TEMPO-derivatives as catalysts in the oxidation of primary alcohol groups in carbohydrates

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Abstract

Primary hydroxyl groups in aqueous starch, pullulan and methyl α-D-glucopyranoside were oxidised to the corresponding carboxylic acid functionalities by TEMPO-(4-X)-derivatives using sodium hypochlorite as the primary oxidant. All the combinations of substrates and nitroxyl radicals resulted in stoichiometric conversions, and the selectivity for oxidation of primary hydroxyls was high. Some depolymerisation occurred throughout the oxidation, especially when 4-acetoxy and 4-mesyl-TEMPO were used. The pH window of the activity of the inexpensive 4-acetamido-TEMPO was found to be substantially lower from that of the other tested TEMPO-derivatives; thus allowing milder reaction conditions. At pH 8, the rate of oxidation was ca. two times higher when 4-acetamido-TEMPO was used compared to the other catalysts. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years, catalytic oxidation of primary hydroxyl groups in polysaccharides to the corresponding carbonyl or carboxyl functionalities by the stable nitroxyl radical TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) has been recognised for the high regioselectivity and reaction rate. Several publications describe procedures for oxidation of various polysaccharides, e.g. starch, inulin, guar gum, pullulan, dextrins, cellulose and chitins [1–4]. Recently, procedures for the oxidation of galactomannans, sucrose, and hyaluronan were published [5–8]. The conversion of primary alcohols to carboxylates proceeds via a reactive aldehyde-intermediate, which is present at low concentration throughout the oxidation [9]. Regeneration of the nitrosonium salt, which is the active oxidising species, takes places in situ by the use of a suitable primary oxidant, e.g. sodium hypochlorite with catalytic amounts of sodium bromide [10]. Hypochlorite processes without bromide have been developed recently [6,11]. Moreover, the use of peroxo-disulfate together with heterogeneous silver catalysts in TEMPO-oxidation was reported recently [12]. In addition to TEMPO, a few other TEMPO-derivatives with different functional groups on the 4 position, i.e. like 4-methoxy-TEMPO and 4-acetamido-TEMPO have earlier been applied as catalyst in the oxidation of alcohols, but the work has been limited to substrates in organic solutions [13–18]. The aim of this study was to extend the use of some TEMPO-(4-X)-derivatives (Fig. 1) to

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X=NHCOCH$_3$ **(1)**

X=OCH$_3$ **(2)**

X=OCOCH$_3$ **(3)**
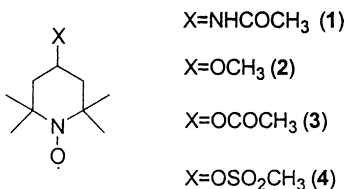
X=OSO$_2$CH$_3$ **(4)**

Fig. 1. The TEMPO-derivatives used as catalysts in the oxidation experiments.

oxidation of carbohydrates in aqueous solution. In addition to 4-acetamido-TEMPO **1**, which is commercially available and cheaper than TEMPO (X = H), the TEMPO-derivatives **2**–**4** were prepared and subsequently employed as catalysts in the oxidation of potato starch, pullulan and methyl α-D-glucopyranoside.

2. Experimental

2.1. Materials and reagents

Potato starch (water content 18%) was obtained from AVEBE (Groningen, The Netherlands). Pullulan (a glucan with repeating trimeric unit: ([→ 6)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 →])) (water content 8%) was obtained from TNO (Food and Nutrition Research, Zeist, The Netherlands). The average molecular mass (M_w) of the pullulan sample was estimated by SEC-MALLS to be ca. 400 kDa. 4-Acetamido-TEMPO and 4-hydroxy-TEMPO was obtained from Degussa-Hüls (Marl, Germany). Sodium hypochlorite (ca. 15%) was obtained from Akzo Nobel (Arnhem, The Netherlands). The active chlorine content was determined by iodometric titration. All other chemicals were of commercial grade and were used without further purification.

2.2. General methods

The molecular mass distribution was 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, M_w 1000–700,000 connected in series with a TSKgel G 6000 PW, M_w 500,000–50,000,000. As eluent NaH$_2$PO$_4$ (50 mM buffered at pH 7.0) was used. Flow rate was set at 0.5 ml/min. Under the given conditions, V_0 is ca. 12 ml. Reversed phase chromatography was carried out with a Pharmacia HPLC ÄKTA EXPLORER 10, equipped with a UV–VIS detector (UV-900). TEMPO-(4-X)-derivatives were monitored at 425 nm. The column was a Phenomenex Luna 3u C18(2) 150 mm × 4.60 mm. As eluent acetonitrile (70%)–water (30%) was used. Flow rate was set at 0.5 ml/min. 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 amperometry mode. The PED detector was equipped with a gold working electrode and a Ag/AgCl reference electrode. Eluent: 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 and 20% 100 mM NaOH + 500 mM NaAc. Quantitative ^{13}C NMR spectra were recorded on a Varian VXR-400 S spectrometer using D$_2$O as solvent. As internal standard, *t*-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 ^{1}H decoupling during acquisition only. ^{1}H NMR spectra were recorded on a Varian VXR-400 S spectrometer. As internal standard *t*-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. Uronates formed in the oxidised materials were determined with the Blumenkrantz assay, which method allows quantitative determination of carboxylic acid groups without interfering with neutral sugars [19] (the latter assay is assumed to represent the carboxylic groups arising from TEMPO-oxidation).

2.3. Synthesis of 4-methoxy-TEMPO (**2**)

Compound **2** was prepared according to the procedure of Miyazawa et al. [13]. To a solution of 4-hydroxy-TEMPO (5 g, 30 mmol) in DMF (45 ml) NaH (0.72 g, 30 mmol) was added. The solution was cooled in ice to 2°C, and CH$_3$I (1.89 ml, 30 mmol)

dissolved in DMF (10 ml) was dropwise added under nitrogen. The temperature was raised to 20°C and the mixture was stirred for 6 h. Then diethyl ether (250 ml) was added and the by-product sodium iodide was filtered off. The reddish solution was extracted with water (3 × 30 ml), dried over anhydrous MgSO$_4$ and the ether was removed in a rotational evaporator. The crude product was purified by column chromatography over silica gel (diethyl ether) and subsequently recrystallised in hexane at −20°C to afford **2** as needle shaped, orange-red crystals in 65% yield (3.5 g), mp 39–39°C. Anal. Calc. for C$_{10}$H$_{20}$NO$_2$: C, 64.48; H, 10.82; N, 7.52; O, 17.18; Found: C, 65.50; H, 10.88; N, 6.47; O, 18.30 (stored at −20°C).

2.4. Synthesis of 4-acetoxy-TEMPO (3)

To a solution of 4-hydroxy-TEMPO in DMF (5 g, 30 mmol) in DMF (50 ml) and pyridine (2 ml), at 35°C, acetic acid anhydride (2.8 ml, 29 mmol) was added. The temperature was kept at 35°C throughout the reaction. After stirring for 60 h, diethyl ether (100 ml) was added and the mixture was extracted with water (3 × 30 ml), dried over MgSO$_4$ and the ether was evaporated off under vacuum. The crude product was purified by column chromatography over silica gel (diethyl ether) and subsequently recrystallised from hexane at room temperature to afford **3** as orange-red crystals in 46% yield (2.9 g), mp 48–52°C. Anal. Calc. for C$_{11}$H$_{20}$NO$_3$: C, 61.65; H, 9.41; N, 6.54; O, 22.40; Found: C, 62.75; H, 9.55; N, 5.80; O, 22.90 (stored at −20°C).

2.5. Synthesis of 4-mesyl-TEMPO (4)

To an ice-chilled solution of 4-hydroxy-TEMPO (3.4 g, 20 mmol) in pyridine (15 ml), methanesulfonyl chloride (2.2 g, 20 mmol) was slowly added dropwise. After 30 min, the temperature was raised to room temperature and the mixture was stirred for 50 h. Diethyl ether (50 ml) was added and the mixture was extracted with water (3 × 30 ml), dried over MgSO$_4$ and the ether was evaporated off under vacuum. The crude product was purified by column chromatography over silica gel (diethyl ether) and subsequently recrystallised from hexane at room temperature to afford **4** as orange-red crystals in 49% yield (3.5 g), mp 76–80°C. Anal. Calc.

for C$_{10}$H$_{20}$NO$_4$S: C, 47.98; H, 8.05; N, 5.59; O, 25.56; Found: C, 48.70; H, 8.17; N, 5.26; O, 25.50 (stored at −20°C).

2.6. General procedure for oxidation of potato starch and pullulan

Potato starch (10 g) was gelatinised in demi-water (450 ml) at 95°C (with effective mechanical stirring) and then slowly cooled to the desired oxidation temperature (10–20°C). Pullulan (5 g) was dissolved in 250 ml water. TEMPO or the analogues thereof (0.051 mmol/g substrate) were dissolved in the solution. NaOCl (2 M) was added in small portions of 2 ml, throughout the oxidation to minimise unwanted side reactions. An amount of NaOCl (1 or 2 mol NaOCl/mol primary alcohol) corresponding to 50 or 100 mol% degree of oxidation (C-6 carbonyls + C-6 carboxyls) was added. The pH was kept at 8.5 throughout oxidation by addition of sodium hydroxide (0.5 M), using a pH-stat apparatus. After completion of the reaction, the remaining aldehyde-intermediates were reduced to the alcohol by NaBH$_4$ (0.2 g). The oxidised materials were precipitated in 2 volumes of ethanol, filtered off and subsequently rinsed with EtOH/acetone. The materials were dried under vacuum at 25°C for 10–15 h. All materials were re-dissolved in water, ion-exchanged (DOWEX 50WX8-100 cation exchanger, Sigma) and subsequently freeze-dried. Representative ^{13}C NMR data of oxidised starch and pullulan samples (400 MHz, D$_2$O) are δ C-1 99.2, C-2-C-5 71.0-74.5, C-6 (ox) 177.8.

2.7. General procedure for oxidation of methyl α-D-glucopyranoside (Me α-Glcp)

To a thermostatted (22°C) solution of Me α-Glc*p* (388 mg, 2 mmol) in 50 ml of demi-water, NaOCl was added (2.0 ml, 2.12 M). pH was adjusted (i.e. range between 5.5–8.5) 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 TEMPO or an analogue thereof (0.4 ml, 32 mM). Throughout the oxidation aliquots were taken and quenched in a water/EtOH solution. The concentration of uronic acid was determined by the Blumenkrantz assay.

3. Results and discussion

3.1. NaOCl/4-X-TEMPO oxidation of starch and pullulan

Formulas of the fully oxidised products of starch and pullulan are given in Fig. 2. Regardless of the TEMPO-derivative used as catalyst, the reactions proceeded virtually stoichiometrically with regard to the primary oxidant NaOCl (Table 1). As ca. 10% of the C-6-groups are estimated to be present as the aldehyde-intermediate at 50% degree of oxidation [9], the carboxyl amount is not expected to exceed ca. 45 mol% (mol C-6 carboxyls/mol primary alcohol). In a few experiments, somewhat lower values were obtained for pullulan. The consumption of NaOH indicated >90% yield in all performed oxidations. The time of completion of the oxidation reactions showed substantial variations. Pullulan required longer reaction times compared to starch, probably as only two out of three glucose units are available for CH_2OH-oxidation. In both the oxidation of starch and pullulan, normal TEMPO gave the highest rate of oxidation at pH 8.5. This might be due to some sterical hindrance of the TEMPO-derivatives. The molecular mass distribution of the oxidised materials was investigated by size exclusion chromatography

Table 1
Oxidation of starch and pullulan by TEMPO and the TEMPO-derivatives with NaOCl as the primary oxidant at pH 8.5 and 10°C[a]

Entry	Substrate	TEMPO-derivative	Reaction time (min)	C-6 carboxyls (mol%)[b]
1	Starch	TEMPO	80	43
2	Starch	1	300	>95
3	Starch	1	90	41
4	Starch	2	230	45
5	Starch	3	220	44
6	Starch	4	230	46
6	Pullulan	TEMPO	120	46
7	Pullulan	1	400	90
8	Pullulan	1	230	36
9	Pullulan	2	330	42
10	Pullulan	3	420	41
11	Pullulan	4	420	39

[a] In entries second and seventh, 2 mol NaOCl/mol primary alcohol was added, corresponding to 100% degree of oxidation. In the other entries, 1 mol NaOCl/mol primary alcohol was added, corresponding to 50% degree of oxidation.
[b] Determined by the Blumenkrantz assay.

(SEC). In comparison to the polysaccharides oxidised by TEMPO, minor depolymerisation seems to have occurred in the oxidation catalysed by the TEMPO-derivatives (Fig. 3).

Following explanations may be considered: Firstly, competitive non-catalytic sodium hypochlorite
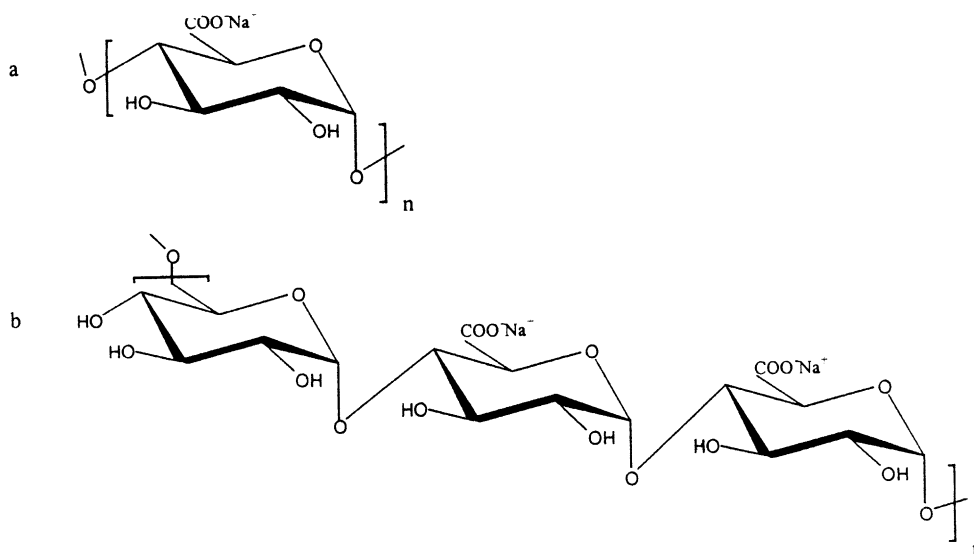

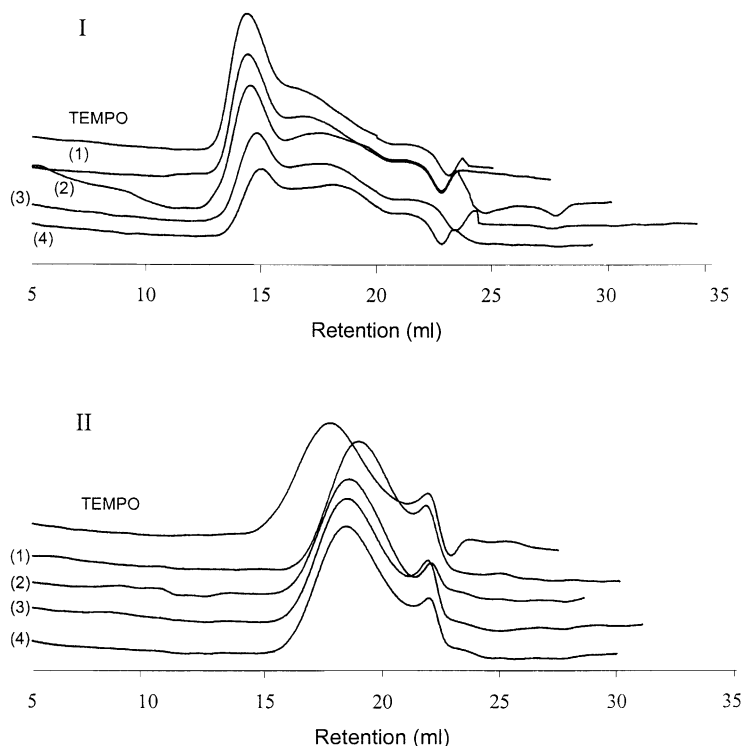Fig. 2. (a) 6-Carboxy-starch and (b) 6-carboxy-pullulan.

Fig. 3. Molecular mass distribution (SEC retention time vs. absorbance at 215 nm) for 4-X-TEMPO/NaOCl oxidised starch (I) and pullulan (II) at 20°C (all the samples were oxidised to 50% degree of oxidation). Catalysts: TEMPO, 4-acetamido-TEMPO (1), 4-methoxy-TEMPO (2), 4-acetoxy-TEMPO (3), 4-mesyl-TEMPO (4).

oxidation, resulting in ring cleavage between vicinal secondary hydroxyl groups may not be negligible [20–22]. Secondly, the high selectivity that is the characteristic feature in the TEMPO-mediated oxidations might be somewhat lower for the TEMPO-derivatives, i.e. also secondary hydroxyl groups of the glucose ring are oxidised. The presence of carbonyl functionalities at C-2, C-3 in starch or C-2, C-3, C-4 in pullulan would facilitate depolymerisation via β-alkoxy-elimination [23,24]. To investigate for the presence of non-selectively oxidised sugars, the starch materials oxidised by TEMPO and 4-mesyl-TEMPO (**4**) were hydrolysed (15 h in 2 M H_2SO_4) and then analysed by high performance anionic chromatography (HPAEC–PED). Besides neutral glucose and glucuronic acid, no other species could be detected (Fig. 4). If dicarboxy starch had been present in the oxidised product, glyoxylic acid and erythronic acid would have appeared after hydrolysis. Analysis

of the oxidised products by ^{13}C NMR further indicated that starch and pullulan were exclusively oxidised in the C-6 position; only the required signals from the repeating sugar units could be observed (see experimental section for assignment of peaks). Apparently, the tested TEMPO-derivatives have the same high selectivity for C-6 oxidation as normal TEMPO, and hypochlorite oxidation is negligible. It seems reasonable to conclude that the depolymerisation stems from β-alkoxy-elimination of the C-6-aldehyde-intermediate throughout the oxidation.

3.1.1. 4-X-TEMPO/NaOCl oxidation of methyl α-D-glucopyranoside (Me α-Glcp)

The activity of the TEMPO-(4-X)-derivatives over the pH range, 5–10 was studied using Me α-Glc*p* as model compound and hypochlorite as primary oxidant. The formation of uronic acid versus time showed a large dependence on pH (Fig. 5(I)), as
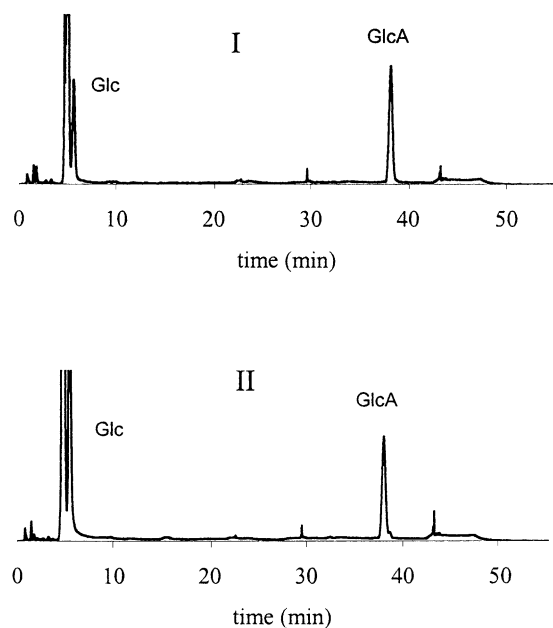
Fig. 4. HPAEC–PED chromatograms of hydrolysed oxidised starch. Catalysts: (I) 4-mesyl-TEMPO and (II) TEMPO. Glc = glucose, GlcA = glucuronic acid.

Fig. 5. Relative reaction rate vs. pH in the oxidation of Me α-Glc$p$ (I) and pullulan (II) by 4-X-TEMPO/NaOCl at 20°C. (◆) 4-Acetamido-TEMPO; (■) TEMPO; (△) 4-methoxy-TEMPO; (▲) 4-acetoxy-TEMPO; (●) 4-mesyl-TEMPO.

previously was shown for the (H)OCl/NaBr/TEMPO system. Except in the oxidation catalysed by **1** (the 4-acetamido-derivative), the highest reaction rate was found at pH ca. 9.0 for the TEMPO-derivatives. At this pH, the TEMPO-derivatives **3** and **4** appeared to be stable as no significant amount of 4-OH-TEMPO, which is the product of hydrolysis, could be detected by reversed phase chromatography in samples taken between 0.5–3 h when subjecting **3** and **4** to pH 9.0–9.2 at 20°C. At pH 11 some hydrolysis was observed.

Surprisingly, in the oxidation catalysed by **1**, the maximum reaction rate was found at one pH-unit lower (i.e. pH 8.0) compared to the other catalysts. At this pH, the reaction rate was 2–3 times higher than that of the other catalysts. The same general trend was observed when pullulan was used as the substrate under otherwise identical conditions (Fig. 5(II)); the effect seems to be independent of the substrate.

In the oxidation process, the magnitude or the ratio of the rate-constants for the different steps in the catalytic process are apparently different when TEMPO or **1** is applied as the catalyst. In a separate set of ex-
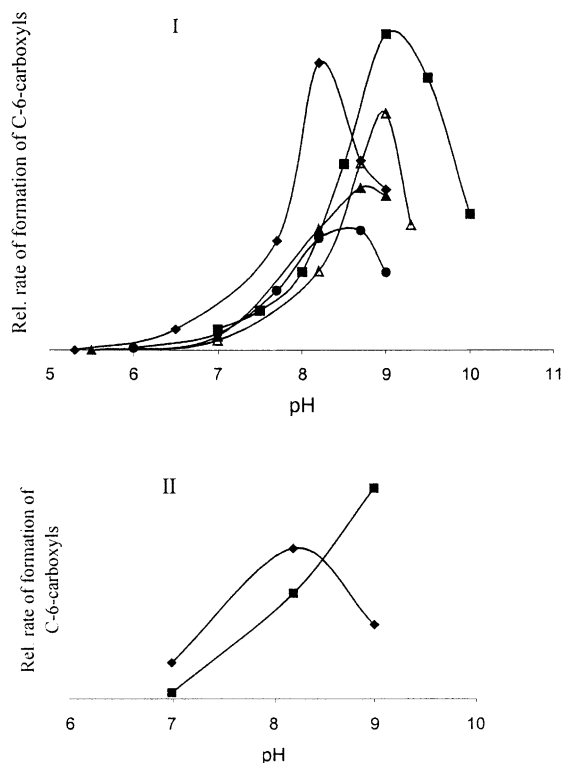
periments, carried out in the absence of the substrate, Me α-Glc$p$, it was seen that the nitrosonium ion of TEMPO was formed faster than the nitrosonium ion of **1** upon oxidation by (H)OCl (step I), (Scheme 1). This trend was most evident between pH 5.5 and 7.0 (Fig. 6). The lower oxidation rate of **1** in comparison to TEMPO is expected to give a lower steady-state concentration of the nitrosonium ion when the Me α-Glc$p$ or polysaccharide substrate is present.

Consequently, the maximum reaction rate will be found at a lower pH compared to a TEMPO catalysed oxidation, as the formation of the nitrosonium ion is suppressed at higher pH. However, since the absolute reaction rate is higher for an oxidation catalysed by **1** at 8.0 (Fig. 5) it is likely that the nitrosonium ion of **1** is a stronger oxidant compared to the corresponding nitrosonium ion of TEMPO (step II). Analogously, the regeneration of the radical (step III) is likely
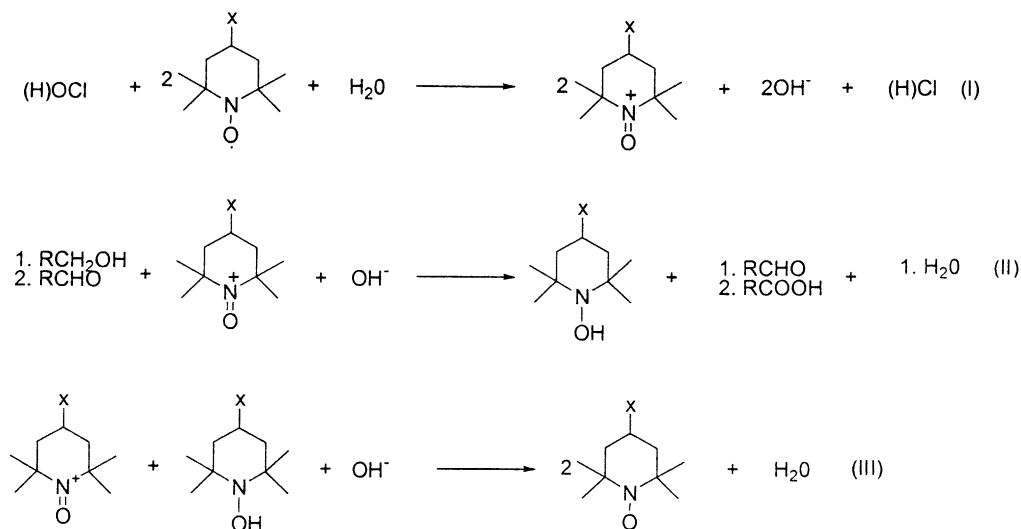
Scheme 1. Reaction steps in the TEMPO-catalysed primary alcohol oxidation: (I) oxidation of TEMPO to TEMPO+; (II) oxidation of the alcohol or aldehyde-intermediate by TEMPO+; (III) regeneration of TEMPO.
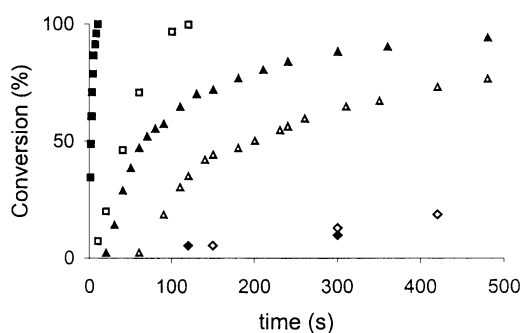

Fig. 6. Oxidation of TEMPO ((■) pH 6.0; (▲) pH 7.0; (◆) pH 8.5) and 4-acetamido-TEMPO ((□) pH 6.0; (△) pH 7.0; (◇) pH 8.5) to the corresponding nitrosonium ions by (H)OCl in the absence of substrate.

to be faster when the nitrosonium ion is a stronger oxidant.

4. Conclusions

The utility of some TEMPO-(4-X)-derivatives in monomeric and polymeric carbohydrate oxidation in aqueous solution is demonstrated. Analysis of the oxidised materials revealed that stoichiometric conversions were attainable, regardless of the TEMPO-derivative and carbohydrate substrate used. A slight depolymerisation of the polysaccharide backbone occurred throughout the oxidation by the TEMPO-(4-X)-derivatives, as was indicated by size exclusion chromatography. The intermediate aldehyde forms are assumed to be vulnerable for depolymerisation. Upon oxidation of Me α-Glc$p$ and pullulan by the 4-acetamido derivative (**1**), the maximum reaction rate was found at one pH-unit lower compared to TEMPO and the other catalysts, thus allowing milder conditions. At this pH (i.e. 8.0), the reaction rate was ca. two times higher compared to TEMPO. 4-Acetamido-TEMPO is less expensive than TEMPO, making it an interesting alternative as catalyst in oxidation reactions.

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