Highly regioselective surface acetylation of cellulose and shaped cellulose constructs in the gas-phase

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Abstract
Gas-phase acylation of cellulose is an attractive method for modifying the surface properties of cellulosics. However, little is known concerning the regioselectivity of the chemistry, in terms of which cellulose positions are preferentially acylated and if acylation can be restricted to the surface, preserving crystallinities/morphologies. Consequently, we reexplore simple gas-phase acetylation of modern-day cellulosic building blocks – cellulose nanocrystals, pulps, regenerated fibre and aerogels. The gas-phase acetylation is shown to be highly regioselective for the C6-OH, is further supported with computational modelling. This contrasts with liquid-state acetylation, highlighting that the gas-phase chemistry is much more controllable, yet with similar kinetics to the uncatalyzed liquid-phase reactions. Furthermore, this method preserves both the native crystalline structure of cellulose and the supramolecular morphologies of even delicate cellulosic constructs (aerogel exhibiting retention of chiral cholesteric liquid
crystalline phases). Therefore, we are convinced that this methodology will lead to more rapid adoption of precisely tailored and cellulosic materials.

Main Text

Cellulose has been the most important polymer for the development of humankind. In its technical form (mainly cotton, chemical wood-based pulps and flax fibre, or other plant-based fibres), it has had a multitude of applications spanning thousands of years - from papermaking to production of textiles, to the first thermoplastics and beyond (Klemm et al. 2005). The concept of the ‘Cellulose gap’ (Hämmerle 2011) has highlighted the need to find replacement feedstocks to cotton, to satisfy our increase in population and standards of living. However, with wood-based chemical pulps as a replacement feedstock, recent trends have shown that existing paper-grade pulp mills are decreasing in profitability, and production is generally below capacity (CEPI - https://www.cepi.org/key-statistics-2020/ 2020). Therefore, significant efforts are being put into bulk valorisation of a range of chemical pulps, other cellulosic materials and their precursor feedstocks. In the short term, there is clear interest in the use of fibrous cellulosic materials, on all length-scales (nano to macrofibrillar), in applications such as improved-strength composite materials (Hubbe and Grigsby 2020). Paper-grade pulp, specifically, is finding rapid adoption in foam-forming (Hjelt et al. 2021). A longer-term goal is the use of paper-grade pulp (Ma et al. 2018; Kuura 2021) and perhaps even lower purity and recycled cellulosics in regenerated fibre production (Haslinger et al. 2019). The topic of nanocellulosates also has significant long-term interest - as substrates for materials applications (Trache et al. 2020), mainly based on the high aspect-ratio that nanocellulose provides in the formation of high-strength composites and colloids (Tardy et al. 2021). However, there are also more interesting intrinsic properties, such as, nanocellulosates axial chirality (Usov et al. 2015) and the potential for asymmetric chemical modification (Heise et al. 2021), that allow for the
preparation of novel materials. These nano-scaled particles have been exploited to form a range of porous materials, where a complex network with various degrees of long-range order is present (France et al. 2017; Tripathi et al. 2019). The implementation of such foams or aerogels in application is currently compromised due to the challenges in altering their surface chemistry, whilst preserving their complex architectures.

A new dimension to develop the intrinsic qualities of celluloses is controllable surface chemical modification, where the crystallinity and morphology of the native materials can be maintained (Tardy et al. 2021). This not only can be used to introduce functional chemical moieties but also for modification of surface properties, for physiochemical interaction with different media, e.g., in nanocomposites and dispersions (Beaumont et al. 2021b; Yang et al. 2021; Tardy et al. 2021). Thus, understanding and controlling the surface chemistry of these materials is critical. The traditional heterogeneous liquid-state modifications of cellulose are already well studied, for those low-cost reactions that are performed industrially (Klemm et al. 1998a, b). One of the most successful commercial reactions is acetylation, typically performed using low-cost acetic anhydride and acetic acid, with sulfuric acid as catalyst (Steinmeier 2004). This method has been shown in key articles to yield cellulose triacetate (CTA), where the outer fibril chains are converted to CTA, followed by a progressive conversion of the inner chains towards the core (Sassi and Chanzy 1995). In the industrial process, lower DS values are only accessible by applying a ‘ripening’ stage, where water is added to the batch reaction to hydrolyse some of the acetate esters. Clearly this method cannot preserve the crystallinity and morphology of the native materials, which are often so critical for application.

Due to the amphiphilic nature of the lattice interactions for crystalline cellulose (Nishiyama et al. 2002), exotic amphiphilic solvents are required for its complete dissolution (Liebert 2010).
However, if the DS of surface modification is high enough the surface chains will strip off into common bulk molecular solvent, depending on the solvent and functionality (Funahashi et al. 2017). This puts limits on our ability to control surface modification, to only the surface chains, using liquid-phase chemistry. Gas-phase reactions, by contrast, potentially offer lower costs than the liquid-state reactions and do not provide a bulk solvating media for stripping of chains. Another important feature is that many delicate shaped cellulose materials, e.g. nano-structured aerogels, do not survive immersion in liquids. In addition, due to the low-cost, high volatility and high reactivity of most common acylating reagents, acylations are an important class of reaction for modification of the properties of cellulosics. Even high boiling aliphatic acylating agents are of use, that can impart significant hydrophobicity to cellulosic materials at low degrees of substitution (DS) (Fumagalli et al. 2013).

The first patent on gas-phase modification of cellulose, using gaseous acetic anhydride (Ac$_2$O), was already published at the beginning of the 20$^{th}$ century (Plauson 1922). Similarly, regenerated cellulose fibres (rayon) have been impregnated with aqueous potassium acetate and acetylated up to an acetate content of 35 % (bulk DS of 2.4), using gaseous Ac$_2$O (Hill 1964). Many more modern reports concern both gas and liquid-phase acylation of cellulose and nanocelluloses, many for the purposes of improving phase compatibility and behaviour in composites or suspensions (Dufresne 2012). While many patents and publications have been made concerning gas-phase acylation of wood and cellulose, there are limited studies concerning the regioselectivity of these surface acylations, in part due to the absence of suitable high-resolution analytics. Consequently, our aim in the present manuscript is to better understand the regioselectivity of gas-phase reactions, using the classical acetylation as a model reaction. Thereafter, we showcase that gas phase reaction between acetyl anhydride can
be tethered to the C6-OH of both cellulose I and cellulose II architectures, without disrupting the supramolecular structures of cellulose as well as the network of nanocelluloses.

Utilising a novel solution-state NMR method for analysis of bulk crystalline celluloses (King et al. 2018; Koso et al. 2020), it was possible to follow the DS and regioselectivity of acetylation of commercial freeze-dried Southern Pine cellulose nanocrystals (FD-CNCs, H₂SO₄-hydrolysed), using a combination of ¹³C-¹H HSQC, ¹H and diffusion-edited ¹H NMR spectroscopy. Reactions were performed in a simple atmospheric-pressure reaction vessel, with dry FD-CNCs exposed to Ac₂O vapour (Figure 1). Considering our FD-CNCs were derived from Southern Pine, the Fernandes et al. 24-chain rhomboid model for softwood serves as the only published softwood model (Fernandes et al. 2011). Using this model, full 6-OH surface acetylation corresponds to a bulk degree of substitution (DS) of 0.33. By gas-phase acetylation of the FD-CNCs over a period of 32 days at room temperature, the combination of ¹H NMR (including peak-fitting to determine DS, SI-Section S2) and diffusion-edited ¹H NMR showed a steady increase to a DS of 0.29 with visibly high regioselectivity for 6-OH acetylation (Figure 1). This value is rather close to the value of 0.24 for the succinylation of hardwood-based cellulose nanofibrils (CNF), using a novel regioselective aqueous-based method (Beaumont et al. 2021 – in press).

![Diagram of cellulose acetylation](image)

**Figure 1.** Diffusion-edited ¹H NMR in [P₄₄₄₄][OAc]:DMSO-d₆ (King et al. 2018; Koso et al. 2020) showing high regioselectivity for 6-OH acetylation and a bulk DS (at 32 d) close to that...
predicted for full 6-OH surface acetylation of a 24-chain rhomboid softwood elementary fibril model (Fernandes et al. 2011).

The 32 d sample was further analysed using 2D $^{13}$C heteronuclear single-quantum correlation (HSQC) spectroscopy. The sample was analysed initially in the $[\text{P}_{4444}]\text{[OAc]}$:DMSO-d$_6$ electrolyte, to further confirm the bulk regioselectivity of acetylation (Figure 2a). The sample was also suspended in DMSO-d$_6$, where only surface chains can be exfoliated into solution (Figure 2b), further allowing determination of regioselectivity. The nanocrystalline nature was also confirmed to be preserved by high-resolution AFM after casting from DMSO (Figure 2c, d) – importantly, the acetylation resulted in partial recovery of the elementary nanocrystals (Figure 2d1) from the typically observed clusters (Figure 2c1). While the cross-sections of the pristine CNCs highlight a clean baseline, with features below 0.3 nm (Figure 2c2), features larger than 2 nm that are not associated with CNC morphology are observed after acetylation (Figure 2d2). The larger features on the baseline indicate exfoliation of the functionalized cellulose chains, subsequently aggregated onto the substrate. The HSQC in $[\text{P}_{4444}]\text{[OAc]}$:DMSO-d$_6$ electrolyte confirmed the high regioselectivity for 6-OH acetylation, by the appearance of a new set of geminal-CH$_2$ 6 (6-OAc) correlations ($^1$H - 4.43 & 4.14 : $^{13}$C - 62.89 ppm). Correlations corresponding to 2-CH (2,3-OAc) and 3-CH (2,3-OAc) are visible, compared with literature assignments (Kono et al. 2015), but only after significant scaling.

When the HSQC of the CNCs dispersed in DMSO-d$_6$ (where only CNC surface chains are in solution – only solvated chains are ‘visible’ using solution-state NMR) was analysed, a similar ratio of geminal-CH$_2$ 6 (6-OAc) and unmodified geminal-CH$_2$ 6 correlations were apparent, from the correlation peak volumes. This ratio would be consistent with the cellulose I$_{\beta}$ crystalline structure (Nishiyama et al. 2002), where rotation of adjacent AGUs in the crystal lattice results in every alternate polymer AGU 6-OH surface-facing. This further supports the
regioselective surface coverage model. As shown in Figure 1, at bulk DS levels close to the complete C6-OH substitution, the regioselectivity starts to decrease. A small amount of 2-CH (2-OAc), consistent with the diffusion-edited $^1$H results, is also visible.

Figure 2. HSQC spectra for: a) 32 d acetylated FD-CNCs in the [P$_{4444}$][OAc]:DMSO-d$_6$ electrolyte, b) 32 d acetylated FD-CNCs dispersed into DMSO-d$_6$. High resolution AFM for: c) pristine unmodified FD-CNCs, & d) 32 d acetylated FD-CNCs (with cross-section profiles).

While it is expected that primary alcohols should react faster, such high regioselectivity was quite unexpected, and has been so far only reported in the case of aqueous-based solid and liquid acylations using N-acylimidazoles (Beaumont et al. 2021b, a, c). In comparison to acetylation in the dissolved state (Abe et al. 2016), selectivity is clearly significantly improved.
To further understand why regioselectivity apparently increases for gas-phase reactions, DFT transition-state (TS) modelling was performed to locate the TS for acetylation of 2-, 3- and 6-OH groups and directly compare their Gibbs free energies, relative to the lowest TS ($\Delta\Delta G^{TS}$).

All calculations were gas-phase (no implicit solvation) performed at the BP86-D3(BJ)-def2-SVP DFT level using the ORCA 4 software package (Neese 2012). This was achieved by initial calculation of the relaxed potential energy surfaces (rPES) for rotation of the dihedral angles where the acetate groups are attached directly to the ring carbon atom (Figure 3). Due to the additional C-C bond between the acetate and sugar unit, for the 6-OAc, barriers to rotation were obviously lower and peaked at around 7 kcal/mol. Several minima were observed. For the 2-OAc, full rotation was presented with an ~30 kcal/mol barrier. For the 3-OAc, full rotation was not achievable and the maxima, before failure of the calculation, approached 40 kcal/mol. The minima were used for calculation of the respective TS. Relative to the 6-OAc TS ($\Delta\Delta G^{TS} = 0$ kcal/mol), the 2-OAc TS $\Delta\Delta G^{TS} = 4.6$ kcal/mol and the 3-OAc TS $\Delta\Delta G^{TS} = 21.3$ kcal/mol. At room temperature, the Boltzmann population of an energy level of 4.6 kcal/mol, relative to 0 kcal/mol, is 0.00042. This clearly indicates that 6-acetylation in the gas-phase should be strongly favoured. In all cases, H-bonding plays a part in stabilisation of each TS, as do steric interactions with adjacent atoms and chains (reduced conformational freedom) in increasing $\Delta\Delta G^{TS}$ values for the 2- and 3-OH acetylations.
Figure 3. DFT rPES for acetate rotation and transition-state modelling of the acetylation mechanism, for 2-, 3- and 6-OH acetylation using acetic anhydride.

To effectively compare whether gas-phase acetylation truly is more regioselective than the alternative liquid-phase methods, an analytical workflow was set up whereby the FD-CNCs were acetylated under different conditions: in gas or liquid Ac₂O, catalysed or uncatalyzed, at two temperatures (RT & 80 °C) with 3 kinetic time points (1, 2 & 6 d) (Figure 4). The bulk DS of acetylation was determined for each by ¹H NMR (peak fitting, SI-Section S2.4). Regioselectivity was visualised using diffusion-edited ¹H NMR and the crystallinity of the 6 d samples were visualised using WAXS. DS values and crystallinity details are in the SI (Table...
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Figure 4. Comparison of regioselectivity and crystallinity changes for gas- vs liquid-phase acetylations (in presence or absence of neutral organic bases).
Finally, we decided to assess the regioselectivity, and provide broad insights on acetylation kinetics, for a series of different cellulose substrates (Figure 5): 1) FD-CNCs, 2) spray-dried CNCs (SD-CNCs), 3) CNC Aerogel, 4) IONCELL fibres (regenerated cellulose II air-gap spun fibres, using an ionic liquid direct-dissolution solvent) (Sixta et al. 2015), 5) pre-hydrolysis hardwood (mainly birch) kraft pulp (P-H kraft Pulp), 6) beech sulphite pulp. All samples were analysed after reaction at RT for 6 d. DS and crystallinity data are given in the supporting info (Table S2).

Figure 5. Cellulose substrate scope showing rough regioselectivity and kinetics through the diffusion-edited $^1$H spectra in [P$_{4444}$][OAc]:DMSO-d$_6$ (King et al. 2018; Koso et al. 2020).

Not surprisingly, the SD-CNCs showed very low reactivity (by ATR-IR, SI Fig S42), compared to the FD-CNCs. It is expected that spray-drying results in much reduced surface area, through aggregation, compared to freeze-drying. XPS analysis (SI Figure S43) of the samples also showed the presence of silicates which is expected to occupy reactive sites and potentially facilitate aggregation during spray-drying. Similarly, the CNC Aerogel sample is expected to maintain a high surface area. Surprisingly, this resulted in a higher DS and high
regioselectivity, compared to the FD-CNCs. Naturally, the BH-PHK and BH-S pulps should have rather low surface areas, due to their much more complex morphology and hornification during the drying procedures. This is reflected in their lower DS values, which however do seem to maintain high regioselectivity. Finally, the IONCELL reaction also gave similar DS values to the pulp samples, indicating a large degree of aggregation and low surface area after drying, preventing further reaction under ambient conditions. In the case of the patent concerning gas-phase acetylation of regenerated cellulose fibres (Hill 1964), much higher degrees of acetylation were possible (clearly indicating non-selective CTA formation), but only after pre-swelling with potassium acetate solution. Thus, specific surface area and porosity of dried materials are of clear importance, for controlling regioselectivity yet reaching high degrees of reactivity. The low reactivity of the fibrillar cellulose samples will likely also be alleviated to some extent using higher temperatures. Initial indications in this study suggest that the regioselectivity may suffer with increasing temperature. However, this can be minimised by targeting lower DS values, as full crystallite surface coverage is likely not required in all cases. This may also be critical in some cases to prevent exfoliation of chains, under solvating conditions.

In conclusion, we have showed that gas-phase reaction with acetate anhydride presents a unique opportunity for selective modification of cellulose I and II substrates. The regioselectivity was more controllable than the corresponding non-, acid- or base-catalysed liquid-phase systems, especially at higher temperatures. This is highly beneficial for controlled surface modification strategies but also for situations where supramolecular 3D morphology should be retained, as with many delicate shaped cellulose objects, e.g., aerogels. It is hoped that this publication will contribute the chemical and analytical know-how required to quickly
expand the scope, and the scale, of application for a wide range of sustainable cellulose materials with complex 3D networks and morphologies.

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