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Selective oxidation of carbohydrates by 4-AcNH-TEMPO/peracid systems

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Abstract

Starch, amylopectin, inulin, pullulan and methyl α-D-glucopyranoside (Me α-Glc*p*) were oxidised by 4-acetamido-2,2,6,6-tetramethylpiperidine-1-oxyl (4-AcNH-TEMPO) as the mediator and peracetic acid or monoperoxysulfate (Oxone®) as the regenerating oxidant. The conversion of primary alcohol groups to the corresponding carboxyl groups proceeded with high yield and selectivity, provided that sodium bromide was added as co-catalyst.

The mass molecular distributions of the oxidised polysaccharides indicated that no major depolymerisation occurred during oxidation. Oxone appeared to be the most efficient oxidant as the reaction rate was 25 times higher than that of peracetic acid in the oxidation of Me α-Glc*p*. On the other hand, oxone produces a larger amount of waste as by-product than peracetic acid. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Starch; Amylopectin; Inulin; Pullulan; Methyl α-D-glucopyranoside; 4-Acetamido-2,2,6,6-tetramethylpiperidine-1-oxyl; Oxone; Peracetic acid

1. Introduction

Selective oxidation of primary alcohol groups in mono- and polysaccharides by stable nitroxyl radicals, e.g. 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) or derivatives thereof as the mediator, to the corresponding carboxyl functionalities has been described in recent years (Chang & Robyt, 1996, 1998; De Nooy, Besemer, & Van Bekkum, 1995a; De Nooy, Rori, Masci, Dentini, & Crescenzi, 2000; Heeres, Van Doren, Gotlieb, & Bleeker, 1997; Isogai & Kato, 1998; Jiang, Drouet, Milas, & Rinaudo, 2000; Lemoine et al., 2000; Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999a,b; Sierakowski, Milas, Desbriéres, & Rinaudo, 2000).

The reaction is characterized by mild conditions, high reaction rate and high regioselectivity. Through a one-electron transfer reaction, the radical is oxidised to the corresponding nitrosonium salt, which is the active oxidant in the primary alcohol oxidation (Bobbit & Flores, 1988; De Nooy, Besemer, & Van Bekkum, 1996).

Regeneration of the oxidising species is accomplished in situ by the addition of a primary oxidant. Over the years, several oxidants have been evaluated for this purpose, such as *m*-chloroperbenzoic acid, copper (II) salts, potassium ferricyanide, sodium bromite, *N*-chlorosuccinimide and [bis(acetoxy) iodo]benzene (Cella, Kelley, & Kenehan, 1975; De Mico, Margarita, Parlanti, Vescovi, & Piancatelli, 1997; Einhorn, Einhorn, Ratajczak, & Pierre, 1996; Inokuchi, Matsumoto, Nishiyama, & Torii, 1990; Mangzhu et al., 1999; Miyazawa & Endo, 1985; Semmelhack, Schmid, Cortés, & Chou, 1984). In addition, electrochemical regeneration has been applied for this purpose (Schnatbaum & Schäfer, 1999).

However, in the vast majority of the studies, hypochlorite (bleach) and hypochlorite/bromide have been utilised as primary oxidants (Anelli, Biffi, Montanari, & Quici, 1987; Bragd, Besemer, & Van Bekkum, 2000; De Nooy, Besemer, & Van Bekkum, 1995a).

In some recent studies, attempts have been made to find cleaner and milder primary oxidants for the regeneration of TEMPO, such as the three component system hydrogen peroxide/methyltrioxorhenium (MTO)/HBr (Hermann, Zoller, & Fischer, 1999) or by oxidative enzymes (laccase, horse radish peroxidase) (Van der Lugt, Jetten, Besemer, & Van Doren, 1999; Viikari, Niku-Pavola, Buchert, Forssell, Teleman & Krus, 1997).

Peracids, e.g. peracetic acid and persulfuric acid are versatile reagents which have been used successfully in several applications, including chlorine free bleaching

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processes (Rucker, 1989; Sundara & Kronis, 1998) or as reagents in organic synthesis (Grudzinski, Roberts, Howard, & Newton, 1978; Hirano, Oose, & Morimoto, 1991; Kennedy & Stock, 1960; Morimoto, Hirano, Ashiya, Egashira, & Zhuang, 1987; Pandell, 1983).

The use of peracetic acid/sodium bromide as regenerating oxidising system in TEMPO-mediated oxidation of sugars was first reported by Van der Lugt et al. (1998).

Recently, the use of oxone (potassium monoperoxypersulfate) as primary oxidant in the TEMPO-process was reported; in bi-phasic media aromatic and aliphatic alcohols, e.g. benzyl alcohol and 1-nonanol, were oxidised to aldehyde or keto functionalities (Bolm, Magnus, & Hildebrand, 2000).

In another study, ammonium peroxydisulfate and catalytic amounts of supported silver were utilised to regenerate TEMPO in the oxidation of methyl α-D-glucopyranoside (Me α-Glc*p*) (Kochkar, Lassalle, Morawietz, & Hölderich, 2000).

In this paper, we report our work on organic and inorganic peracids as primary oxidants in the 4-AcNH-TEMPO oxidation of aqueous solutions of starch, amylopectin, inulin, pullulan and Me α-Glc*p* to produce the corresponding 6-carboxy-analogues.

2. Experimental

2.1. Materials and reagents

Potato starch was obtained from AVEBE (Groningen, The Netherlands). Pullulan (a glucan with repeating trimericunit: (1 → 6)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 →])) (water content 8%) was obtained from Hayashibara (Okayama, Japan). Waxy maize starch (99% amylopectin) was obtained from ZBB de Bijenkorf (Koog a/d Zaan, The Netherlands). Inulin HP (DP 30) was obtained from Orafti (Oreye, France). 4-Acetamido-TEMPO (4-AcNH-TEMPO) was obtained from Degussa–Hüls (Marl, Germany). Sodium hypochlorite (ca. 2 M) was obtained from Akzo Nobel (Arnhem, The Netherlands).

Oxone® ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) was obtained as a solid material from Aldrich (consists of 2 mol active oxidant ($KHSO_5$)/mol of molecules) and peracetic acid (CH_3COOOH) was obtained from FLUKA as a solution in water comprising of peracetic acid (38%), acetic acid (45%) and hydrogen peroxide (ca. 10%). Sodium peroxydisulfate was obtained from Aldrich as a solid material. All other chemicals were of commercial grade and used without further purification.

2.2. General methods

Molecular mass distributions were determined with a Pharmacia HPLC ÄKTA EXPLORER 10, equipped with a UV-Vis detector (UV-900). Uronic polysaccharides were monitored at 215 nm. The SEC-column system was a TOSOHAAS TSKgel G 4000 PW, MW 1000–700 000, connected in series with a TSK-gel G 6000 PW, MW 500 000–50 000 000. As eluent sodium phosphate (50 mM buffered at pH 7.0) was used. Flow rate was set at 0.5 ml/min. Under these conditions, the void volume (V_0) is ca. 12 ml.

HPAEC-analysis was performed with a Dionex DX-300 instrument. The column was a CarboPac PA-1. Amperometric detection was performed with a Dionex pulsed electrochemical detector (PED) in the pulsed amperometric mode. The PED-detector was equipped with a gold working electrode and an Ag/AgCl reference electrode. The eluent was time-programmed: 0–5 min; 100 mM NaOH, 5–10 min; linear gradient to 80% 100 mM NaOH and 20% 100 mM NaOH + 500 mM NaAc, >10 min; 80% 100 mM NaOH and 20% 100 mM NaOH + 500 mM NaAc.

Reversed phase chromtography was carried out with a Pharmacia HPLC ÄKTA EXPLORER 10, equipped with a UV–Vis detector (UV-900). 4-AcNH-TEMPO was monitored at 425 nm. The column was a Phenomenex Luna 3u C18(2) $150 \times 4.60\ \text{mm}^2$. As eluent acetonitrile (70%)–water (30%) was used. Flow rate was set at 0.5 ml/min.

Quantitative ^{13}C NMR spectra were recorded on a Varian VXR-400 S spectrometer using D_2O as solvent. As internal standard *tert*-butanol was used (31.2 ppm). The spectra were recorded with a 0.33° flip angle, an acquisition delay of 10 s, 50 000 datapoints and ^1H decoupling during acquisition only. ^1H NMR spectra were recorded on a Varian VXR-400 S spectrometer. As internal standard *tert*-butanol was used (1.4 ppm). The spectra were recorded with an acquisition time of 5 s, an interpulse delay of 5 s and 100 datapoints.

The concentration of active oxidant and the concentration of hydrogen peroxide in the peracid solutions was determined using the procedure developed by Greenspan and Mackellar (1948). Uronates formed in the oxidised materials were determined with the Blumenkrantz assay, which allows quantitative determination without interference with neutral sugars (Blumenkrantz & Asboe-Hansen, 1973). The latter assay is assumed to represent the content of primary carboxylic groups in the oxidised materials.

2.3. General procedure for the oxidation of polysaccharides

Potato starch (3.66 g (18% moisture), 18.5 mmol anhydroglucose units) or waxy maize starch (3.33 g (9% moisture), 18.5 mmol anhydroglucose units) was gelatinised in demineralised water (200 ml) at 95 °C with effective mechanical stirring. Pullulan (1.08 g (5% moisture), 6.1 mmol anhydroglucose units) was dissolved in 50 ml water. Inulin (3.16 g (5% moisture), 18.5 mmol) was dissolved in 200 ml water at 60 °C. TEMPO or 4-AcNH-TEMPO (0.096 mmol/g substrate) and NaBr (0.08 g/g substrate) were added and dissolved. The solution was cooled on ice and the

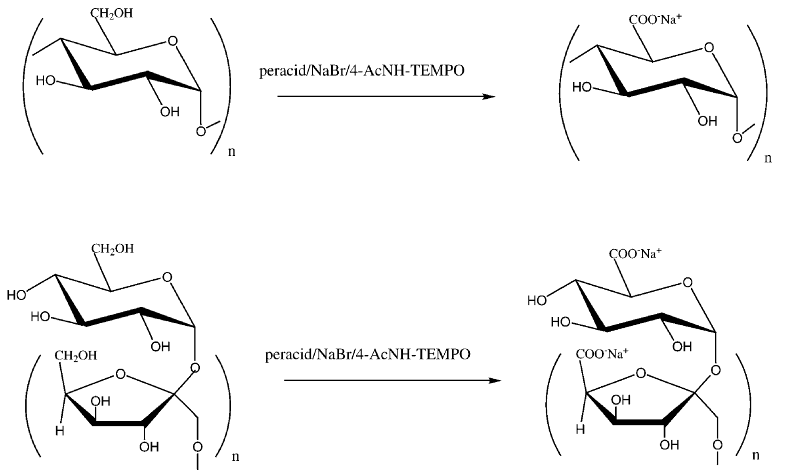


Fig. 1. Scheme of the oxidation of starch (amylose component) and inulin to the corresponding 6-carboxylated derivatives by the 4-AcNH-TEMPO/peracid/NaBr system.

temperature was maintained ≤ 5 °C throughout the reaction. The actual peracid (2 mol/mol primary alcohol) was added in small portions throughout the oxidation to minimise unwanted side reactions. After each addition, pH was raised to the desired value and then kept constant by the addition of 0.5 M NaOH using a pH stat apparatus.

After completion of the reaction, the remaining aldehyde-intermediates were reduced to the starting alcohol using 150 mg of $NaBH_4$. After 1 h pH was adjusted to ca. 6.0 with 0.5 M HCl and the oxidised polysaccharide was precipitated in two volumes of ethanol, filtered off, and re-dissolved in 50 ml of water. Finally, the materials were ion-exchanged in the H-form (Sigma DOWEX 50WX8-100 cation exchanger) and freeze-dried. Representative ^{13}C NMR data (400 MHz, D_2O) of oxidised starch, amylopectin and pullulan samples are δ C-1 99.2, C-2-C-5 71.0–74.5, C-6 (ox) 177.8, C-6 (non ox.) 62.0; Inulin (fructose unit) δ C-1 64.1, C-2 105.1 C-3-C-5 71.0–74.5, C-6 (ox) 179.0, C-6 (non ox.) 62.0.

The materials to be analysed by size exclusion chromatography (SEC) were subjected to membrane filtration (cut-off size 10 000 Da) instead of the ion-exchange procedure, in order to minimise depolymerisation. After the latter

Table 1
Oxidation of polysaccharides and Me α-Glc*p* with the peracid/4-AcNH-TEMPO/bromide system (see Section 2 for a detailed description of the experimental conditions)

Entry[a]	Substrate	pH	Oxidant	Temperature (°C)	Reaction time[b] (h)	Conversion[c] (mol%)
1	Starch	7.5	Oxone	15	5–8	85
2	Starch	8.2	Oxone	< 5	8–10	87
3	Starch	9	Oxone	< 5	8–10	90
4	Starch	8.2	Oxone	10	10–12	60
5	Amylopectin	8.2	Oxone	< 5	5–8	85
6	Pullulan	8.2	Oxone	< 5	6–12	85
7	Inulin	8.2	Oxone	< 5	5–10	60
8	Me α-Glc*p*	8.2	Oxone	< 5	5–10	85
9	Me α-Glc*p*	8.2	Oxone	< 5	10–12	58
10	Starch	8.2	Peracetate	< 5	10–12	85
11	Amylopectin	8.2	Peracetate	< 5	10–15	60
12	Inulin	8.2	Peracetate	< 5	10–15	80
13	Me α-Glc*p*	8.2	Peracetate	< 5	10–12	90
14	Me α-Glc*p*	8.2	Peracetate	< 5	10–12	< 5
15	Me α-Glc*p*	8.2	Perborate	< 5	10–12	–
16	Me α-Glc*p*	8.2	Percarbon-ate	< 5	10–12	–
17	Me α-Glc*p*	8.2	Peroxydi-sulfate	< 5	10–12	< 10

[a] In entries 4, 9 and 14 no bromide was added as co-catalyst.

[b] Represents the time when the primary oxidant was consumed (except for entries 14–17 where some oxidant remained when the oxidation was stopped).

[c] Determined by the Blumenkrantz assay (taken as C-6-oxidation).

treatment, the salt content (mainly NaCl and NaBr) was ca. 2–5% of the dry material.

The presence of residues of the 4-AcNH-TEMPO species in the oxidised materials was determined using the following procedure: after precipitation and membrane filtration, the oxidised material (1 g) (re-dissolved in 30 ml water) was treated with a hydrophobic resin (Sigma Amberlite XAD-16) (2 g). After 2 h of stirring, the resin was filtered off and the 4-AcNH-TEMPO species was subsequently desorbed from the resin by acetonitrile (20 ml). The filtrate was concentrated under reduced pressure to ca. 1 ml and analysed by reverse phase chromatography.

2.4. Oxidation of methyl α-D-glucopyranoside (MGP)—kinetic experiments

To a thermostatted (22 °C) solution of MGP (388 mg, 2 mmol) in 50 ml of demiwater, the peracid oxidant (2.2 mol/mol primary alcohol) was added. The pH was adjusted to the desired value (i.e. between 7.5–9.5) and kept constant throughout the oxidation by addition of NaOH (0.5 M), using a pH-stat. The reaction was initiated by the addition of 4-AcNH-TEMPO (0.0384 mmol), which was taken from a 32 mM stock solution. The progress of the reaction was monitored by taking aliquots and determining the concentration of uronic acid by the Blumenkrantz assay.

3. Results and discussion

3.1. Peracid/4-AcNH-2,2,6,6-tetramethylpiperidine-1-oxyl/bromide oxidation of polysaccharides

Peracetate and oxone were used as primary oxidants in the 4-AcNH-TEMPO mediated oxidation of various polysaccharides, i.e. starch, amylopectin, pullulan and inulin, to afford the corresponding 6-carboxy derivatives (Fig. 1).

In most of the oxidations performed in the presence of catalytic amounts of NaBr high conversions were attainable (Table 1). Use of oxone alone in combination with 4-AcNH-TEMPO resulted in less oxidation (≤60%) at the conditions tested (entries 4 and 9).

When peracetate without bromide was employed as the oxidant, the conversion was low (entry 14). Oxone, unlike peracetate, was able to convert TEMPO to the active nitrosonium salt without the bromide co-catalyst. Thus, the oxone/4-AcNH-TEMPO system represents an interesting alternative when a halogen free process is desired. When bromide was used as co-catalyst in the peracetate/4-AcNH-TEMPO oxidations, the extent of conversions was comparable to that of the oxone/bromide/4-AcNH-TEMPO system.

In both the presence and absence of bromide, the reaction rate was higher for oxone compared to peracetate, which further indicated that oxone is the strongest oxidant of the two.

The regioselectivity of the oxidation reactions appeared to be high (>95%) as only carboxyl groups arising from the oxidation of primary hydroxyls could be seen by quantitative ^{13}C NMR spectroscopy.

When taking into account the reaction rate, oxone is clearly superior to the peracetate oxidant, but its use in industrial processes is limited largely due to the inevitable handling of large amount of solids. The *E*-factor, which is the amount of waste produced divided by the amount of product formed (Sheldon, 1994), amounts for oxone oxidation of starch to 3.3 kg/kg, whereas for peracetate oxidation of starch the *E*-factor amounts to just 1.8 kg/kg.

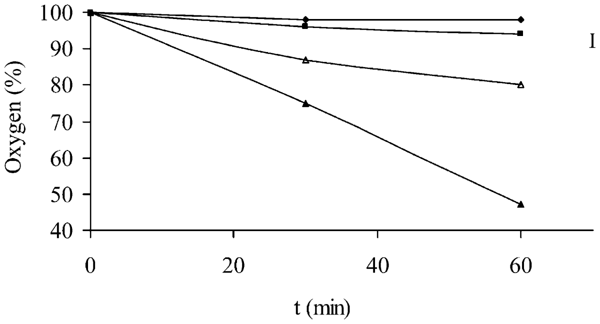
There are several alternatives for the preparation of persulfuric acid (also known as Caro's acid) but the most general method involves mixing of sulfuric acid and high concentration hydrogen peroxide, to afford the product in equilibrium with the reactants at approximately 70% conversion (Wang, Chang, Jameel, & Amini, 1994). Preparation of pure and stable monoperoxysulfate, which is the corresponding salt of persulfuric acid has never been succeeded. For the preparation of peracetic acid, a strong acid catalyst (sulfuric acid) is added to acetic acid and hydrogen peroxide to attain equilibrium in reasonable time (45% conversion). In order to reduce manufacturing and transportation costs, processes for on-site generation of peracids have been developed, especially for bleaching applications in the paper industry (Wang et al., 1994). The peracids used as primary oxidants in TEMPO oxidation processes might be prepared analogously.

In addition to peracetate and oxone, other peracids, i.e. sodium peroxydisulfate, sodium perborate and sodium percarbonate were tested as co-oxidants in TEMPO-oxidation, but only low or negligible conversions of Me α-Glc*p* were achieved.

In the work by Kochkar et al. (2000) Me α-Glc*p* was oxidised to the corresponding acid with high selectivity (99%) at 78% conversion by TEMPO/ammonium peroxydisulfate together with catalytic amounts of silver supported on alumina.

Further, Kochkar et al. tested oxone together with the silver catalyst, but only a low conversion (6%) was reported. However, in our experiments, the oxone/sodium bromide or peracetate acid/sodium bromide systems were superior. Since peroxydisulfate salts (peroxydisulfates are often named as persulfates in literature) hydrolyses at alkaline pH and disproportionate to monoperoxysulfate and sulfate, the actual oxidant of TEMPO may in fact be the monoperoxysulfate species (Stephanou, 1957). Moreover, since earlier studies have revealed that amines generally undergo oxidation in the presence of TEMPO, it cannot be ruled out that the ammonium cation of the peroxydisulfate oxidant is oxidised in a competitive side reaction (Hunter, 1984).

In our study, complete conversion of the polysaccharides was not attained under the conditions used (Table 1). This was probably due to the accompanying decomposition of the peracids that occurred throughout the oxidation. We investigated the magnitude of the side reactions at different pH at ambient temperature in the absence of the other



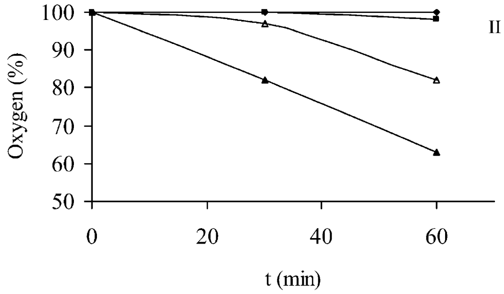


Fig. 2. Decomposition of (I) oxone (2.0 mM) (◆) pH 5.0, (■) pH 7.0, (△) pH 9.0 and (▲) pH 10.0 and (II) peracetate (2.0 mM) ((◆) pH 5.0, (■) pH 7.0, (▲) pH 8.2 and (△) pH 9.0 in water at 22 °C. Aliquots were taken at 0, 30 and 60 min and the active oxygen content was determined by the method of Greenspan (see Section 2).

reactants (Fig. 2). The results clearly indicate that decomposition is not negligible, particularly in alkaline conditions. In earlier studies it was shown that the most significant loss of active oxidant in peracetic acid and oxone involves attack of the peracid on the corresponding peracetate anion (Ball & Edwards, 1956; Rucker & Cates, 1988). Further, the rate maxima were found close to pK_a values, where the concentration of the peracid and that of its anion are equal (peracetic acid has a pK_a of ca. pH 8.2 and oxone ca. pH 9–10 (refers to the second weakly acidic proton of HSO_5^-). Decomposition of oxone may explain why Kochkar et al. were not successful using oxone as primary oxidant, as the experiments were carried out at higher pH (i.e. 9.5).

In order to suppress the decomposition, the oxidant should be added slowly in small doses, which are allowed to react prior to the next addition. The temperature should be kept as low as possible.

The isolated oxidised starch, amylopectin (waxy maize starch) and pullulan samples were analysed by size exclusion chromatography (SEC–UV–VIS). Since no relevant calibration standards were available, the data obtained was solely used for comparison of the relative mass molecule distribution for each oxidised polysaccharide. The elution curves of the material were assessed as to the shape and peak elution volume. The various conditions applied for the oxidation of starch apparently had a large impact on the distribution pattern. Thus, a somewhat higher pH (i.e. 9.3) (Fig. 3, curve 3) leads to more degradation than oxidation at pH 8.2. (Fig. 3, curve 1 and 2). Oxidation using oxone in the absence of bromide effected more severe depolymerisation, possibly due to the prolonged reaction time (Fig. 3, curve 4). In order to minimise depolymerisation the reaction should be carried out at <5 °C and pH < 9.0, which is also the case in the hypochlorite/bromide system (Bragd et al., 2000). Alkaline pH and higher temperature should be avoided as the C-6 aldehyde-intermediate formed in the course of the oxidation easily undergoes β-alkoxy elimination (Whistler & BeMiller, 1958). In addition to the peak at 12–14 ml and the shoulder at 15–20 ml, which are believed to constitute the amylopectine and amylose fractions, respectively, some small fractions were

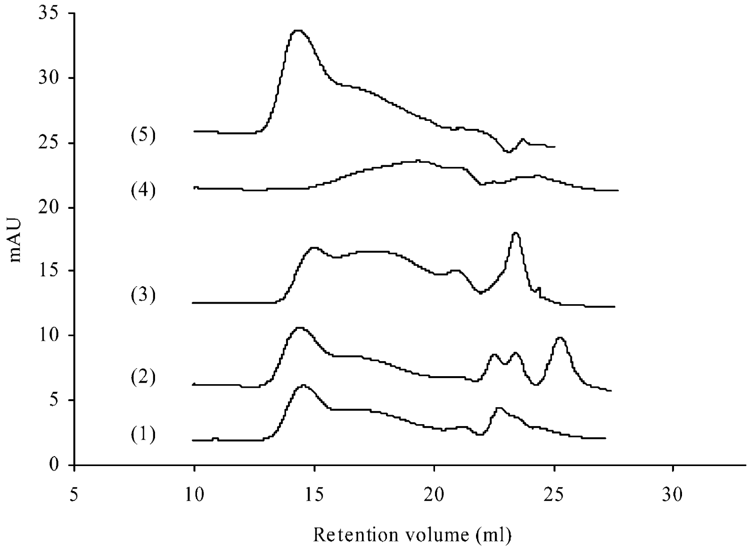


Fig. 3. Molecular mass distribution (SEC retention time vs. absorbance at 215 nm) for 4-AcNH-TEMPO/peracid/NaBr oxidised potato starch at 2 °C and pH 8.2: (1) oxone, (2) peracetate, (3) oxone, pH 9.3, (4) oxone without NaBr, (5) sodium hypochlorite/TEMPO (reference).

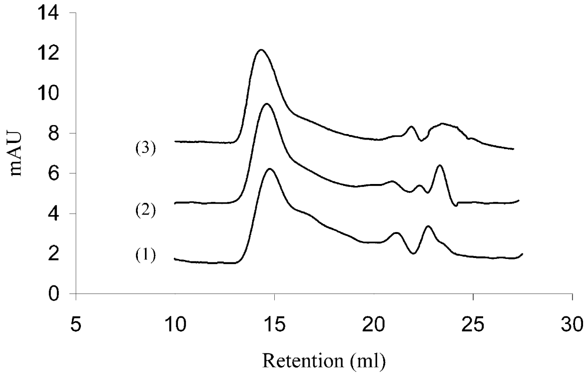


Fig. 4. Molecular mass distribution (SEC retention time vs. absorbance at 215 nm) for 4-AcNH-TEMPO/peracid/NaBr oxidised waxy maize starch at 2 °C and pH 8.2. Oxidants (1) oxone, (2) peracetate, (3) hypochlorite/TEMPO (reference).

obtained at 22–25 ml. These peaks consisted largely of salts such as NaCl and NaBr, indicated by the high conductivity of these fractions. As no detectable amounts of 4-AcNH-TEMPO were found in the oxidised materials (see Section 2 for the analytical testing procedure), the presence of this species in the low molecular weight fractions can be ruled out.

It must be taken into account that the differences in the degrees of oxidation may influence the overall elution time as a more oxidised material is larger due to a higher degree of hydration and will elute earlier than a sample with lower degree of oxidation.

For comparison, a starch sample oxidised by 4-AcNH-TEMPO/sodium hypochlorite was added as a reference (DO = 75%) (Fig. 3, curve 5). In addition, the latter sample was analysed by SEC-MALLS, which enables absolute molecular weight determination. In the latter analysis the average molecular weight (M_w) was determined to be 2500 kDa, M_w/M_n was 6.5 and the radius of gyration (rGz) was 75 nm. As the elution profiles for curve 1 and curve 2 (Fig. 3) are quite comparable to the reference sample (slightly shifted towards higher elution volumes), we reasoned that the overall average molecule weight (M_w) for these materials are in the same range as the hypochlorite oxidised material. Since the average molecule weight (M_w) for the amylopectin component in native potato starch is in the order of magnitude of 500 000 kDa, (Young, 1984) the degradative side reactions occurring throughout the 4-AcNH-TEMPO oxidation process cannot be neglected, probably regardless of the reaction conditions and the primary oxidant used.

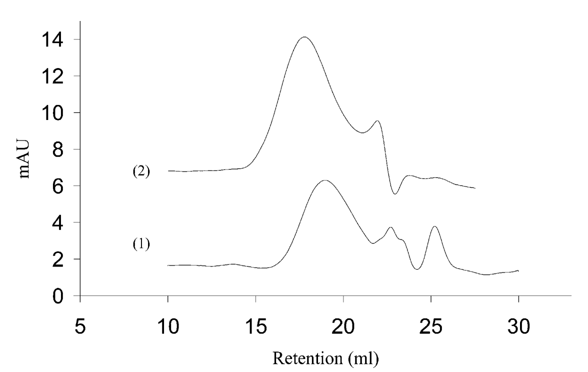


Fig. 5. Molecular mass distribution (SEC retention time vs. absorbance at 215 nm) at 2 °C and pH 8.2: (1) 4-AcNH-TEMPO/oxone/NaBr oxidised pullulan, (2) sodium hypochlorite/TEMPO oxidised pullulan (reference).

The oxidised amylopectin and pullulan samples were compared to the corresponding 4-AcNH-TEMPO/hypochlorite oxidised material (Figs. 4 and 5). Similar to starch, somewhat more degradation appeared to have occurred for the peracid oxidised materials. The pullulan reference sample was analysed by SEC-MALLS; the average molecule weight (M_w) was determined to be 38 000 kDa, M_w/M_n was 1.7 and rGz was 36 nm.

3.2. Oxidation of methyl α-D-glucopyranoside

The peracids in combination with catalytic amounts of sodium bromide and the nitroxyl radical were employed in the oxidation of Me α-Glc*p* to investigate the reaction kinetics for the system under various conditions. Me α-Glc*p* was selected since this compound is well documented from previous reports comprising the TEMPO-oxidation process. The generally accepted reaction mechanism for TEMPO mediated oxidations is depicted in Fig. 6.

The course of the reaction using peracetate, oxone and hypochlorite is depicted in Fig. 7. The reaction rate differed significantly for the three oxidants at pH 8.2; the highest reaction rate was obtained for hypochlorite, followed by oxone. These data support the results from the polysaccharide oxidations (Section 3.1). In contrast to the other oxidants, an initial phase where no formation of uronates could be seen occurred in the peracetate oxidation. The unreactive period might be due to the concomitant reaction of the nitrosonium salt with hydrogen peroxide, which is present in the peracetate solution (ca. 10%) in equilibrium with peracetate and acetic acid. By this reaction, the nitrosonium ion reverts to the radical while peroxide is oxidised to oxygen (Sen, Golubev, Kulyk, & Rozantsew, 1976). In the early phase when the peroxide concentration still is high this rapid side reaction cannot be neglected; especially under the alkaline conditions applied here.

The overall rate-constant (k_{obs}) for the oxidation of the C-6 hydroxyl groups was calculated using Eq. 1, assuming steady state concentration of the aldehyde-intermediate and the nitrosonium ion (De Nooy et al., 1995b). Linear correlations were obtained at pH 8.2, indicating first-order kinetics (the rate constant for the peracetate process was approximated using data *after* the initial low reactive period)

$$-\frac{d}{dt} = k_{obs}([\mathrm{Me\ Glc}p]_0 - [\mathrm{Carboxylate}]_t) \qquad (1)$$

Deviations from the first-order kinetics were found under

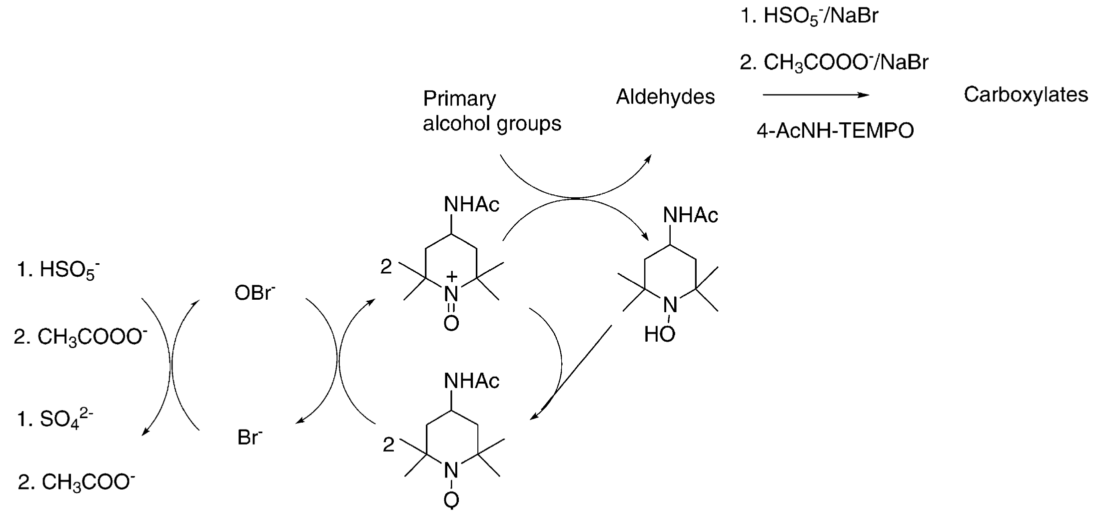


Fig. 6. Simplified reaction mechanism for the peracid/NaBr/4-AcNH-TEMPO oxidation of glucopyranosides. The conversion of the aldehyde-intermediate to the carboxy-product proceeds similarly.

more alkaline conditions (Fig. 8), which also previously was seen in the TEMPO/hypochorite process (Bragd et al., 2000). This is probably due to the instability of the nitrosonium salt at pH > 9, which results in a decrease of the overall reaction rate (Endo, Miyazawa, Shiihashi, & Okawara, 1984). Moreover, the formation of the nitrosonium salt is suppressed under more alkaline conditions which results in a lower concentration of the latter species (Bragd, Besemer & Van Bekkum, 2001).

Of particular interest was the influence of the pH on the reaction rate for the different peracid oxidants. The rate of formation of 6-carboxyls was found to be largely dependent of pH for both oxone and peracetate. A distinct maximum in the reaction rate was found at pH 9 for oxone and at pH 8 for peracetate. Since the rate-maximum coincides with pK_a for both the oxone and the peracetate systems, it is tempting to conclude that the reaction mechanism involves the peracid as well as its anion. The maximum rate constant for the oxone oxidation was 20–25 times higher than that for the peracetate oxidation (Fig. 9).

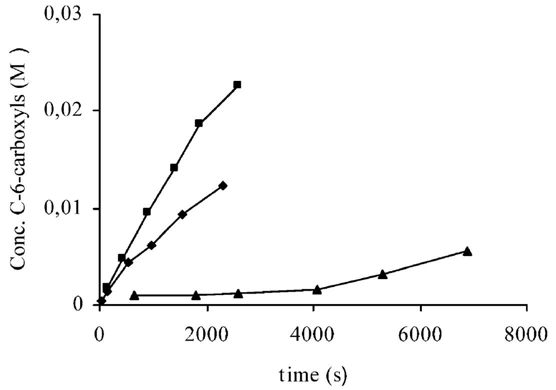


Fig. 7. The rate of formation of C-6 carboxyls vs. time at pH 8.2 and 20 °C in the 4-AcNH-TEMPO/NaBr/Peracid catalysed oxidation of Me α-Glc*p* (■) hypochlorite, (◆) oxone/NaBr, (▲) peracetate/NaBr.

The rate-constants (k_{obs}) were found to be independent of the concentration of oxone and peracetate, which implies that the reaction of the nitrosonium ion with the carbohydrate substrate is the rate-limiting step (Fig. 10). However, at lower concentrations of peracetate (perac./Me α-Glc*p* < 2) (Fig. 10(II) the reaction rate decreased, possibly as the formation of the active nitrosonium salt becomes rate-limiting; thus giving a lower steady state concentration of this species. By contrast, no such trend was seen for the stronger oxidant oxone. Instead, the rate constant decreased at higher molar ratios (oxone/Me α-Glc*p* > 5) (Fig. 10(I)).

Kochkar et al. reported that the conversion of the oxidation of Me α-Glc*p* was dependent on the concentration of peroxydisulfate; an optimal peroxydisulfate/TEMPO ratio of ca. 50 was found (Kochkar et al., 2000). The observed effect was believed to be due to complex formation of the cationic nitrosonium ion and sulfate anion, which at higher concentration of the oxidant would lead to a decreased cationic charge on the nitrogen and consequently a less favourable substrate reaction. However, the protonated nitrosonium complex as it is presented in the report is unlikely to exist at the applied weakly alkaline conditions.

Jiang et al. (2000) recently reported on the TEMPO/bromide/hypochlorite oxidation of hyaluronan (Jiang et al., 2000). It was demonstrated that extra addition of the co-catalyst sodium bromide or any other salt decreased the rate of oxidation. The addition of salts was believed to shield the Coulombic attraction between the cationic nitrosonium ion and the carboxylated substrate, thus lowering the local concentration around the substrate. When we performed the oxidation of Me α-Glc*p* with additional sodium sulfate and sodium chloride added, similar effects were observed (Fig. 11). Since oxone produces a high salt concentration (sulfate anion) as byproduct in the oxidation reaction, it seems reasonable to conclude that the

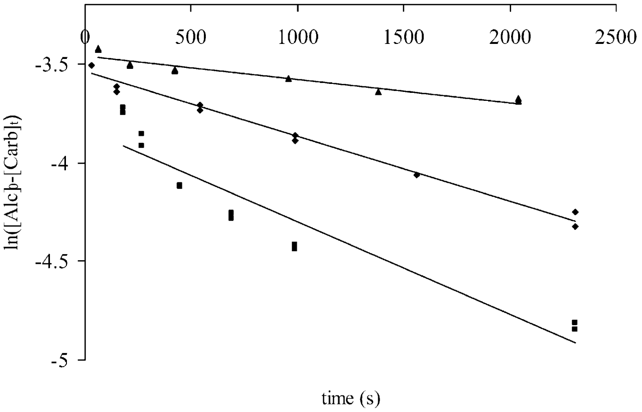


Fig. 8. In ($[\mathrm{Alc}]_0$-$[\mathrm{Carb}]_t$) vs. *t* in the 4-AcNH-TEMPO/NaBr/oxone oxidation of Me α-Glc*p* at 20 °C (▲) pH 7.5, (◆) pH 8.2, (■) 9.5.

rate-decreasing effect at high oxone concentrations is mainly due to electrostatic interactions.

4. Conclusions

In the oxidation of various polysaccharides by the peracid/NaBr/4-acetamido-TEMPO system, in general, high conversion of primary hydroxyl groups to carboxylate groups was attainable with high selectivity and at high reaction rate. Both oxone and peracetic acid were found to be suitable as primary oxidants, but to attain high conversion bromide had to be added in catalytic amounts as a co-catalyst.

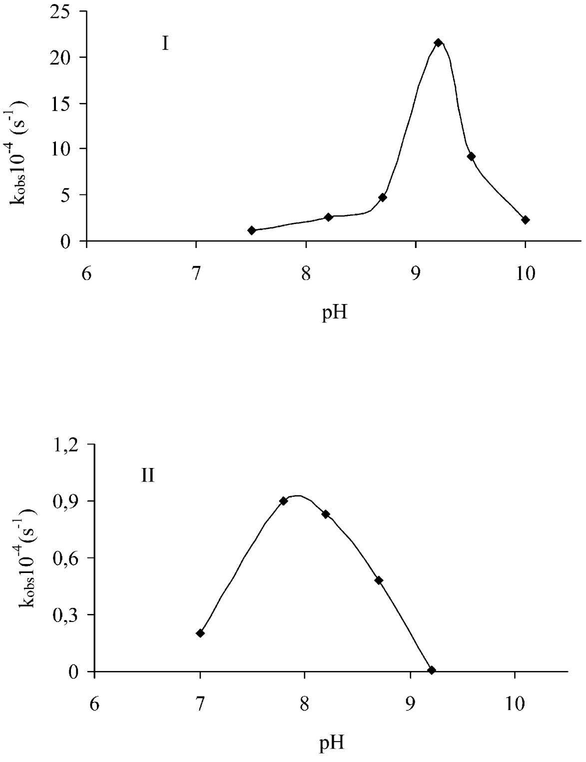


Fig. 9. The rate constant k_{obs} vs. pH for the 4-AcNH-TEMPO/peracid/NaBr oxidation of Me α-Glc*p* at 20 °C: (I) oxone, (II) peracetate.

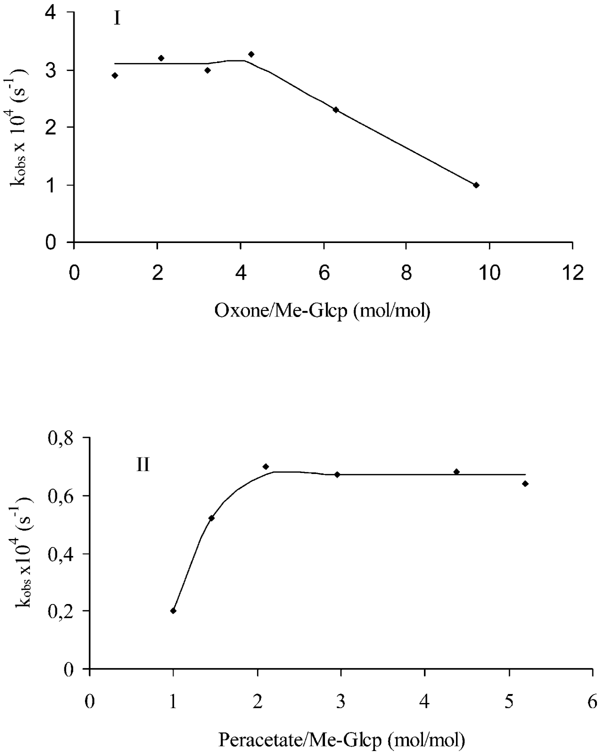


Fig. 10. The rate-constant k_{obs} vs. the ratio of oxone/Me α-Glc*p* (I) and peracetate/Me α-Glc*p*, respectively, in the 4-AcNH-TEMPO/peracid/NaBr oxidation of Me α-Glc*p*.

Provided that the temperature was kept low (< 5–10 °C) the depolymerisation was minor and the relative mass molecular distribution was rather similar to that obtained in hypochlorite/4-AcNH-TEMPO oxidation. Unlike peracetic acid, oxone had sufficient oxidising power to regenerate 4-AcNH-TEMPO without the presence of bromide, but this completely halide-free system resulted in a lower reaction rate and less conversion (ca. 60%). The reaction was also acompanied by more severe depolymerisation.

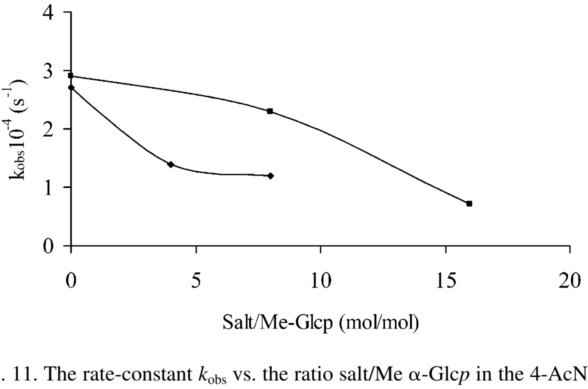


Fig. 11. The rate-constant k_{obs} vs. the ratio salt/Me α-Glc*p* in the 4-AcNH-TEMPO/oxone/NaBr oxidation of Me α-Glc*p* at 2 °C and pH 8.2 (◆) Na_2SO_4, (■) NaCl.

The rate of oxidation was approximately 25 times higher for oxone than for peracetic acid in the oxidation of Me α-Glc*p*. The results indicated that the rate of reaction step involving the oxidation of 4-AcNH-TEMPO to the corresponding nitrosonium salt is lower when peracetic acid is the oxidant, thus resulting in a lower steady state concentration of the latter species and consequently reaction rate. At higher concentrations of salt, the reaction rate decreased, which may be due to poorer interaction of the cationic nitrosonium ion and the carboxylated substrate.

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