed using DESI-MS occasionally got soaked, especially if the spray was focused on one spot for a period of time. The wetting of an area and not just a small spot occasionally meant that when DESI-MS was performed, particularly on aged samples that contained yellowed degradation products, barely visible tidelines[34] formed on the paper where the experiment was completed. Tidelines formed due to the migration of degradation products to the wet–dry

interface. During the control study, only the 26 856 h sample developed tidelines. The visual impact of tidelines can be minimized if edges of sheets are analyzed, smaller areas are scanned or if areas are scanned rapidly with little or no dwelling of solvent spray in one spot.
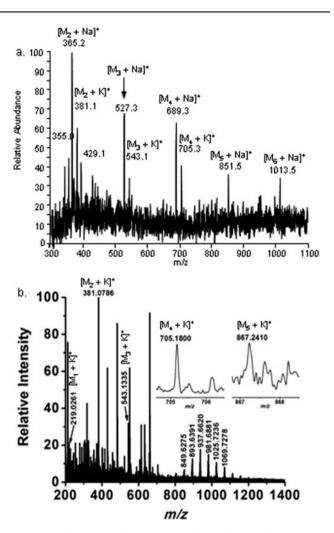
Naturally aged papers

Establishing that DESI-MS and LAESI-MS could be used to track the progress of the generation of degradation products in artificially aged cotton paper *in lieu* of ESI-MS, it became important to determine if the same degradation products would be observed in naturally aged rag papers. If both artificially and naturally aged cellulose showed the same degradation products, then these techniques could be used in the field of art conservation, where the absence or presence of oligosaccharides in a piece of paper might be used as markers for degradation of books and work of art on paper. Also, printing papers are known to contain sizes and additives, as well as have varying surface textures which might affect the detection of degradation products using DESI-MS or LAESI-MS. Therefore, it was important to determine whether oligosaccharide degradation products could be analyzed using these same two techniques in spite of the potential added complexity of manufactured paper.

Papers from two naturally aged books were selected for DESI and LAESI analyses. Since making paper using cotton became common in the 1800s (up until the American Civil War), most likely due to the invention of the cotton gin in 1794,[35] both books chosen for analysis were published in the early1800s. Polarized light microscopy of individual fibers pulled from at least three sheets of paper in each book showed that the papers in both books were made from a mixture of cotton and linen.

Fig. 7 shows the results of both DESI-MS and LAESI-MS analyses of paper taken from the book published in 1833. The DESI-MS result (Fig. 7a) showed sodiated oligosaccharides ranging from cellobiose (m/z 365.2) to cellohexaose (m/z 1013.5) as well as potassiated oligosaccharides ranging from cellobiose (m/z 381.1) to cellotetraose (m/z 705.3). The signal response here was low as seen by the S/N ratio. Sodium bound dimers were not observed.

The LAESI mass spectrum (Fig. 7b) was different from the DESI mass spectrum. Potassiated oligosaccharides from glucose (m/z 219.0) to cellopentaose (m/z 867.2) were observed. Evidence of a synthetic, chlorinated polymer with a repeat unit of m/z 44 was also present in the spectrum, ranging from m/z 849.6 to m/z 1069.7. The presence of the polymer was reproducible across the sheet that was analyzed using LAESI-MS. As the book was published in 1833, the polymer could not have been used in the original manufacture. The page of the book analyzed using LAESI may have been exposed to the polymer during its use or storage or contaminated during handling. Other ions in this mass spectrum with significant relative abundance are of unknown origin. The synthetic polymer was not observed in the DESI experiment but a different sheet from the same book was used in those experiments. Ions other than oligosaccharide degradation products were detected by LAESI-MS. A detailed analysis of those ions is beyond the scope of this work. However, these ions are worth characterizing in the future when more papers are analyzed.
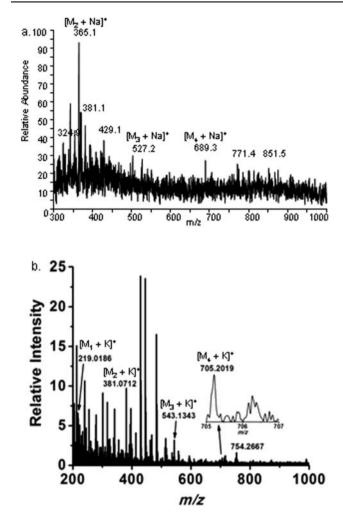

Fig. 7 Analysis of paper from a book published in 1833 using (a) DESI-MS and (b) LAESI-MS (Na—sodium adduct; K—potassium adduct; 1, 2, 3, 4, 5, 6—glucose, cellobiose, cellotriose, cellotetraose, cellopentaose and cellohexaose, respectively).

Fig. 8 shows the results of both DESI-MS and LAESI-MS analyses of a page taken from a book published in 1857. The DESI-MS results (Fig. 8a) showed sodiated oligosaccharides ranging from cellobiose (m/z 365.1) to cellotetraose (m/z 689.3) as well as the potassiated oligosaccharide cellobiose (m/z 381.1). The LAESI mass spectrum (Fig. 8b) showed potassiated oligosaccharides ranging from glucose (m/z 219.0) to cellotetraose (m/z 705.2).

Fig. 9 compares the normalized total relative abundances of the oligomers detected in the pages from the two books using the two different techniques. The figures show the abundances for the naturally aged papers in the book published in 1833 and 1857, respectively. It is clear that both naturally aged books have undergone degradation and that both techniques show similar results regardless of the adducts generated.

Naturally aged books showed that regardless of possible additives or coatings applied, oligosaccharides were observed. These results indicate that both DESI-MS and LAESI-MS can be used to study the qualitative degradation of celluloses in
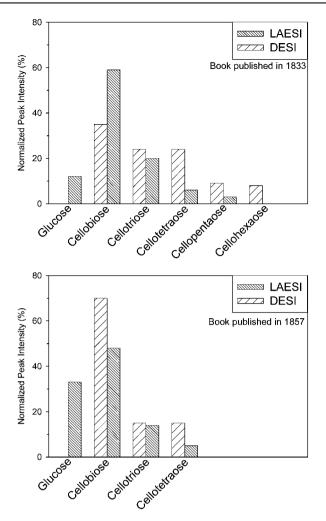
Fig. 8 Analysis of paper from a book published in 1857 using (a) DESI-MS and (b) LAESI-MS (Na—sodium adduct; K—potassium adduct; 1, 2, 3, 4—glucose, cellobiose, cellotriose and cellotetraose, respectively).

Fig. 9 Normalized total peak intensities of oligosaccharides generated in naturally aged papers as determined by DESI-MS and LAESI-MS.

books. Also, since both techniques can be employed with minimal impact on the samples themselves—pages of a book or an artifact can be set next to a DESI sprayer or laid down on a table for LAESI analysis with microscopic sampling—both may be used to study rare artifacts with minimal impact on the artifact itself.

Conclusions

Two atmospheric pressure mass spectrometry ionization techniques, DESI and LAESI, were found to be useful for *in situ* analysis of cellulose degradation products in three different absorbent paper substrates, one of which was made of cotton, two of which were made of a mixture of cotton and linen. A control study of thermally aged 100% cotton paper showed that both techniques were able to track the course of cellulose degradation. As degradation proceeded, increased amounts and a larger variety of degradation products in the form of oligosaccharides were formed. Degradation products formed ranged from glucose to cellodecaose. The higher molecular weight oligosaccharides were observed with prolonged aging while low molecular weight oligosaccharides were observed at all aging

times examined. Analysis of the mass spectra showed that, regardless of aging time or technique employed, the most common degradation products seen were cellobiose, cellotriose and cellotetraose.

Analysis of two books published in the 19th century and comprised of linen and cotton showed evidence of oligosaccharides ranging from glucose to cellohexaose. This indicated that during natural and thermal aging similar degradation products are formed. These positive results allow for further analysis of naturally aged papers to look for differences in degradation in stained areas *versus* unstained areas, to see whether different degradation byproducts are generated during degradation due to hydrolysis and oxidation, or to examine the chemical interaction between paper and the media printed on it. Finally, it might be possible to use these techniques to look for the additives used during the manufacturing process. The advantage of the DESI and LAESI techniques over an earlier used sampling technique[5] was that these surface techniques are non-destructive while the sample preparation for ESI-MS results in damage to, or complete destruction of, the sample.

While it was shown here that DESI-MS and LAESI-MS can be used to track oligosaccharide products formed during cellulose

degradation in paper, it is proposed that the same techniques may be used to study the degradation of cellulose in other materials, objects or organisms, or the decomposition of any other polymer that generates ionizable, low molecular weight products during aging. In these other applications, adjustments to the experimental parameters would be necessary to optimize the ionization of the degraded material.

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