Autocatalytic oxidation of primary hydroxyl functions in glucans with nitrogen oxides

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Abstract

The selective oxidation of the primary hydroxyl groups in the glucans cellulose, amylose and pullulan with nitrogen oxides has been studied. The polymers were dissolved in 85% phosphoric acid and sodium nitrate was used as the stoichiometric oxidant. A catalytic amount of sodium nitrite was added to reduce the induction time. With this reaction system, where the oxidising nitrogen oxides are formed in situ, the primary hydroxyl groups could be completely oxidised ($> 95\%$) to carboxylic acids. Undesired ketones due to secondary hydroxyl group oxidation were subsequently reduced with sodium borohydride. Especially for the α-glucans, this oxidation–reduction sequence of secondary hydroxyl functions apparently gave epimerisation. Degradation of the polymers was slow provided the oxidation was performed at 4 °C. Thus, pullulan with $\langle M_w \rangle \approx 170$ kg/mol yielded a polyuronate with $\langle M_w \rangle \approx 100$ kg/mol. A study of this reaction system with β-cyclodextrin as the substrate clearly showed that the reaction was autocatalytic. © 1997 Elsevier Science Ltd. All rights reserved.

Keywords: Selective oxidation; Primary alcohols; Sodium nitrate; Pullulan; β-Cyclodextrin

1. Introduction

Selective oxidation of primary hydroxyl groups in polysaccharides may yield polyelectrolytes with thickening, gel forming and metal sequestering properties. Natural polycarboxylates of this type, e.g., alginates [1] and pectates [2], are widely used in various industries for these properties. It might be of commercial interest to oxidise polysaccharides like starch and cellulose to obtain polyelectrolytes with related structures. Recently, we reported such a selective primary hydroxyl-group oxidation with hypochlorite and catalytic amounts of TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and sodium bromide [3,4]. Several water-soluble polysaccharides, e.g., starch and inulin, could be completely oxidised with high selectivity. However, for non-water-soluble polysaccharides like cellulose, much longer reaction times were necessary which caused degradation of

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the polymer due to β-elimination and oxidative degradation [5].

For the selective oxidation of water-insoluble polysaccharides, we turned our attention to the nitrogen oxides oxidation. It has been known for more than half a century, that treatment of cellulose with gaseous dinitrogen tetraoxide principally yields 6-carboxycellulose [6]. This product is commercially available and has been successfully used in wound healing to prevent post-surgical adhesions [7]. An improvement of the nitrogen oxides oxidation, with respect to the degree of oxidation and the molar mass distribution of the products, could be made by first dissolving the polymeric substrate in 85% phosphoric acid and adding a stoichiometric amount of sodium nitrite as the oxidant [8–13]. In this acidic environment, the oxidising nitrogen oxides are formed in situ from nitrite. The oxidation with nitrogen oxides is not completely selective and some ketones are introduced due to oxidation of secondary hydroxyl groups [14]. These can be reduced through treatment of the obtained polymers with sodium borohydride [10–13].

In this paper, we present an advantageous variant of the nitrogen oxides oxidation in 85% phosphoric acid. Instead of sodium nitrite, sodium nitrate is used as the stoichiometric oxidant and a catalytic amount of sodium nitrite is added to reduce the induction time. One advantage of this variant is readily seen. It has been shown that the oxidising nitrogen oxides are reduced to NO [10,15–17], which itself is not an oxidant for these substrates. Hence, the stoichiometry of the overall reactions is given by:

$$RCH_2OH + 4\,HNO_2 \rightarrow RCOOH + 4\,NO + 3\,H_2O$$
$$(1)$$

$$3\,RCH_2OH + 4\,HNO_3 \rightarrow 3\,RCOOH + 4\,NO + 5\,H_2O$$
$$(2)$$
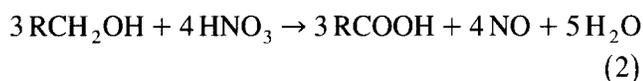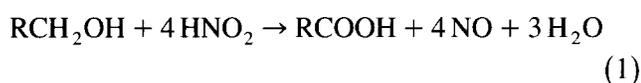
For the oxidation of primary hydroxyl groups, three times the amount of nitrite is required when compared to nitrate. Moreover, three times as much of the toxic radical NO would be formed when the reaction is performed with nitrite instead of nitrate.

We have applied this nitrate oxidation in 85% phosphoric acid to the glucans cellulose, amylose and pullulan (a linear water-soluble polysaccharide consisting of the following repeating trimer: [→ 6)-α-D-Glcp-(1 → 4)-α-D-Glcp-(1 → 4)-α-D-Glcp-(1 →]). The reaction has been optimised with respect to the degree of oxidation and the molar mass distribution. Furthermore, experiments have been performed with

β-cyclodextrin as the substrate to obtain more insight in this reaction system.

2. Results and discussion

Oxidation of the polymers.—In all cases, it was observed that, in order to obtain high oxidation grades, a clear solution of the polysaccharide in phosphoric acid had to be obtained before the oxidant was added. The substrates were dissolved for several hours (ranging from 1–6, depending on the temperature and substrate) until a clear, viscous solution was obtained. The polymer concentration that could be attained in 85% phosphoric acid differed substantially for the used polysaccharides. In 30 mL 85% phosphoric acid, 4 g of amylose and 5 g of pullulan could be dissolved, whereas with cellulose as the substrate, this was only about 1 g. After addition of nitrate and a catalytic amount of nitrite, a white to light green foam developed with a volume depending on the substrate and reaction conditions. A modest excess of sodium nitrate (1.8 mol/mol primary hydroxyl function, theoretically 1.3 mol/mol primary hydroxyl function according to Eq. (2)) proved to be sufficient for the complete oxidation of primary hydroxyl groups in the polymers studied. The catalytic amount of sodium nitrite added was set at 0.2 g for all reactions. In Table 1, the influence of the reaction time and temperature on the degree of oxidation with cellulose as the substrate is shown. The products were analysed after a sodium borohydride reduction with respect to the oxidation grade according to the 3-hydroxybiphenyl assay for uronic acids [18], which has proven earlier to be satisfactory for these 6-carboxy-polymers [4]. The reaction at 20 °C was faster than that at 4 °C and essentially complete oxidation (> 95%) was achieved when the reaction at 20 °C was quenched after approximately 20 h. The reaction at 4 °C required approximately 40 h.

In Fig. 1, the ^{13}C NMR spectra of the oxidised polymers (4 °C, 40 h) are shown after reduction with sodium borohydride. Clearly, the primary hydroxyl groups are virtually completely oxidised to yield uronates as can be seen from the disappearance of the C-6 carbon resonances at 61–62 ppm. This was confirmed with the 3-hydroxybiphenyl assay, which showed absorbances in agreement with complete conversion to uronates. It is noteworthy that cellulose can also be oxidised to completion with sodium nitrite in phosphoric acid [11], whereas complete oxidation of amylose was not achieved using this

Table 1
Influence of the reaction time and reaction temperature on the degree of oxidation with cellulose as the substrate[a]

Entry	Temperature (°C)	Dissolving time (h)	Oxidation time (h)	DO (%)[b]	Yield (%)[c]
1	20	2	3	23	77
2	20	2	6	72	85
3	20	2	9	88	82
4	20	2	20	99	74
5	4	5	6	< 10	–
6	4	5	24	90	85
7	4	5	40	97	88

[a]Conditions: 1.0 g cellulose in 30 mL 85% phosphoric acid, 0.95 g sodium nitrate, 0.2 g $NaNO_2$.
[b]Degree of oxidation (DO) of the products after reduction with $NaBH_4$ as measured with the 3-hydroxybiphenyl assay.
[c]Yield as calculated with the monomer molar masses depending on the DO as given in the previous column.

method [12]. This has been attributed [12] to the fact that only a limited amount of sodium nitrite could be efficiently added to the solution of the polysaccharide in phosphoric acid. Since a much higher concentration of amylose, compared to cellulose, in phosphoric acid is needed to obtain a solution that is sufficiently viscous to retain the developing gaseous nitrogen oxides, amylose could not be oxidised to completion using nitrite as the stoichiometric oxidant.

Although the ^{13}C NMR spectra show the complete oxidation of primary hydroxyl groups, obviously side products are obtained after oxidation–reduction. As mentioned in the introduction, the nitrogen oxides oxidation is not completely selective for primary

Fig. 1. ^{13}C NMR spectra of the sodium salts of oxidised–reduced cellulose (above), pullulan (middle) and amylose (below). t-Butanol was used as an internal reference. Reaction conditions were as described in Section 3.

hydroxyl groups. In the order of 10–20% of the anhydroglucose units are also oxidised at the secondary hydroxyl functions to give ketones [11,12,14], which make the polymer labile under alkaline conditions due to β-elimination [19]. Therefore, after the oxidation, the polymers were reduced with sodium borohydride. The reduction is performed under alkaline conditions and an excess of sodium borohydride was used to increase the reaction rate of the reduction compared to the competing β-elimination. The proportion of non-selective oxidation was confirmed with the uronic acid assay, which showed absorbances (proportional to the uronic acid concentration) in the order of 20% higher after the reduction. The ^{13}C NMR spectra shown in Fig. 1 seem to indicate that epimerisation has occurred during this oxidation–reduction sequence. This is especially true for the spectra of the oxidised–reduced α-glucans amylose and pullulan as can be seen from the second anomeric carbon resonance arising at ~ 95 ppm. It may be noted that this is an exceptionally high field value for a glycosidically linked anomeric carbon [20]. However, from quantitative ^{13}C NMR analysis, this resonance was indeed shown to belong to the anomeric carbon. To determine whether this anomeric carbon in these α-uronates arose from the manno- or the allo-configuration, from C-2 or C-3 epimerisation, respectively, we hydrolysed oxidised–reduced amylose in 1 M HCl for 20 min at 155 °C [21]. The resulting yellow solution was analysed with HPAEC and compared to standard solutions of D-glucuronic acid and D-mannuronic acid. Regrettably, due to only a small difference in retention times of the various uronic acids, we were not able to definitively conclude which configuration was obtained.

For the determination of the effect of the reaction conditions on the molar mass distribution of the products, pullulan was chosen as a substrate. This
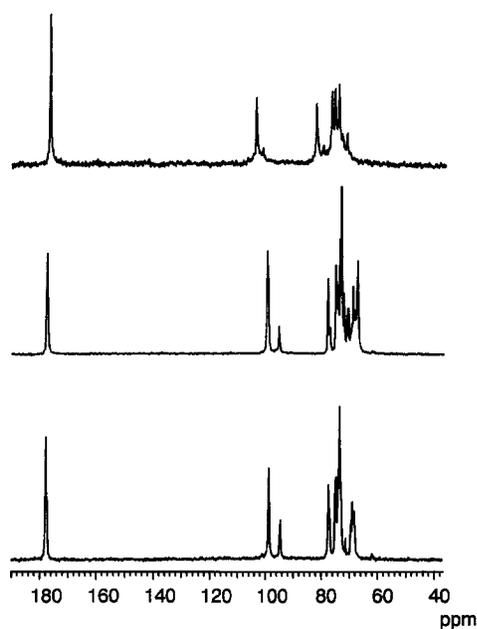
linear polymer is water-soluble and the molar mass distribution of the oxidised pullulans could be compared to that of the parent polymer. The molar mass distribution was determined with size-exclusion chromatography (SEC) with on-line multi-angle-laser light scattering (MALLS) detection. The applied temperature appeared to exert a substantial influence on the degradation during the oxidation. In Fig. 2, the plots of the molar mass distribution of pullulan and two completely oxidised pullulans (4 °C, 40 h and 20 °C, 20 h) are shown. Although longer reaction times are necessary at 4 °C, markedly less degradation occurs at this temperature. In fact, degradation by hydrolysis is surprisingly slow in 85% phosphoric acid at low temperature, even after 40 h. This low rate of hydrolysis may possibly be due to the low water activity in this solvent. The molar mass distribution of the oxidised products at 4 °C is comparable to that of the products obtained from the TEMPO-mediated hypohalite oxidation [5].

Autocatalysis.—When sodium nitrate is added to a solution of polysaccharide in 85% phosphoric acid, the oxidation only starts after several hours. This points to an autocatalytic reaction, for which an induction time is a characteristic [22]. Autocatalytic nitric acid oxidations are very common and it is well-known that the reaction is initiated by the presence of lower valent oxynitrogen species such as nitrite or nitric oxide. Nitric acid as well as sodium nitrate are always contaminated to some extent with those lower valent oxynitrogen species. The initial concentration of these species in an autocatalytic nitric acid oxidation determines the length of the
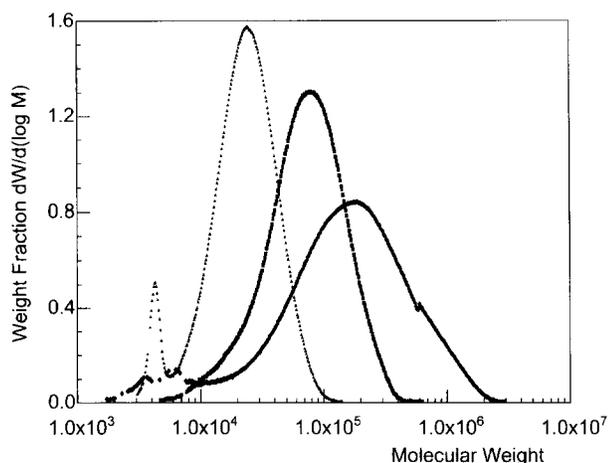
Fig. 3. Influence of the initial concentration of $NaNO_2$ on the reaction rate as measured with the 3-hydroxybiphenyl assay. Conditions: 0.70 g β-cyclodextrin in 5 mL 85% phosphoric acid, 0.70 g sodium nitrate and $NaNO_2$: ▼ 0 mg; +20 mg; ▲ 40 mg; ● 60 mg. Lines are drawn to guide the eye.

induction time [22]. This implies that small amounts of nitrite can be advantageously added, giving the above-mentioned reaction system, which is in fact a mild version of commonly applied nitric acid oxidations in concentrated nitric acid or sulphuric acid [23]. To obtain more insight in this reaction system, some experiments with β-cyclodextrin as the substrate were performed because the polysaccharide solutions were far too viscous and formed foams which made it impossible to properly take aliquots. During the oxidation and during contact with a pipet, gaseous nitrogen oxides escaped from the oversaturated, intensely green solution and aliquots had to be carefully weighed. The amount of uronic acid was determined with the 3-hydroxybiphenyl assay [18] and experiments were performed at ambient temperature.

In Fig. 3, the influence of the initial concentration of sodium nitrite on the rate of oxidation is shown. The maximum absorbance of ~ 0.80 found with the uronic acid assay corresponds to $> 90\%$ primary hydroxyl group oxidation. This was shown after borohydride reduction and workup of a sample. When no sodium nitrite was added to the reaction mixture, it took more than 3 h for the reaction to start. The oxidation of β-cyclodextrin is substantially faster than that of the polymers described above, probably

Fig. 2. Differential molecular weight plots of pullulan (◆, right peak) and oxidised–reduced pullulans at 4 °C (■, middle peak) and at 20 °C (▲, left peak) as obtained by SEC–MALLS.

due to the high viscosity and the heterogeneous nature (foam) of the reaction mixture when polymers are the substrates. The initial concentration of nitrite does not influence the reaction rate, but has a severe influence on the induction time. As mentioned earlier, this is typical for autocatalytic nitric acid oxidations, as is the sigmoidal shape of the curves [22]. Further evidence for autocatalysis could be obtained by performing the reaction in a petri dish without stirring. When sodium nitrite was added to a solution of β-cyclodextrin and sodium nitrate in 85% phosphoric acid, a light green reaction front developed which moved in several minutes to the sides of the petri dish. Had the reaction not been autocatalytic, this would have taken in the order of hours since diffusion in the relatively viscous 85% phosphoric acid is a slow process. The autocatalytic nature of the reaction system can be qualitatively explained by examining the relevant reactions [15–17]:

$$HNO_2 + H^+ \rightleftarrows NO^+ + H_2O \qquad (3)$$

$$RCH_2OH + NO^+ \rightarrow RCHO + H^+ + HNO \qquad (4)$$

$$HNO + HNO_2 \rightleftarrows 2 NO + H_2O \qquad (5)$$

$$2 NO + HNO_3 + H_2O \rightleftarrows 3 HNO_2 \qquad (6)$$
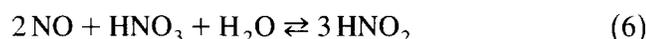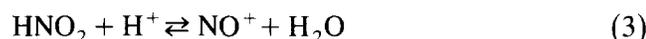
which gives the following overall reaction:

$$RCH_2OH + HNO_3 \rightarrow RCHO + HNO_2 + H_2O \qquad (7)$$

The only role of nitric acid is to reoxidise NO, hence, it is seen from the overall Eq. (7) that nitrous acid is the reaction product. This species rapidly forms NO^+ (Eq. (3)) under the applied conditions [24], which explains the autocatalytic behaviour of this reaction system. Note that we assume that NO^+ is the actual oxidant, as has been found from extensive kinetic studies [15–17]. In the reaction mixture, other nitrogen oxides are formed (e.g., dinitrogen trioxide and dinitrogen tetraoxide), which are not important for this analysis since the exchange processes between the various nitrogen oxides are all equilibria. Furthermore, the oxidation of the hydroxyl function, takes place in the liquid phase, where these nitrogen oxides are known to form NO^+ under the applied conditions.

The influence of the initial nitrate concentration on the rate of oxidation is shown in Fig. 4. The initial nitrate concentration does not seem to influence the induction time or have much influence on the reaction rate. This is characteristic for autocatalytic nitric acid oxidations of alcohols [16], since Eq. (4) is the rate determining reaction. For the complete oxidation of the primary hydroxyl groups, theoretically, 0.43 g
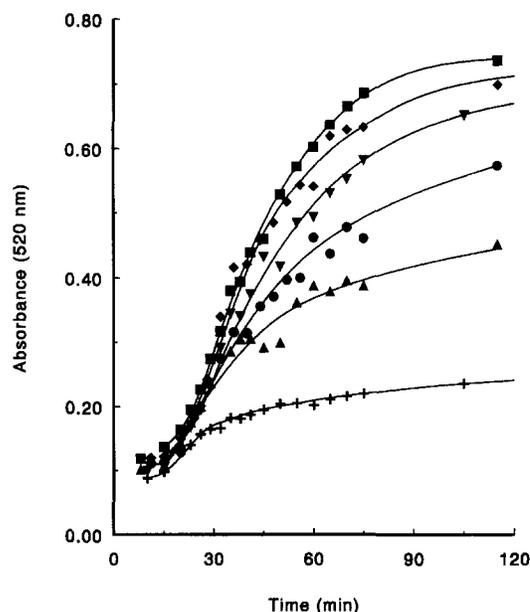
Fig. 4. Influence of the initial concentration of sodium nitrate on the reaction rate as measured with the 3-hydroxybiphenyl assay. Conditions: 0.70 g β-cyclodextrin in 5 mL 85% phosphoric acid, 40 mg $NaNO_2$ and sodium nitrate: +0.10 g; ▲ 0.20 g; ● 0.30 g; ▼ 0.40 g; ♦ 0.50 g; ■ 0.70 g. Lines are drawn to guide the eye.

sodium nitrate would be necessary according to the overall Eq. (2). We found that essentially complete oxidation could be achieved with 0.5 g sodium nitrate, implying that, although nitrogen oxides are lost either as escaping gas or due to non-selective oxidation, most nitrogen oxides are used for the oxidation of primary hydroxyl groups. On the other hand, the influence of the small amount of sodium nitrite added is neglected and oxygen was not excluded from the reaction mixture, which is known to oxidise HNO and nitrogen monoxide to give more oxidant. These features make an exact quantitative analysis of this reaction system difficult.

As stated above, from earlier investigations [15–17], it has become more than likely that NO^+ is the actual oxidant and the mechanism involves an α-hydride abstraction yielding a protonated aldehyde and HNO. However, the nature of the hydride abstraction is still uncertain. The fact that primary alcohols are more rapidly oxidised than secondary ones, which has not been considered in those studies, should be accounted for. Thus, a direct hydride abstraction by NO^+ [15,17] seems unlikely, since in this case secondary alcohols are expected to react more easily than primary ones due to stabilisation of the carbocation. For this reason, we prefer a reaction sequence starting with an electrophylic attack of NO^+ on the

oxygen of the hydroxyl function [16] and subsequent elimination of HNO to give a protonated carbonyl. To envision this rate limiting elimination, however, remains difficult.

3. Experimental

Materials.—Amylose V and β-cyclodextrin were gifts from Avebe (Veendam, The Netherlands). Pullulan was obtained from Hayashibara (Yokohama, Japan), and microcrystalline cellulose (Avicel) from Merck (Darmstadt, Germany). All other chemicals were analytical-grade commercial products and were used without prior purification.

Optimised oxidation method.—The polysaccharide (cellulose: 1.0 g; amylose: 4.0 g; pullulan: 5.0 g) was dissolved at 4 °C in 85% phosphoric acid (30 mL). After approximately 4–6 h slowly stirring, a viscous solution is obtained and sodium nitrate (for cellulose: 0.95 g; amylose: 3.8 g; pullulan: 3.2 g; 1.8 mol/mol primary hydroxyl function) is added. After a further 30 min stirring, $NaNO_2$ (0.2 g) was added and the solution was stirred for 1 h in a fumehood. Stirring was stopped and the mixture was left for 40 h at 4 °C during which a foam developed. The oxidised polymer was subsequently precipitated with cold EtOH (−20 °C, 200 mL) under vigorous stirring with a spatula. Beware that during this action, toxic gaseous nitrogen oxides escape. The white precipitate was filtered and washed several times with cold 20:80 water–EtOH and dissolved in cold water (200–400 mL). The solution was neutralised with Na_2CO_3 and excess $NaBH_4$ (∼0.3 g/g polymer) was rapidly added. After stirring overnight at room temperature, the mixture was brought to pH 5–6 by adding glacial CH_3COOH. The product was purified and isolated by extensive dialysis, ultrafiltration and freeze drying. Degrees of oxidation were higher than 95% and yields were generally in the order of 80%.

Oxidation of β-cyclodextrin.—All measurements were performed at room temperature. β-Cyclodextrin (0.70 g, 13% moisture, 3.7 mmol anhydroglucose units) was dissolved in a solution of sodium nitrate in 85% phosphoric acid (5 mL). At higher sodium nitrate concentrations, the salt was partly insoluble, however, during the reaction non-dissolved sodium nitrate disappeared. After 30 min, at time = 0, $NaNO_2$ was added. The mixture was stirred during the oxidation. Aliquots were taken with a pipet and weighed. This was important since gases escaped during the taking of the aliquots. The amount of oxidation was

subsequently determined according to the 3-hydroxybiphenyl assay for uronic acids [18].

SEC–MALLS.—Pullulan samples were dissolved in 0.1 M sodium nitrate and were fractionated with three PW TSK columns (G6000PW-G5000PW-G3000PW, Toyo Soda) in series. Absolute molar masses were determined on-line with a DAWN-DSP-F (Wyatt Technology) MALLS-detector. An interferometric refractive index detector (Optilab, Wyatt Technology) was used as the concentration detector. The refractive index increment, dn/dc, was measured with the Optilab refractometer. For pullulan, a value of 0.152 mL/g and, for oxidised pullulan, 0.143 mL/g was found.

Acknowledgements

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