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## Debugging periodate oxidation of cellulose: Why following the common protocol of quenching excess periodate with glycol is a bad idea

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### ABSTRACT

Periodate oxidation of cellulose to produce “dialdehyde cellulose” (DAC) has lately received increasing attention in sustainable materials development. Despite the longstanding research interest and numerous reported studies, there is still an enormous variation in the proposed preparation and work-up protocols. This apparently reduces comparability and causes reproducibility problems in DAC research. Two simple but prevalent work-up protocols, namely glycol quenching and filtration/washing, were critically examined and compared, resulting in this cautionary note. Various analytical techniques were applied to quantify residual iodine species and organic contaminations from quenching side reactions. The commonly practiced glycol addition cannot remove all oxidising iodine compounds. Both glycol and the formed formaldehyde are incorporated into DAC's polymeric structure. Quenching of excess periodate with glycol can thus clearly be discouraged. Instead, simple washing protocols are recommended which do not bear the risk of side reactions with organic contaminants. While simple washing was sufficient for mildly oxidised celluloses, higher oxidised samples were more likely to trap residual (per)iodate, as determined by thiosulfate titration. For work-up, simple washing with water is proposed while determining potential iodine contaminations after washing with a simple colorimetric test and, if needed, removal of residual periodate by washing with an aqueous sodium thiosulfate solution.

### 1. Introduction

In 1928, Léon Malaprade developed the oxidative cleavage of vicinal diols by periodic acid or its salts, which later was named after him: Malaprade reaction or periodate oxidation (Malaprade, 1928). According to the current state of knowledge, the oxidation involves the formation of a cyclic periodate ester which enables regioselective cleavage of the carbon-carbon bond between two “neighboring” hydroxy groups, thereby generating two aldehyde groups (Scheme 1) (Buist et al., 1971). For steric reasons, in order to form the cyclic intermediate, these hydroxyl groups need to be vicinal (at two adjacent carbon atoms) and—if a ring structure such as pyranoses or furanoses are to be oxidised—in *cis*-configuration.

The periodate oxidation proceeds at low temperatures and in aqueous solvents. Because of its simplicity, quantitative conversion, high selectivity, and mild reaction conditions, the Malaprade reaction, still almost 100 years after its discovery, has remained the most common protocol for the oxidative cleavage of glycols. Carbohydrate scientists quickly recognised the potential of periodate oxidation in polysaccharide research: first as an analytical tool (Abdel-Akher et al., 1952; Aspinall & Ross, 1963; Bobbitt, 1956; Guthrie, 1962) to determine the position of vicinal (*cis*-) hydroxyls and later to selectively cleave and derivatise the typical ring structures of carbohydrates (Kristiansen et al., 2010).

In cellulose chemistry, the Malaprade reaction is used to selectively cleave the glucopyranose units' C2-C3 bond, formally generating two

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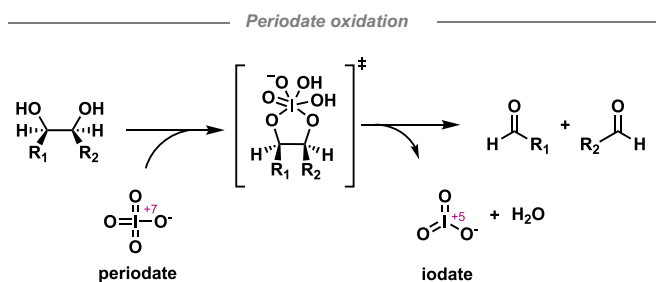
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**Scheme 1.** Periodate oxidation (Malaprade reaction) of a 1,2-diol via a cyclic five-membered periodate intermediate, forming two aldehydes after C-C-bond cleavage.

aldehyde groups per unit (Jackson & Hudson, 1937; Amer et al., 2016; Nypelö et al., 2021). The oxidised cellulose is usually referred to as “dialdehyde cellulose” (DAC). The aldehyde groups are known to form hydrates, hemiacetals, and hemialdals subsequently, which are present in dynamic, water-dependent equilibria. Usually, only a minute amount of the generated carbonyl groups are present in the free form, which explains that typical analytical techniques for carbonyl detection (NMR, IR, Raman) do not report the aldehyde content determined by chemical means (titration) (Amer et al., 2016; Kim et al., 2000; Simon et al., 2022; Spedding, 1960).

DAC has received much attention in recent years in the field of sustainable materials development. It has been studied in drug delivery applications (Abou-Yousef et al., 2021; Chen et al., 2018; Koshani, Tavakolian, and van de Ven, 2021), in the medical field (Dong & Li, 2018; Hou et al., 2018; Syamala Devi et al., 1986), in sensors (Doughan et al., 2015; Nypelö et al., 2018; Shen et al., 2014), for energy storage (Nematdoust et al., 2020; Ruan et al., 2018), and in packaging applications (Chen et al., 2021; Koshani, Zhang, et al., 2021; Plappert et al., 2018). In addition, DAC is not only used directly but also converted into derivatives by follow-up chemistry. The aldehyde groups were further reduced to dialcohol cellulose (Kasai et al., 2014; Larsson et al., 2014a; Lei & Feng, 2020), oxidised to dicarboxy cellulose (Maekawa & Koshijima, 1984; Sharma et al., 2017; Varma & Chavan, 1995a), crosslinked with biopolymers (Kanth et al., 2009; Kim et al., 2017; Koshani, Tavakolian, and van de Ven, 2021; Münster et al., 2018), aminated (Dash et al., 2012; Guigo et al., 2014; Lindh et al., 2016; Simon et al., 2023; Sirviö et al., 2013), and derivatised by Schiff-base reactions (Keshk et al., 2015; Kim & Kuga, 2001).

Despite the variety of applications and the rich follow-up chemistry of the aldehyde moieties, periodate oxidation has the drawback of generating equimolar amounts of iodate, in addition to remaining, non-reacted or excess periodate as potentially toxic waste (National Center for Biotechnology, 2022; National Center for Biotechnology Information, 2022). Efficient recycling of the periodate and properly chosen work-up of the reaction mixture is necessary to eliminate this problem. Sodium periodate can be regenerated electrochemically (Arndt et al., 2020; Janssen & Blijlevens, 2003), with hypochlorite (Liimatainen et al., 2013) or—most environmentally compatible—by ozone treatment under alkaline conditions, which in addition removes low-molecular-weight organic contaminants (Koprivica et al., 2016). Already in 1956, the problem of separating iodate and periodate salts from carbohydrates was described by Bobbitt in his comprehensive review (Bobbitt, 1956). When applied to polymers, such as cellulose, complete removal of the reactive iodate and periodate salts is crucial to prevent contamination of the generated DAC, but of course, non-trivial because of absorption and adsorption phenomena. Owing to the complex and sometimes hardly predictable chemistry of oxidised iodine compounds, including the potential formation of radicals and rich disproportionation behavior (Ritchie, 2008; Yun et al., 2017), their presence can result in various unwanted subsequent reactions and byproduct formation. This can lead to contamination of the generated materials, depolymerisation,

reproducibility problems (for instance through side reactions with  $\text{NH}_2\text{OH}$  during oximation for DO determination (Subbotina et al., 2022)), or undesirable property changes, such as coloring of the DAC at storage or elevated temperatures due to condensation reactions and chromophore formation (Ahn et al., 2019).

The abundance of research on periodate oxidation of other carbohydrates to dialdehyde analogs (e.g., dialdehyde starch), which, like DAC, have gained increasing attention, is staggering. The published oxidation procedures differ greatly in the reaction conditions and protocols used. Various work-up methods for separating DAC from the iodine species are popular in the cellulose community. In this report, several studies on such work-up variants were examined and compared to highlight the importance of thorough DAC purification, point out pitfalls and present alternative workflows to monitor and, if needed, remove unwanted contaminations effectively.

## 2. Experimental section

### 2.1. Chemicals and reagents

All reagents and chemicals were purchased from commercial suppliers in the highest purity available and used without further purification unless otherwise noted. Details about the cellulosic pulps used in this study (Pulp A, B, and C; Fig. 2) are summarised in previous work (Hettegger et al., 2022; Table S3).  $^{13}\text{C}$ -labeled formalin solution was prepared by heating paraformaldehyde- $^{13}\text{C}$  (254 mg) in distilled water (10 mL) for 4 h at 60 °C. Insoluble particles were filtered off and aqueous formalin- $^{13}\text{C}$  was used for NMR labeling experiments.

### 2.2. Characterization

Carbon ( $^{13}\text{C}$ ) cross polarisation/magic angle spinning (CP/MAS) NMR spectra were recorded on a Bruker Avance III HD 400 spectrometer with a resonance frequency of 400.34 MHz and 100.67 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively, equipped with a 4 mm dual broadband CP/MAS probe. Data were acquired at a spinning rate of 12 kHz, with a CP contact time of 2 ms and SPINAL 64  $^1\text{H}$  decoupling. Chemical shifts were referenced externally against the carbonyl signal of glycine with  $\delta = 176.03$  ppm.

Carbon nuclear magnetic resonance ( $^{13}\text{C}$  NMR) was recorded using a Bruker Avance II 400 spectrometer (resonance frequencies 400.13 and 100.63 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively) equipped with a liquid  $\text{N}_2$ -cooled cryoprobe head (Prodigy) with z-gradients at room temperature with standard Bruker pulse programs. Chemical shifts are reported in parts per million (ppm) relative to acetone added as internal standard:  $\delta_{\text{H}} = 2.22$  ppm and  $\delta_{\text{C}} = 30.9$  ppm.

Elemental analysis (EA) was performed on a Thermo Flash Smart CHNSO Elemental Analyzer (combustion EA). For each sample, 1 to 3 mg was weighed into tin foil cups after freeze-drying. The Elemental analyzer was calibrated beforehand by linear calibration using sulphanylamine as the standard. All measurements were done at least in triplicate and averaged. C, H, N and S were analysed directly. DS calculations were based on the nitrogen values.

Ultraviolet-visible (UV-Vis) spectra to track the periodate consumption were recorded on a JASCO V-560 double-beam spectrometer equipped with a temperature thermostat and automated cell changer and were evaluated with Spectra Manager™ software.

The thermal behavior was analysed using an STA 449 F3 Jupiter device. The sample was investigated in the temperature range of 40 to 600 °C with a heating rate of 10 K/min (dynamic method) under a helium flow of 70 mL/min. The results were processed using Netzsch Proteus software. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) thermograms were examined.

Conductivity measurements were performed in triplicate using a sensION+ EC7 conductivity meter (Hach).

### 2.3. Standard procedure for the periodate oxidation of cellulose and other polysaccharides

A round bottom flask was charged with sodium periodate and deionised water. Cellulose or another polysaccharide was added, and the mixture was stirred in the dark to limit side reactions. The temperature, periodate concentration, and reaction duration were varied to control the DO. The work-up protocols were altered to investigate the formaldehyde and iodate contamination when quenched excess periodate by ethylene glycol and insufficient purification.

### 2.4. Determination of the degree of oxidation

The DO was monitored during periodate oxidation by UV–Vis spectroscopy from the absorbance at 222 nm previously described (Han et al., 2010; Simon et al., 2022). After isolation of the periodate-oxidised polysaccharide, the DO was determined accurately based on the quantitative oximation reaction with hydroxylamine hydrochloride. The DO was either calculated from the nitrogen content using a previously reported formula (Simon et al., 2023) or by potentiometric titration of the formed hydrochloric acid following our previously adjusted protocol (Simon et al., 2022; Zhao & Heindel, 1991). Each DO was determined at least in duplicate.

### 2.5. Gravimetric analysis of residual formaldehyde with dimedone reagent

A stock solution of 5,5-dimethyl cyclohexane-1,3-dione (dimedone, 14.02 g) in 1 L of a 1:1 (v/v) mixture of 1 mM aqueous HCl and absolute ethanol was prepared. For quantitative determination of the formaldehyde content, the aqueous washings from an oxidation run were combined and brought to a defined volume (500 mL or 1 L). A 50 mL aliquot was mixed under stirring with 100 mL of the dimedone stock solution. The mixture was stirred at room temperature for 90 min. The white precipitate (dimedone-formaldehyde condensation product) was filtered off and washed twice with an ice-cold mixture of distilled water and absolute ethanol (v/v = 3:1, 20 mL). The precipitate was dried at room temperature under vacuum to constant weight. Three independent determinations were performed and averaged. The standard deviation was below 2.5 % throughout. The total amount of formaldehyde contained in the washings was calculated from the gravimetrically determined content in the 50 mL aliquot.

### 2.6. Determination of residual ethylene glycol by gas chromatography

The quantity of trapped glycol corresponds to the difference between the free glycol detected by gas chromatography and the total amount used. For quantitative determination of the remaining glycol content, the aqueous washings from an oxidation run were combined and brought to a defined volume (500 mL or 1 L). A 50 mL aliquot was taken and evaporated *in vacuo* at room temperature. The residue was mixed with dichloromethane (5 mL) and anhydrous calcium sulfate (0.2 g) was added. After stirring for 10 min, the solids were filtered off and the solvent was removed *in vacuo*. The residue was derivatised according to the literature procedure (Becker, Liebner, et al., 2013; Becker, Zweckmair, et al., 2013). In short, the residue was dissolved in 200  $\mu$ L of pyridine and incubated at room temperature for 30 min. A solution of the silylation catalyst (1.5 mg/mL DMAP in pyridine, 200  $\mu$ L and silylation agent *N,O*-bis(trimethylsilyl)-trifluoroacetamide containing 10 % trimethylsilyl chloride, 200  $\mu$ L) was added to the mixture, which was stirred at 70 °C for 2 h. After cooling to room temperature, the derivatised samples were kept at –20 °C until analysis. The derivatised samples were diluted with ethyl acetate (600  $\mu$ L) and filtered before injection. Aliquots of 0.2  $\mu$ L were injected in splitless mode and analysed on an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass selective detector and Agilent GC Sampler 120, with analysis

parameters based on the protocol of Sundberg et al. (1996).

### 2.7. Extraction of formaldehyde from dialdehyde cellulose

Aliquots of formaldehyde-loaded DAC (DO of 25 %) obtained by periodate oxidation of microcrystalline cellulose were kept in closed vials between Teflon stoppers to limit the evaporation of trapped formaldehyde. The samples were kept either never-dried or after lyophilisation at room temperature or 110 °C for 4 days. Periodically (after 1, 24, 48, and 96 h), aliquots were Soxhlet-extracted with aqueous trifluoroacetic acid (pH 4 to 5) for 24 h. The pH was adjusted to the initial pH (4 to 5) every 3 h if necessary. The extracted formaldehyde was determined gravimetrically as the dimedone adduct (see above). Each sample was prepared and measured in duplicate.

### 2.8. NMR study of formalin-<sup>13</sup>C treated dialdehyde cellulose

DAC (degree of oxidation of 8 %; 400 mg) obtained from microcrystalline cellulose was stirred in freshly prepared formalin-<sup>13</sup>C solution (10 mL) for 1 h at room temperature. The treated DAC was filtered off, split into three aliquots, and stored for 96 h in closed vials at room temperature, at 60 °C or 110 °C in the oven. All three samples were investigated together with the untreated DAC by solid-state NMR spectroscopy.

### 2.9. Determination of residual iodate by redox titration

To roughly quantify the contaminations with residual IO<sub>3</sub><sup>-</sup> impurities, DAC suspensions were titrated with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, following and adapting a procedure for iodised table salt. DAC samples were prepared by stirring microcrystalline cellulose (5 g) in H<sub>2</sub>O (200 mL) for 24 h with varying stoichiometric amounts of NaIO<sub>4</sub> (0.125 equiv. to 1.0 equiv.) and different reaction temperatures (room temperature or 50 °C). Residual NaIO<sub>4</sub> was removed by the addition of ethylene glycol (10 mL) followed by stirring for 1 h at room temperature. The solid residue was separated by vacuum filtration (sintered glass filter, porosity 1 or 2) and thoroughly washed with distilled water (500 mL). The still wet DAC sample was transferred to a 250 mL volumetric flask and filled with distilled water to yield a stock suspension (approx. 20 g DAC/L). To break up agglomerates, the mixture was flushed through the holes of an open Büchner funnel. An aliquot of the suspension (50 mL) was transferred to an Erlenmeyer flask using a graduated cylinder before aqueous stock solutions of NaI (2 mL; 5 wt%), H<sub>2</sub>SO<sub>4</sub> (5 mL; 1 M) and potato starch (2 mL; 1 wt%; the starch was used after boiling and filtration of residual particles) were added. The presence of oxidising iodine compounds was evidenced by a deep purple color of the iodine-starch complex. The analyte was diluted with distilled water (50 mL) and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> stock solution was added with a burette until the coloration ceased. Approximate contents of NaIO<sub>3</sub> were calculated from the Na<sub>2</sub>SO<sub>3</sub> consumption (cf. Tables 3 and S1).

### 2.10. Quick colorimetric test for the presence of oxidising iodine compounds

After separating solid DAC by filtration, centrifugation, or decantation, an aliquot was transferred to a test tube and suspended in distilled water. Approx. 1 mL of each of the aqueous stock solutions of NaI (5 wt %), H<sub>2</sub>SO<sub>4</sub> (1 M), and potato starch (1 wt%) were added. The presence of oxidising iodine compounds is indicated by the deep purple color of the iodine-starch complex. The coloration usually occurs immediately. However, if only small quantities of contaminations are present or the sample was dried, it is advised to agitate the suspension (30 min) or manually break up agglomerates using a spatula (Fig. S3).

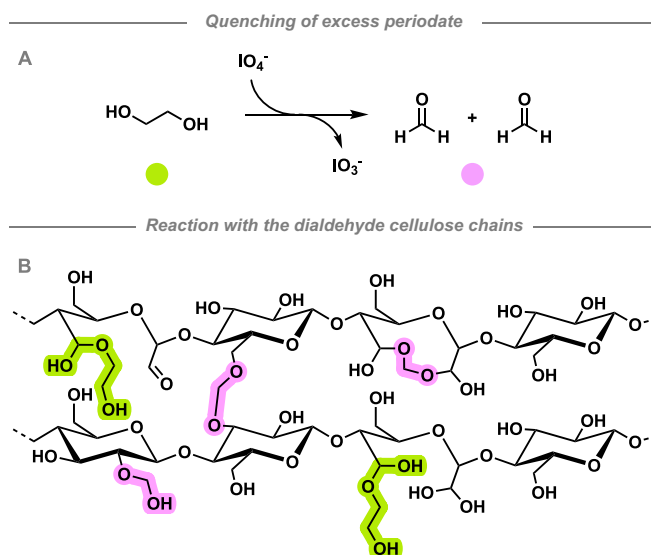
### 2.11. Reductive decontamination of dialdehyde cellulose

If the colorimetric test for oxidising iodine compounds is positive, the bulk of DAC is transferred to a reaction vessel and resuspended in distilled water. The suspension is acidified by addition of glacial acetic acid ( $\text{H}_2\text{SO}_4$  is applicable as well but might result in unwanted side reactions) before an aqueous solution of  $\text{Na}_2\text{S}_2\text{O}_3$  (determination of the appropriate amount: see above) is added and the reaction mixture is agitated. The reaction is usually completed in <5 min. However, iodine species trapped in agglomerates proved to be less accessible and might require longer times to react. Thus, it is important to break up agglomerates formed during filtration or centrifugation or preferably to limit their formation by resorting to decantation. The addition of catalytic  $\text{NaI}$  before the reductant can accelerate the reaction and allows for visible tracking of severe contaminations over a brown triiodide complex ( $\text{NaI}$  addition is not necessary as iodide ions are also formed during the reduction). The amounts of reagents needed for complete removal can vary significantly depending on sample size or degree of contamination. To avoid wasting chemicals, it is advised to gradually add the reductant and control the reaction by removing aliquots and applying the colorimetric test (see above). Excessive addition of  $\text{Na}_2\text{S}_2\text{O}_3$  can also result in the precipitation of fine particles of elemental sulfur, which are difficult to remove from the reaction mixture. The purified DAC is separated by filtration, centrifugation or decantation and thoroughly washed with distilled water. For samples with iodine compounds being difficult to remove by washing, it is likely that also the chemicals used during the decontamination and their reaction products will be trapped in the DAC. This needs to be considered in the planned applications or follow-up chemistry, although their influence can be expected to be less critical than the presence of oxidising iodine compounds.

### 3. Results and discussion

One of the most common work-up steps after DAC preparation includes the addition of ethylene glycol (Han et al., 2010; Kim et al., 2000; Münster et al., 2017; Sickinga et al., 2022; Soni et al., 2022) or glycerol (Llàcer Navarro et al., 2021; Lucia et al., 2019) to quench excess periodate and thereby to stop periodate side reactions or further cellulose conversion before washing or dialysis. Quenching with glycol has already been introduced in the 1950s (Gibbons & Boissonnas, 1950), and since then, only a few studies have considered possible side reactions of the formaldehyde formed. Sickinga et al. (2022) mentioned the possibility of imine formation of formaldehyde with the diamine dyes used in their study, but like others, did not consider a possible reaction of formaldehyde with cellulose or DAC. Ironically, the formaldehyde-cellulose reaction has been used for over a century—particularly in the textile industry, since the formaldehyde modification improves the wet strength of fibers (Eschaliér, 1906). In addition, the crosslinking behavior of the aldehyde groups in DAC has been heavily discussed in the literature (Amer et al., 2016; Kim et al., 2000; Simon et al., 2022; Spedding, 1960). Based on this knowledge, it is reasonable to assume that the formaldehyde generated upon glycol quenching of excess periodate, and excess glycol itself, could participate in side reactions with either hydroxyl or aldehyde groups of the DAC. The simplest reaction would be the hydroxymethylation of cellulosic hydroxyl groups, forming metastable *O*-hydroxymethyl moieties (hemiacetals of formaldehyde). Also acetal formation, resulting in the formation of methylene bridges between hydroxyl groups, and thus intramolecular or intermolecular crosslinking is conceivable (Scheme 2). At elevated temperatures, an aldol-type condensation reaction of formaldehyde and the aldehyde groups of DAC is conceivable. After all, formaldehyde is the most reactive aldehyde there is.

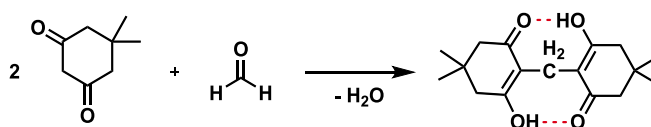
To investigate these hypotheses, excess sodium periodate after oxidation of cellulose (*i.e.*, microcrystalline cellulose, pulp, bacterial cellulose and cotton linters) and also other carbohydrates for comparison (*i.e.*, xylan, alginate, polyglucuronic acid, chitosan and amylose)



**Scheme 2.** Quenching reaction of excess periodate with ethylene glycol, generating iodate and formaldehyde (A). Side reactions of DAC with formaldehyde and/or ethylene glycol: acetal and hemiacetal formation (intra- and intermolecular) by reaction of DAC's hydroxy and aldehyde groups with formaldehyde and ethylene glycol (B).

was quenched with glycol. The generated formaldehyde was analysed as the dimedone adduct by gravimetric determination (Scheme 3), as a relatively simple yet robust and nicely reproducible method (Spencer & Henshall, 1955). Furthermore, dialdehyde celluloses with varying degrees of oxidation were treated afterward with glycol and/or aqueous formaldehyde to investigate the trapping reaction and consumption potential by gravimetric titration and CP-MAS <sup>13</sup>C NMR spectroscopy.

Another ubiquitous protocol in DAC purification—either following or instead of glycol quenching—consists of simple filtration of the generated DAC and thorough washing with deionised water (Chen & van de Ven, 2016; Larsson et al., 2014b; Sirviö et al., 2011). The reported amounts of water used and the number of conducted washing steps vary significantly, and potential residual contaminations are not even considered in many cases. Notably, purification of the DAC using dialysis (Leguy et al., 2018; Llàcer Navarro et al., 2021) may result in an entirely iodine-free DAC, but it is time-consuming and inapplicable on a larger or even industrial scale. Especially when conducting simple washing procedures in a Büchner funnel and working with DAC samples of a high DO, trapping of iodate and periodate compounds in the oxidised material seems very plausible. The intensive morphological changes upon periodate oxidation often result in a gel-like material known to clog the filter rapidly (Lindh et al., 2014; Siller et al., 2015). This makes the filtration overly time-consuming and tedious and negatively influences washing efficiency. To address this work-up variant, dialdehyde celluloses with varying degrees of oxidation were generated to prove the trapping of generated iodine compounds in DAC. The residual iodate was quantified by titration, adapting a simple protocol from table salt analytics. Furthermore, conductivity measurements were included to study the amounts of water and the number of washing steps necessary to remove the iodine-containing compounds as completely as possible.



**Scheme 3.** Gravimetric determination of formaldehyde as the dimedone adduct (“dimedone adduct”).

### 3.1. Quenching of periodate with glycol: the fate of the formed formaldehyde

To investigate the fate of the generated formaldehyde upon quenching excess periodate with glycol during the work-up of DAC, microcrystalline cellulose was oxidised, excess sodium periodate was quenched, and the formaldehyde was quantified gravimetrically as the dimedone precipitate (Scheme 3) (Spencer & Henshall, 1955). In the ideal case, two equivalents of formaldehyde will be produced from one equivalent of glycol, and this formaldehyde amount should be present in the washings. The gravimetric determination of the formaldehyde with dimedone is one of the oldest, yet most robust, unambiguous and reliable techniques to determine this reactive C<sub>1</sub>-species, which has been widely used also for biological samples. As a highly methylene-active

1,3-diketone, two equivalents of dimedone bind one equivalent of formaldehyde in a highly sensitive and selective Knoevenagel-type condensation reaction (Scheme 3). Three independent measurements were averaged in our work. A series of dialdehyde celluloses (degrees of oxidation of 20, 40, 60, and 80 %) was prepared using a varying excess of sodium periodate. Each reaction was quenched with twice the equivalents of glycol relative to the amount of sodium periodate remaining in the reaction solution after the targeted DO was achieved (according to online tracking by UV-Vis spectroscopy). For example, in the experiment to generate 20 %-oxidised DAC, a residual amount of 0.1 equivalents of non-reacted sodium periodate was determined, which was quenched with 0.2 equivalents of ethylene glycol. Theoretically, gravimetric analysis of the reaction solution after quenching with glycol is expected to yield 0.2 equivalents of formaldehyde (2 mol of

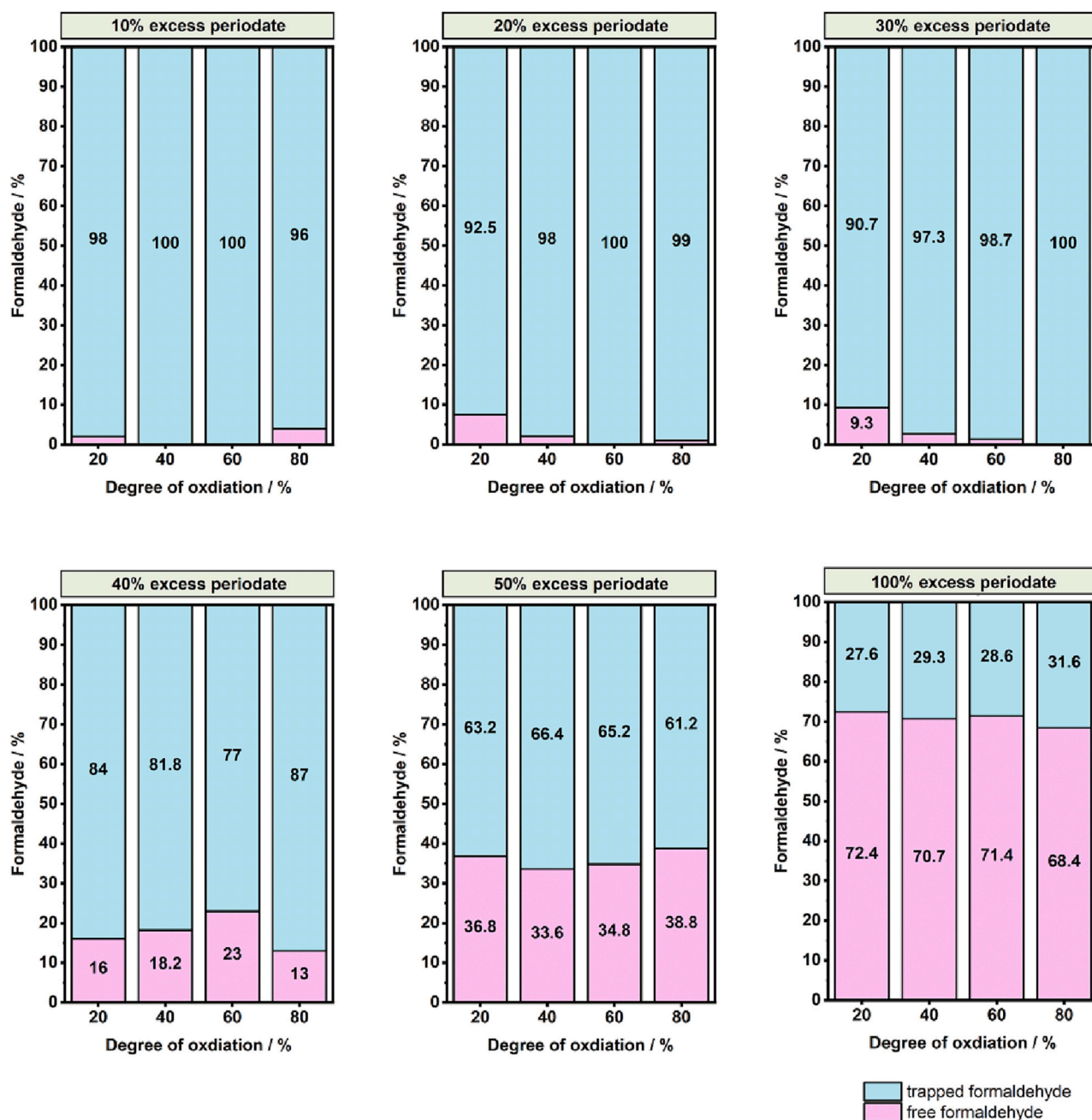


Fig. 1. Quenching excess sodium periodate with ethylene glycol in periodate oxidation of cellulose: trapped and free formaldehyde quantified gravimetrically as the dimedone precipitate after production of dialdehyde celluloses with varying degrees of oxidation (20, 40, 60, and 80 %) and varying periodate excess. The theoretical amount of generated formaldehyde is set to 100 %.

formaldehyde per 1 mol of sodium periodate and 1 mol of glycol). This theoretical value neglects side-reactions of sodium periodate, such as light-induced decomposition, and formaldehyde's side reactions. Interestingly, no significant amounts of formaldehyde were determined in the case of 10 % excess sodium periodate (Fig. 1).

Also, for 20 and 30 % of excess sodium periodate, the detectable amounts of formaldehyde were negligible, independent of the DO of the DAC. For the samples synthesised with a periodate excess of 40 % and more, the formaldehyde equivalent of 28 to 35 % of periodate excess was trapped by the DAC (Fig. 1) and thus not contained in the washings. The theoretical amount of generated formaldehyde (Fig. 1) is set to 100 %. For example, 84 % of the theoretically generated formaldehyde was trapped by 20 %-oxidised DAC with a periodate excess of 40 %, which equals 33.6 % of the periodate equivalents used. These results demonstrate that using glycol to remove excess periodate leads to formaldehyde formation as expected, but this product is not free and interacts to a large extent with the polymer structure of DAC. The fraction of formaldehyde "lost" by reaction with the DAC was roughly one-third of the theoretically formed amount at a periodate excess of 40 % and above. However, based solely on the free formaldehyde data in the washings, it remains unclear whether the DAC traps the formaldehyde or whether it reacts with iodine compounds in the reaction mixture, for instance, under oxidation to formic acid. To address the fate of the formaldehyde, three dialdehyde celluloses with varying degrees of oxidation (20, 40, and 60 %) were synthesised from microcrystalline cellulose and the isolated dialdehyde celluloses were intensively washed with water to remove all iodine-containing species. The possibility of follow-up reactions of the generated formaldehyde with residual iodine compounds could thus be reliably excluded. The isolated dialdehyde celluloses were then treated with excess formaldehyde at either pH 7 or pH 4 at different times to examine whether formaldehyde would react and be trapped in a reaction with DAC, followed by another intensive washing treatment of the DAC. The free formaldehyde in the combined washings was again determined gravimetrically as the dimedone precipitate (Table 1).

Also, the possible binding of ethylene glycol to DAC was examined, as the hydroxy groups of ethylene glycol are theoretically capable of reacting with the aldehyde groups of DAC to form hemiacetals. Even the formation of acetals, with the formation of 1,3-dioxolan ring structures being especially entropically favored in the case of glycol as the co-reactant, would be conceivable, especially at lower pH values (<4). Thus, the isolated dialdehyde celluloses were treated with excess ethylene glycol at either pH 7 or 4 (Table 1). The free ethylene glycol in the washings—and thus any loss by reaction with DAC—was determined by gas chromatography/mass spectrometry (GC/MS) according to a previously reported oximation/silylation protocol (Becker, Liebner, et al., 2013; Becker, Zweckmair, et al., 2013).

For the series at pH 4, which is in the range of the pH determined at the end of unbuffered periodate oxidation (Varma & Kulkarni, 2002), the amounts of formaldehyde that were trapped by DAC agreed astonishingly well with the results of the formaldehyde consumption after

**Table 1**

Amounts of formaldehyde and glycol trapped by dialdehyde cellulose with varying degrees of oxidation (20, 40, and 60 %). Reaction for 1 h at two different pH values at room temperature.

DO	Trapped formaldehyde/% <sup>a</sup>		Trapped glycol/% <sup>b</sup>	
	pH 4	pH 7	pH 4	pH 7
20	36	16	6	2
40	34	18	4	3
60	30	17	6	3

<sup>a</sup> % of the total amount of formaldehyde used. The non-reacting formaldehyde remaining in the washings was quantified gravimetrically as the dimedone precipitate.

<sup>b</sup> % of the total amount of glycol used. The non-reacting glycol in the washings was determined by gas chromatography (see Experimental).

quenching excess sodium periodate with ethylene glycol (Fig. 1). Independent of the DO, around two-thirds of the formaldehyde initially present remained detectable gravimetrically after stirring the DAC for 1 h in formalin. The other third interacted with DAC: between 30 and 36 % of the added formaldehyde remained trapped in the DAC. For the series at pH 7, the amount of trapped formaldehyde was significantly smaller (16 to 18 %). This outcome is readily explained by the higher reactivity of formaldehyde (and aldehydes in general) and the favored formation of hemiacetals and acetals at pH 4 compared to pH 7. Also aldol-type reactions—and similar acid-catalysed condensations involving formaldehyde—would generally proceed faster at pH 4 than under neutral conditions.

Furthermore, the GC/MS data (Table 1) demonstrated that also ethylene glycol was bound by DAC, although the trapped amounts were significantly smaller than in the case of the highly reactive formaldehyde. Ethylene glycol would only react with (masked) aldehyde functionalities in DAC to hemiacetals or possible acetals, while formaldehyde can react with various functionalities, such as (masked) aldehydes and hydroxyl groups, as well. The DO had no systematic influence on the trapped glycol—for all three DAC samples, 2 to 3 % were trapped at pH 7 and 4 to 6 % at pH 4.

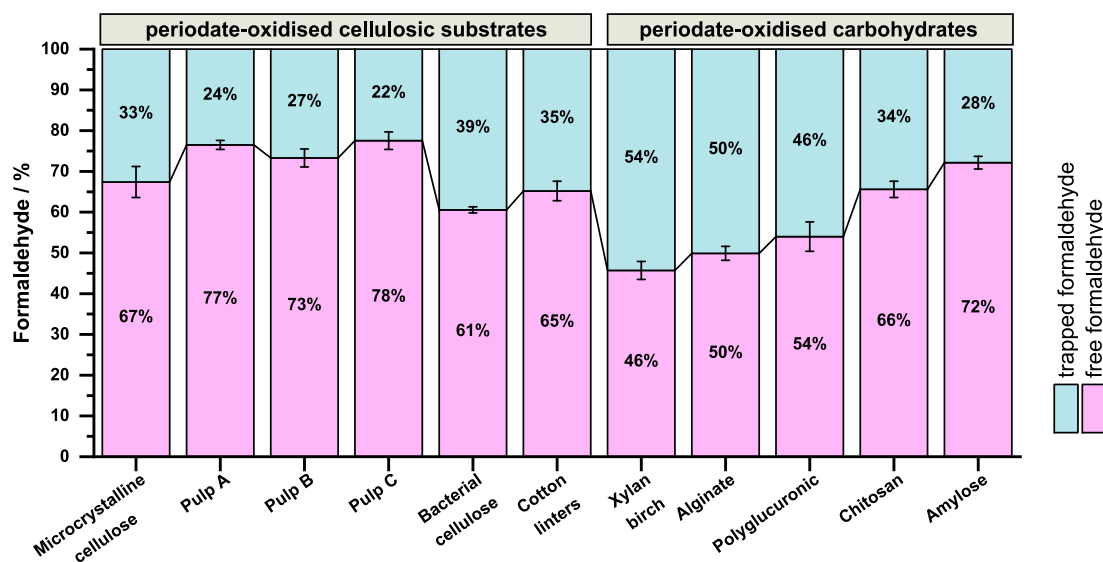
Quenching of excess periodate with ethylene glycol is not only commonly used in cellulose modification but is also frequently used for other polysaccharides (Palasingh et al., 2022; Sarker et al., 2014; Wright et al., 2014). We were interested in whether also these oxidised polysaccharides partly trap the generated formaldehyde—without intending to study those cases exhaustively. Different periodate-oxidised polysaccharides (DO of approx. 25 %) were generated and residual sodium periodate (0.25 equivalents) was quenched with ethylene glycol (0.5 equivalents, which is 2 equivalents relative to residual periodate). The amounts of formaldehyde retained in the oxidised polysaccharides were determined, once again by quantifying the free formaldehyde in the washings as dimedone precipitate (Fig. 2).

All oxidised polysaccharides tested trapped significant amounts of formaldehyde, which suggests that glycol quenching of excess periodate is discouraged not only in the cellulose case but for periodate-oxidised polysaccharides in general. The different celluloses (Fig. 2) bound between 22 and 39 % of the generated formaldehyde, with the values for chitosan and amylose being in the same range. There was a tendency towards higher formaldehyde retention—between 46 and 54 %—for polysaccharides that contained carboxylic acid groups, such as xylan (4-O-methyl-β-D-glucopyranosiduronic acid moieties), alginate (guluronic and mannuronic acids) and polyglucuronic acids. The molecular reasons for this higher binding capacity have not yet been studied.

### 3.2. Analysis of the interactions of formaldehyde and dialdehyde cellulose

Following the quantitation of trapped formaldehyde in cellulose and other polysaccharides, the underlying chemical interactions of formaldehyde and DAC were further evaluated. Oxidised DAC (25 %) was treated with ethylene glycol, filtered off, washed with distilled water, and transferred to reaction vessels sealed with Teflon stoppers. The samples were stored at room temperature or heated to 110 °C for up to 96 h. Aliquots were taken and it was examined to which extent Soxhlet-extraction with aqueous trifluoroacetic acid can recover the initially trapped formaldehyde (Table 2). To investigate the influence of drying on the re-extractable amount of formaldehyde, both a never-dried and lyophilised dialdehyde cellulose were prepared.

At room temperature, most of the formaldehyde remained trapped, and between 6 and 9 % could be recovered with aqueous trifluoroacetic acid. The re-extractable amount decreased with increasing time and even more when the DAC material had been dried before extraction. We did not recover significant amounts of formaldehyde at elevated temperatures after 96 h. In a solid-state <sup>13</sup>C NMR study, DAC obtained from microcrystalline cellulose (DO of 8 %) was treated with <sup>13</sup>C-labeled formalin, filtered, thoroughly washed and kept at different temperatures



**Fig. 2.** Trapped and free formaldehyde of various periodate-oxidised cellulosic substrates and other polysaccharides (DO of approx. 25 % throughout) after quenching residual sodium periodate (0.25 equivalents) with ethylene glycol (0.5 equivalents). The part of the formaldehyde which was not bound by the oxidised polysaccharide, remained in the aqueous washings and was quantified gravimetrically as the dimedone precipitate. The theoretical amount of generated formaldehyde is set to 100 %.

**Table 2**

Re-extractable formaldehyde at room temperature and after heat treatment from dialdehyde cellulose<sup>a</sup> by Soxhlet extraction with aqueous trifluoroacetic acid.

	Re-extractable formaldehyde/% Never-dried		Re-extractable formaldehyde/% After lyophilisation	
	At rt <sup>b</sup>	At 110 °C <sup>b</sup>	At rt <sup>b</sup>	At 110 °C <sup>b</sup>
After 1 h	8.8 (±0.4)	n.d.	6.4 (±0.2)	n.d.
After 24 h	7.7 (±0.1)	2.7 (±0.3)	6.0 (±0.2)	1.0 (±0.1)
After 48 h	6.8 (±0.2)	1.0 (±0.2)	5.1 (±0.3)	0.1 (±0.1)
After 96 h	5.9 (±0.1)	0.3 (±0.1)	3.7 (±0.3)	0.0 (±0.0)

<sup>a</sup> Oxidised dialdehyde cellulose (25 %) obtained from microcrystalline cellulose was treated with ethylene glycol (two equivalents) after periodate oxidation, and the trapped formaldehyde was re-extracted afterward.

<sup>b</sup> Determined gravimetrically as the dimedone precipitate in duplicate after Soxhlet-extraction for 24 h with aqueous trifluoroacetic acid.

(room temperature, 60 °C, and 110 °C) in sealed vessels for 96 h (Fig. S1). In comparison to the untreated sample, the spectra of formalin-<sup>13</sup>C treated DAC at room temperature and at 60 °C showed two distinct <sup>13</sup>C signals around 82.6 ppm and a stronger one around 89.2 ppm, evidencing the incorporation of formaldehyde. The assignment of the peaks to a species covalently bound to the cellulosic material was, however, not conclusive. On the one hand, both peaks were in the range of either formalin (CH<sub>2</sub>(OH)<sub>2</sub> – 82.6 ppm) or a formaldehyde dimer (89.6 ppm) observed in previously reported solution-state NMR studies (Rivlin et al., 2015; Fig. S2). On the other hand, also hemiformals, R-O-CH<sub>2</sub>OH from alcohols R-OH, e.g., from methanol, produce shifts around 90 ppm (Hahnenstein et al., 1994), which would indicate covalent modification of DAC by hemiformal formation with hydroxyl groups. Considering the shift variations owing to solution effects, the lower resolution of the solid-state NMR techniques, and the peak superposition with the cellulose backbone, we were not able to unambiguously distinguish between trapping through absorption phenomena or through covalent bonds, based on the NMR results. In accordance with the Soxhlet extraction data, both peaks disappeared for the sample treated at 110 °C. However, there were no evident new peaks pointing to either condensation or degradation reactions. Only a very broad signal in the aliphatic region appeared. In addition, it seems plausible that the “disappearance” of formaldehyde observed in both NMR and Soxhlet

extraction was, at least in part, caused by insufficient sealing and evaporation during the sample preparation. As small losses were already observed after freeze drying or longer treatment at room temperature for longer periods (Table 2) this deserves special caution: after glycol quenching, formaldehyde—a volatile and highly toxic compound—is not only trapped in the DAC, but apparently also slowly and gradually released into the environment, so that the facet of material and occupational safety must be included in the considerations.

### 3.3. Contaminations with iodine species owing to insufficient washing

After highlighting the potential shortcomings of glycol quenching, the deficiencies of common work-up protocols in removing oxidising iodine compounds were further investigated. Formally, periodate quenching results in the quantitative transformation to iodate (Scheme 1). Also the resulting iodate is a strong oxidant and can generate radicals. Furthermore, the quenching is often combined with rather simple filtration and washing protocols, for which the complete removal of contaminations is questionable. To get further insight, a series of periodate-oxidised dialdehyde celluloses was prepared and purified by quenching/washing protocols. The residual iodate impurities were analysed by iodometric titration with sodium thiosulfate (Table 3).

**Table 3**

Residual sodium iodate in dialdehyde cellulose after quenching excess sodium periodate with glycol<sup>a</sup>.

	DO <sup>b</sup> /%	T/°C	NaIO <sub>4</sub> /eq	Residual NaIO <sub>3</sub> <sup>d</sup> /ppm
1	7	rt <sup>c</sup>	0.25	– <sup>e</sup>
2	10	50	0.25	– <sup>e</sup>
3	13	rt <sup>c</sup>	0.50	– <sup>e</sup>
4	20	50	0.50	71 ± 2
5	29	rt <sup>c</sup>	1.0	161 ± 2
6	56	50	1.0	20,600 ± 500

<sup>a</sup> Microcrystalline cellulose was oxidised to dialdehyde celluloses with varying degrees of oxidation and excess sodium periodate was quenched by the addition of ethylene glycol.

<sup>b</sup> Calculated from nitrogen content (elemental analysis) after oximation.

<sup>c</sup> Reaction performed at room temperature (20 °C).

<sup>d</sup> Given as mg [NaIO<sub>3</sub>] per kg of dry DAC; calculated from Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> consumption.

<sup>e</sup> Below the detection limit of the titration.



The titration showed that the amount of residual iodate depends on the DO of the prepared DAC. While the applied purification protocol was adequate for low-oxidised samples, minor contaminations were determined in the medium range (20 to 30 %) and a significant iodate content remained in the prepared 56 %-oxidised microcrystalline cellulose (Table 3). Notably, it was impossible to establish any meaningful relationship between the DO and iodate content from this and several other titration trials (Table S1). Also, potential chemisorption phenomena were ruled out, as titration results of DAC suspensions and their supernatant liquids were similar (Table S2). In turn, the morphological changes in highly oxidised derivatives and their clogging behavior of the used filter seemed to have an influence (Siller et al., 2015). For these gel-like samples, filtration leads to the formation of DAC agglomerates in the filter cake, in which the iodate-containing solution seemed to be rather tightly entrapped. As a result, also extensive washing was incapable of completely removing the contaminations. To further assess the necessary steps to purify DAC in the case of highly oxidised samples (*i.e.*, DO >20 % and especially when gel-like), contaminations were tracked by conductivity measurements (Binghui et al., 2006; Misra & Das, 1979). Monitoring the supernatant liquid after vacuum filtration might be misleading since the water might not completely penetrate the sample. Thus, the conductivity measurements were combined with a centrifugation protocol to ensure sufficient mixing. The experiments were performed using DAC (DO of 90 % according to UV-Vis monitoring) from microcrystalline cellulose to determine the amount of water and the number of centrifugation steps needed for complete removal of ionic iodine-species (Fig. 3). The conductivity measurements showed that at least three centrifugation steps—preferably four or five—are necessary to remove almost all iodine-containing ions (mainly sodium iodate and periodate). The amounts of water did not significantly influence the separation drastically and could presumably be further reduced (see Experimental section).

### 3.4. Side reactions from contaminations and how to get rid of them

The unwanted follow-up chemistry of residual iodate and periodate, *e.g.*, by disproportionation to iodine or by oxidation reactions, also leads to simple practical problems, such as yellowing of the (per)iodate-contaminated DAC, in particular upon heating (Fig. 4).

The decreased thermal stability of iodate-contaminated DAC, evident from the discoloration of the substrate, was observed upon thermal

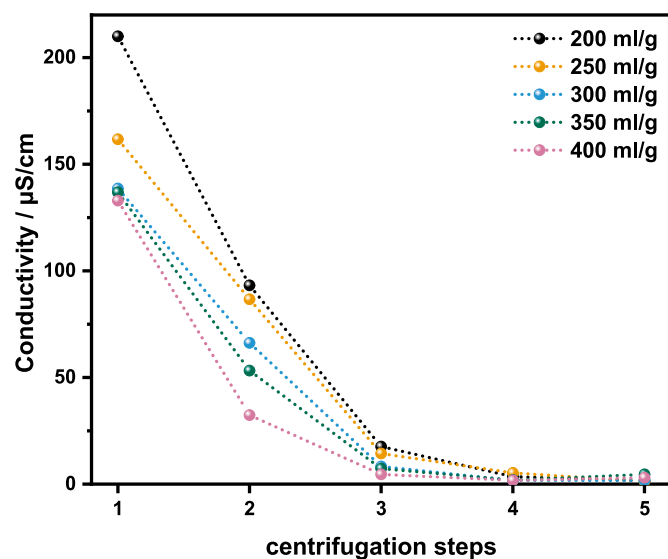


Fig. 3. Sodium periodate concentration of the supernatant after centrifugation with different amounts of water per gram of dialdehyde cellulose, determined from conductivity measurements.

analysis (Fig. 5). Microcrystalline cellulose was oxidised with sodium periodate (DO of approx. 50 %), excess sodium periodate quenched with glycol, and the DAC filtrated and washed with distilled water. Residual iodate was determined with a colorimetric test and removed by a reductive protocol (see below). While the purified, iodate-free DAC showed a TGA curve similar to that reported for mildly oxidised samples (DO around 25 %) (Kim & Kuga, 2001; Varma & Chavan, 1995b), the contaminated sample showed an earlier onset of degradation, comparable to DAC with higher DO (TGA; Fig. 5). The  $T_{95}$  value—the temperature of 5 % mass loss—of iodate-contaminated DAC (176 °C) was 47 °C below the  $T_{95}$  value of the same purified sample (223 °C). There were also differences in the remaining solid residue at 600 °C, with 24.3 % for the purified and 31.6 % for the contaminated sample. In the DSC thermogram, the earlier onset of degradation of the iodate-containing sample related to an exothermic event at around 143 °C (DSC; Fig. 5). Such a peak has not yet been reported. However, to the best of our knowledge, only very mildly oxidised dialdehyde celluloses have been investigated by DSC so far (Vicini et al., 2004), for which the washing procedures were found to be sufficient to remove contaminations. In agreement with the strongly decreased tendency for yellowing, we assign that peak to reactions associated with iodate degradation.

Regarding sustainability, it is preferable not to introduce additional chemicals during the work-up. Therefore, if simple filtration/washing is sufficient and does not lead to clogging of the filter, this represents the best, recommended approach. To control for residual iodine compounds, a quick colorimetric test that used a small aliquot of the prepared DAC proved advantageous (see Experimental section). Under acidic conditions, remaining iodate and periodate react with catalytic amounts of added iodide to iodine, which can be determined quite sensitively as iodine-starch complex. Occurrence of its violet coloration indicates the presence of iodate/periodate and the necessity of further purification steps (Fig. S3).

The previous experiments showed that vacuum filtration and washing with water were sufficient to remove oxidising iodine compounds for low-oxidised dialdehyde celluloses. For more highly oxidised DAC samples, several centrifugation cycles were necessary which—like dialysis protocols—are time-consuming, labor-intensive, and thus not practical on larger scales. Nonetheless, quenching the reaction is, in certain cases, needed to stop the periodate oxidation at a particular reaction time. Otherwise, excess sodium periodate continues to react with the substrate during work-up, especially when more time-consuming protocols are used. As glycol quenching was shown to be insufficient (see above), an alternative and more efficient protocol is needed for these special cases.

If filtration should prove to be too time-consuming or otherwise unsuitable to remove periodate contaminations, we suggest deactivating residual sodium periodate in the DAC with an excess of sodium thiosulfate (see the Experimental section for the optimized protocol), which is also the reactant recommended by safety datasheets and databases (*e.g.*, GESTIS database) to deactivate sodium periodate before disposal (Asakai & Hioki, 2014; Rauscher et al., 2011). For this procedure to be exhaustive, it is essential to break up agglomerates, *e.g.*, by pressing the solid through an open Büchner funnel or by using a blender. Conducting the decontamination reaction in diluted acetic acid after the addition of catalytic amounts of sodium iodide allows visually following the reaction *via* the brown triiodide complex, which can be particularly useful if small agglomerates persist. The decontamination reaction with thiosulfate obviously introduces new impurities (*e.g.*, thiosulfate, tetrathionate, sulfate, and iodide). However, unlike periodate, these are non-toxic, eco-compatible, and did not affect the treated DAC in our experiments. The thiosulfate-decontaminated samples showed a significantly decreased tendency for yellowing (Fig. 4) and exhibited increased thermal stability (Fig. 5). Importantly, the protocol did not have any significant influence on the DO values, as determined by elemental analysis (Table S2). Also ascorbic acid (vitamin C) was successfully applied in periodate decontamination trials. While vitamin C might



Fig. 4. Yellowing effect of iodate-contaminated microcrystalline cellulose at room temperature (A), after drying in the oven for 24 h at 80 °C (B), iodate-contaminated solubilized dialdehyde cellulose at 100 °C after heating for 35 min (C), 45 min (D), and 80 min (E).

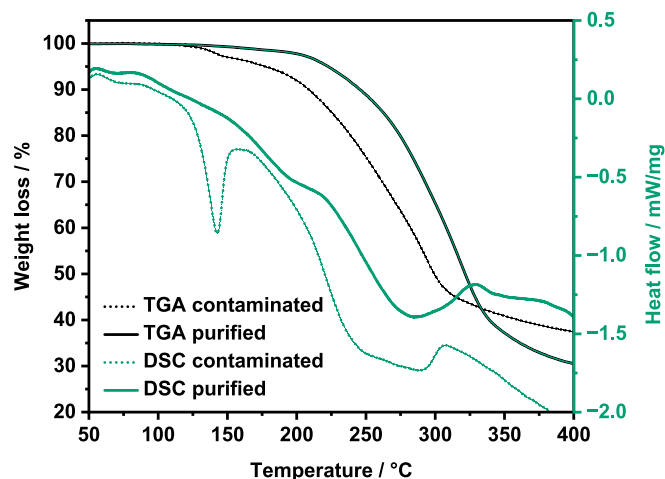


Fig. 5. Thermogravimetric analysis (TGA) data and differential scanning calorimetry (DSC) thermograms of iodate-contaminated and purified DAC (DO of approx. 50 %).

crosslink with DAC and lead to organic impurities, it might represent a valuable alternative in the case of substrates for which the sulfur content is an important analytical marker (e.g., sulfated cellulose nanocrystals; Nypelö et al., 2021).

Filtration alone is not recommended for highly-oxidised celluloses, especially if the morphology is intensively affected and the substances have turned gel-like, or for other periodate-oxidised carbohydrates. In these cases, we suggest resorting to decantation as a faster work-up step,

which does not affect the morphology and avoids the trapping of contaminations in agglomerates. The periodate can be easily recycled from the supernatant phase by ozone treatment under alkaline conditions (Koprivica et al., 2016). If highly concentrated periodate solutions are used, adding water to the decanted DAC slurry, sufficient mixing, and repetition of the decantation step is advisable. This will slow down unwanted oxidation to insignificant levels and reduce the concentration of oxidising iodine species. Afterward, an aqueous sodium thiosulfate solution can be added to the isolated substrate to remove the last potential traces of periodate contaminations (Fig. 6). Thereafter, the DAC can be recovered, e.g., by simple centrifugation which can be extended by dialysis or further centrifugation.

#### 4. Conclusions

In this work, common purification protocols in DAC chemistry were critically revisited, and their shortcomings and pitfalls were highlighted. Especially, glycol quenching proved to be an unnecessary step, insufficient for its intended purpose and likely causing problems by trapping harmful organic impurities and influencing DAC's chemical structure. Instead of periodate quenching, simple washing proved to be sufficient, at least in the case of mildly oxidised DAC samples. DAC samples with higher oxidation degree and gel-like samples are treated with sodium thiosulfate to reduce oxidising iodine species and are further purified best by centrifugation. A fast colorimetric test for oxidising iodine species helps to quickly monitor the purity of the substrate and the efficiency of purification steps.

The remaining contaminations from glycol quenching can lead to various problems. Especially in the context of the often-proposed packaging and biomedical applications of DAC materials, the toxicity

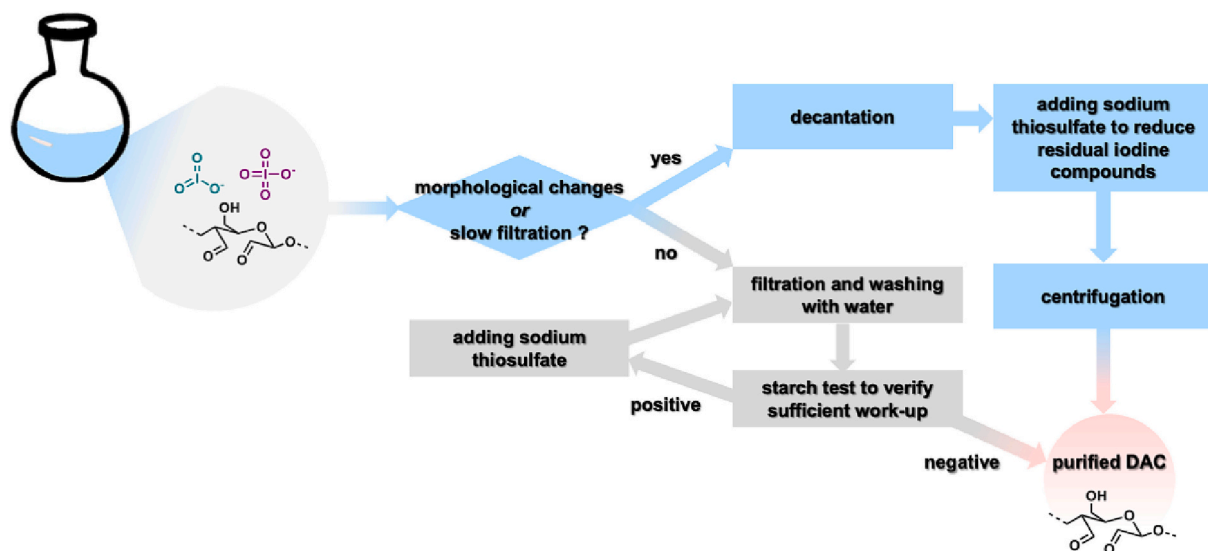


Fig. 6. Adjusted protocol to purify dialdehyde cellulose after periodate oxidation: If the morphology of the isolated dialdehyde cellulose did not change significantly, purification by vacuum filtration and washing with water is sufficient. Gel-like and hard-to-isolate dialdehyde celluloses should be rather decanted and residual oxidising iodine compounds reduced with sodium thiosulfate. Quenching excess periodate with ethylene glycol is generally discouraged.

of trapped and slowly released formaldehyde or oxidising iodine compounds represents a considerable risk. With this cautionary note, we hope to raise awareness for these frequently encountered but often overlooked problems occurring when working with periodate oxidation chemistry and the underlying chemistry.

### CRedit authorship contribution statement

JS and LF contributed equally. JS, LF, MH, AP and TR contributed to the study conception and design. Material preparation, data collection and data analysis were performed by all authors. NMR studies were performed by MB. MH and TR secured the funding. The first draft of the manuscript was written by JS, LF and TR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### 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.2023.120691>.

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