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Degradation of pullulans of narrow molecular weight distribution—the role of aldehydes in the oxidation of polysaccharides

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Abstract

Degradation of pullulan samples of narrow molecular weight distribution was studied in O_2 and N_2 atmosphere at 180 °C and in air at 80 °C, 65% RH. At higher temperatures, size exclusion chromatographic analyses provided evidence for non-random degradation and formation of cross-links, a phenomenon depending on the concentration of carbonyl groups. Initial degradation rates in oxidative and inert environments were comparable, while at longer reaction times extensive cross-linking was typical of degradation in oxygen.

At 80 °C, in a moderately alkaline environment, the degradation remains random, as demonstrated by the mono-modal distributions of molecular weights. Furthermore, there was no evidence of cross-linking. Correlations between the concentration of aldehyde end-groups in the pullulan samples, rates of degradation and the content of peroxides after a pre-oxidation treatment, as determined by the use of chemiluminometry, were demonstrated. This leads to the conclusion that aldehyde groups, via the peroxide intermediates, have the decisive influence on the rates of oxidative degradation of polysaccharides.

Keywords: Pullulan; Cellulose; Oxidation; Size exclusion chromatography; Chemiluminescence

1. Introduction

The thermal degradation of polysaccharides, especially cellulose, is an intensively studied subject. It is of interest in life-assessment of power transformers (Emsley & Stevens, 1994b), where paper is used as an insulator, and particularly in studies of degradation of library and archival materials (Strlič & Kolar, in print), where due to the variety of relevant experimental parameters the approach to degradation studies may become quite complex (Barański, 2002).

In the cellulose degradation below 100 °C, several sets of mechanisms have been outlined, depending on the macromolecular environment (Nevell & Zeronian, 1985). In an acidic environment, the well-known acid-catalysed hydrolysis of glycosidic bonds is the predominant chain-scission reaction route and leads to a rapid loss of mechanical properties of the material (Zou, Uesaka, & Gurnagul, 1996a,b). In a moderately alkaline environment, two reaction mechanisms are dominant: autoxidation and alkaline degradation (Kolar, 1997). The presence of atmospheric oxygen is a pre-requisite for oxidation; however, alkaline degradation may also proceed in its absence, at places where carbonyl groups had formed. The β-alkoxy elimination and a series of rearrangement reactions (Nevell & Zeronian, 1985) are typical for this type of degradation.

In studies of cellulose degradation, viscometry is the routinely used technique (e.g. ISO 5351/1), however, in order to obtain more mechanistic information size exclusion chromatography (SEC) is also often used. During thermal degradation of acidified cellulose in air at 90 °C (Zou, Gurnagul, Uesaka, & Bouchard, 1994), SEC of carbanilated derivates was performed in tetrahydrofuran. It was found out that acid-catalysed hydrolysis proceeds in a random fashion as demonstrated by the constancy of the ratio DP_z/DP_w.

SEC of non-derivatised samples in LiCl/*N*,*N*-dimethylacetamide is also often used despite the difficulties associated

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with cellulose dissolution and systematic errors (Strlič, Kolenc, Kolar, & Pihlar, 2002). In a recent study of cellulose samples subjected to degradation at 120–160 °C in oil (in order to model paper degradation in power transformers) or air (Emsley, Ali, & Heywood, 2000), molecular weight distribution (MWD) was found to change from initial mono-modal to a multi-modal distribution, eventually returning to mono-modal, as the degree of polymerisation (DP) of the material under study reached a limiting value of 150–200. The positions of peaks in the distributions of molecular weights led the authors to believe that bond scission preferentially occurs near the centres of molecules.

Another technique, useful in studies of degradation mechanisms of polymers, is chemiluminometry. Measurements of extremely weak light emitted during chemical reactions have been used to study the degradation of synthetic polymers for decades (Zlatkevich, 1989), however, chemiluminescence during oxidative degradation of polysaccharides has been explored in more detail only recently. Several degradation phenomena leading to chemiluminescence (particular to polysaccharides) were identified (Strlič, Kolar, Pihlar, Matisová-Rychlá, & Rychlý, 2000) and a general cellulose degradation model was proposed (Rychlý, Matisová-Rychlá, Strlič, & Kolar, 2002).

In a study of degradation of cellulosic materials at 80 °C, 65% RH (Malešič, Kolar, & Strlič, 2002) it was shown that the content of carbonyl groups in pulp samples correlates with the extent of degradation during accelerated ageing. However, the exact role of carbonyls is not yet known, as it is difficult to obtain well-defined cellulosic materials. On the other hand, pullulan standards of narrow MWD are freely available and, being linear polymers, may serve as cellulose models for this particular purpose. Thermal degradation of pullulan samples with a defined content of carbonyl groups (aldehyde end-groups) was therefore studied at elevated temperatures (180 °C) in oxidative and inert atmosphere and at 80 °C in air, using SEC and chemiluminescence in order to investigate the role of aldehyde groups into more detail.

2. Experimental

2.1. Sample preparation

Pullulan samples of narrow MWD (set of SEC standards with peak molecular weight, M_p : 5800, 12,000, 24,000, 48,000, 100,000, 186,000, 380,000, 1,600,000 g mol^{-1}, Fluka, Switzerland) were used in the present study.

Degradation at 180 °C was performed in the following way: the samples in powder form (as obtained) were inserted into a glass reactor, subjected to a flow of dry atmosphere (O_2 or N_2, 3 l h^{-1}), and sampled periodically (after 15, 30, 45, 60, 90, 130, 150, 180, 210, 240, 300, 360 min). The reactor was heated in a fan-assisted laboratory drying oven. For the SEC analyses, 1 mg of each sample was dissolved in 1 ml 0.05 mol l^{-1} $NaNO_3$ solution in purified water (MilliQ system, Millipore, France)

For experiments at 80 °C and 65% RH in the climatic chamber (VC 0020, Vötsch, Germany), pieces of glass filter sheets (Macherey Nagel, Germany) were first prepared. The filters were immersed into a solution of $Ca(HCO_3)_2$ (0.01 mol l^{-1}) and dried to provide a moderately alkaline macromolecular environment during the degradation experiment. Pullulan standards of different peak molecular weight were dissolved in purified water (0.04 g ml^{-1}) and 50 μl (2 mg of pullulan) of the resulting solutions were dried on pieces of glass filters. The individual samples on filter sheets were taken out of the climatic chamber periodically (after 1, 2, 3, 5, 7, 10, 13 days) and extracted into 1 ml of 0.05 mol l^{-1} $NaNO_3$ solution in purified water. The dispersions of fibres were centrifuged and the resulting supernatant solutions of degraded pullulans were used for chromatographic analyses.

2.2. Sample analyses

SEC was performed using an Agilent 1100 system consisting of a binary pump (mobile phase: 0.05 mol l^{-1} $NaNO_3$ in water; flow 0.5 ml min^{-1}), automated injector (injection volume 100 μl), column thermostat (columns were thermostatted at 20 °C) and RID detector (detection cell thermostatted at 40 °C). Three columns were used in series: PSS Suprema XL guard column, PSS Suprema XL and PSS Suprema M (Polymer Standards Service, Germany).

For the construction of calibration curves, solutions of non-degraded pullulan samples in concentrations equal to the concentrations of analytes were used. Third-order polynomial column calibration was performed.

Chemiluminometry was carried out using a Lumipol-2 chemiluminometer (Polymer Institute of the Slovak Academy of Sciences, Slovakia). Aqueous solutions (50 μl of a 0.02 mg ml^{-1} solution) of individual pullulan samples were dried in aluminium pans (diameter 1 cm), which were inserted onto a heated oven in the reaction chamber, through which a flow of atmosphere (nitrogen or oxygen) was established (3 l h^{-1}). The light emitted by the sample during degradation is collected by a photomultiplier and signal processing is performed using the photon-counting technique.

The experiments were performed in the following way: a pullulan sample was first oxidised in a flow of oxygen for 60 min at 100 °C. Following this, the atmosphere was abruptly changed to nitrogen and the sample was allowed to cool down to 40 °C for 15 min. The temperature was then steadily increased at the rate of 2.5 °C min^{-1} until the temperature of 200 °C was reached.

3. Results and discussion

3.1. Degradation experiments at 180 °C in nitrogen and oxygen atmosphere

In 1936 (af Ekenstam, 1936), a kinetic model for the degradation of linear polymers was proposed:

$$\mathrm{DP}_t^{-1} - \mathrm{DP}_0^{-1} = kt.$$

The model is applicable if the polymer chain is linear and of high molecular weight; if the polymer is mono-disperse and the products are themselves long chain molecules; and if there is no loss of monomer units during scission. The application and further elaboration of the model was explored by Emsley et al. in a number of publications (Emsley & Stevens, 1994a; Emsley, Heywood, Ali, & Eley, 1997). A computer model of the degradation of linear polymers was proposed (Emsley & Heywood, 1995), in which it was shown that linear DP^{-1} vs. t plots can only be obtained if the degradation process is random and all degradation products are included in the calculation.

Examination of the MWDs during degradation at 180 °C of the pullulan standard of initial peak molecular weight 186,000 g mol^{-1} (Fig. 1) reveals that the process is too complex for the Ekenstam model to be used without limitations. The chosen frequency of sampling enables us to study the initial degradation processes more closely. After approximately 60 min at 180 °C, the distribution changes from initially mono-modal to bi-modal and even tri-modal, which is more evident in the case of ageing in oxygen atmosphere. It can be concluded, that the chain-scission process is not random. This phenomenon was observed also in experiments with cellulose (Emsley et al., 2000).

The MWDs extend well beyond the one of the initial material. Formation of degraded material of higher molecular weight is especially interesting, since it points to the existence of cross-linking reactions. It should be stressed that the proportion of cross-linked material, having a structure different to the initially linear macromolecular material, may be misinterpreted by the SEC method, as the molecular shape plays a very important role in column calibration. Besides, as can be seen in Fig. 2, it has a more pronounced effect on the weight average molecular weight ($\bar{M}_\mathrm{w}$) than on the number average molecular weight ($\bar{M}_\mathrm{n}$). Extensive cross-linking during advanced degradation phases was not reported to take place in cellulose, although, considering the MWDs during degradation experiments presented in (Emsley et al., 2000), the phenomenon is typical for both polysaccharides.

In Fig. 2, changes in number and weight average molecular weights are presented during the degradation of the pullulan standard of initial peak molecular weight 48,000 g mol^{-1}. During a short initial period of 15 min, cross-linking causes an initial increase in $\bar{M}_\mathrm{w}$, a phenomenon presumed to take place during degradation of cellulose, as well (Emsley et al., 2000). Following this short period (period a in Fig. 2), both $\bar{M}_\mathrm{w}$ and $\bar{M}_\mathrm{n}$ start to decrease. During this 60-min period (period b), the MWD remains mono-modal, so the Ekenstam model might be applied. For the subsequent period (period c), formation of peaks in the MWDs is typical. Furthermore, formation of cross-links becomes a prominent feature of degradation in oxygen atmosphere, as demonstrated by the increasing $\bar{M}_\mathrm{w}$. In nitrogen atmosphere, cross-linking is a reaction associated with the initial degradation period, only. The initial increase in $\bar{M}_\mathrm{w}$ in nitrogen atmosphere is even more pronounced than in oxygen atmosphere.

A calculation of the first order degradation rate constants during period b (Fig. 2) of degradation of different pullulans in oxygen and nitrogen atmosphere according to Ekenstam demonstrates the validity of the model (Table 1). Namely, the rates are independent of the initial molecular weights. A comparison of degradation rates in oxidative and inert atmospheres reveals that the rates are almost identical, a feature that is not comparable to cellulose. According to the literature, the rates of cellulose degradation in nitrogen and

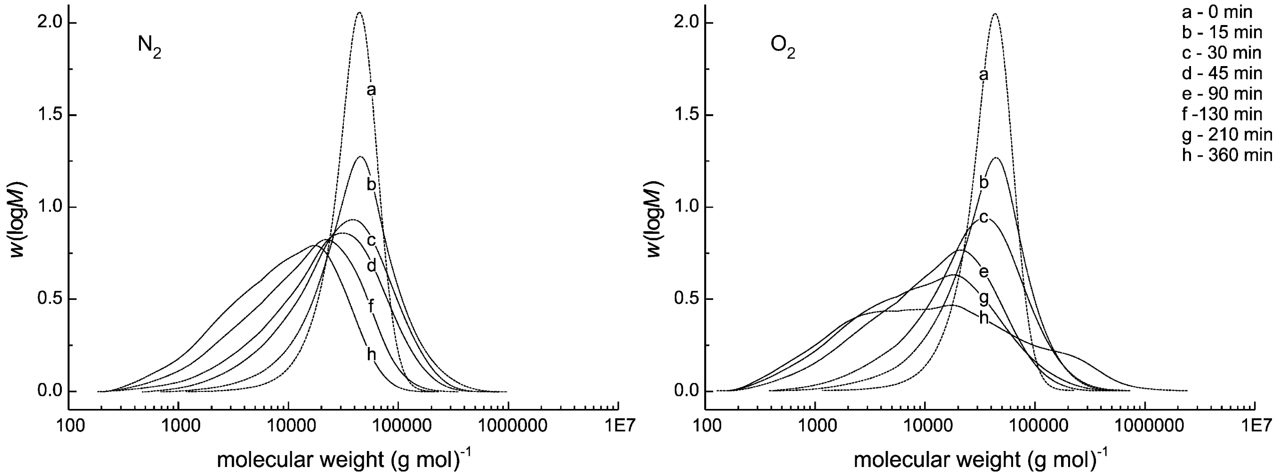

Fig. 1. Molecular weight distributions of the pullulan standard with initial peak molecular weight 48,000 g mol^{-1}, thermally degraded at 180 °C; in nitrogen and oxygen atmosphere.

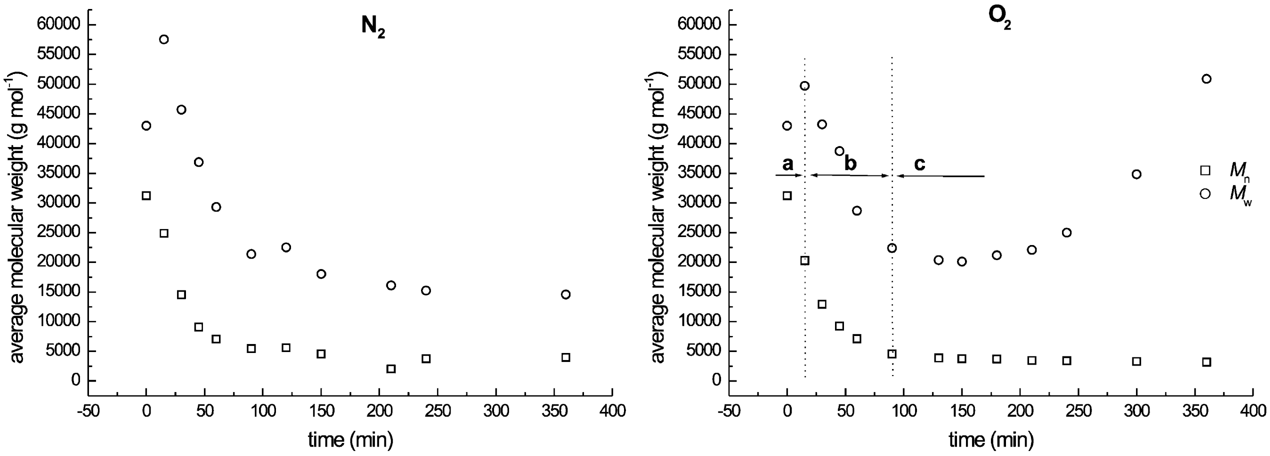

Fig. 2. Molecular mass averages of the pullulan standard with the initial peak molecular weight 48,000 g mol^{-1}, thermally degraded at 180 °C; in nitrogen and oxygen atmosphere. During period b both number and weight average molecular weights decrease and the Ekenstam model may be applied.

air become comparable only at temperatures higher than 300 °C (Shafizadeh & Bradbury, 1979). This may indicate that the stability of the 1 → 6 glycosidic bond in pullulan is lower than the one of the 1 → 4 glycosidic bond in cellulose, which would lead to dissociation of the bonds, regardless of the atmosphere, predominantly via the transglycosidation mechanism of the less stable bond.

The rate constants for the pullulan of the initial peak molecular weight 5800 g mol^{-1} could not be calculated, as $\bar{M}_w$, in both oxygen and nitrogen, appears to increase throughout the duration of the experiments, as can be seen in Fig. 3. Interestingly, the changes in number and weight average molecular weights for all pullulans seem to follow the same pattern, which confirms the existence of the so-called limiting value DP. After long times of degradation all materials converge to this value-in the case of pullulan of initial $M_p = 5800$ g mol^{-1}, this is demonstrated by the steady increase of $\bar{M}_w$.

Convergence to the limiting DP and equality of degradation rates (Table 1) are features common to all pullulan samples regardless of the initial molecular weight. The limiting DP values are 20 ± 3 in O_2 atmosphere and 22 ± 2 in N_2 atmosphere. The values are statistically not different, and on the other hand, they are very different to those usually estimated for cellulose (150–200). A possible explanation for the small limiting DP values of pullulan may be a small size of crystallites, which are known to decompose only after very long times of degradation.

Similar to cellulose (Emsley et al., 2000), formation of typical fragments takes place during degradation of pullulans. From the data presented in Table 2 it can be concluded that the typical fragment molecular weight is independent of the initial molecular weight of the polymer and in the case of pullulan, also on the atmosphere used. This is consistent with the hypothesis that transglycosidation is the predominant degradation reaction at the conditions used. Furthermore, only preferential chain-scission and inter-fragment cross-linking can lead to the formation of the typical fragments. That inter-fragment cross-linking during the advanced period of degradation (period c, Fig. 2) indeed takes place can be concluded in the case of pullulans of initial M_p lower than the M_4 (Table 2).

During the initial period of cellulose degradation at 180 °C, examined in (Strlič, Kolar, Pihlar, Rychlý, & Matisová-Rychlá, 2001), the presence of carbonyl groups was supposed to play an important role. In fact, from Fig. 3, it can be seen that the extent of initial cross-linking of pullulan during the period a (well seen in graphs of $\bar{M}_w$ vs. t) depends on the starting concentration of carbonyl (aldehyde) groups. The results presented in Fig. 3 suggest, that the presence of oxidised (carbonyl and aldehyde) groups formed during degradation in oxygen atmosphere leads to a more pronounced formation of cross-links (possibly also to the inter-fragment cross-linking) after long times of degradation (period c), as opposed to degradation in nitrogen atmosphere, where cross-linking occurs only during the very initial period of heating, when aldehyde

Table 1
First order degradation rate constants for pullulan samples of different initial peak molecular weight calculated for degradation phase b (as delimited in Fig. 2) for nitrogen (k_{N_2}) and oxygen (k_{O_2}) atmosphere

	M_p (g mol^{-1})				
	24,000	48,000	100,000	380,000	1,600,000
k_{N_2} (mol mol$^{-1}_{monomer}$ min^{-1})	2.2×10^{-6}	2.3×10^{-6}	2.4×10^{-6}	2.2×10^{-6}	2.3×10^{-6}
k_{O_2} (mol mol$^{-1}_{monomer}$ min^{-1})	1.8×10^{-6}	2.4×10^{-6}	2.6×10^{-6}	2.7×10^{-6}	2.7×10^{-6}

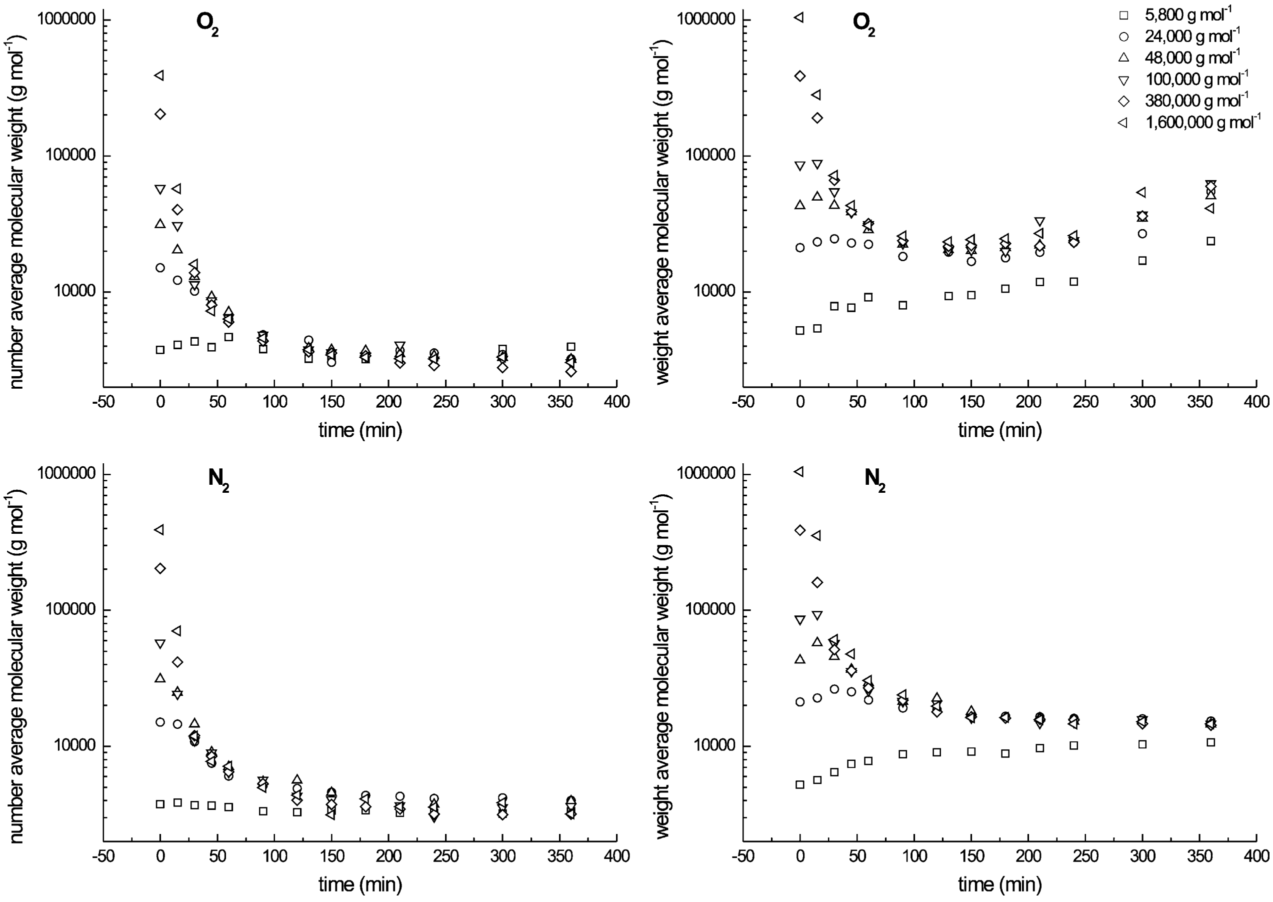

Fig. 3. Number and weight average molecular weights of pullulan standards of different initial peak molecular weights (see legend), thermally degraded at 180 °C; in nitrogen and oxygen atmosphere.

groups from the starting material are still present. None of the degradation reaction mechanisms so far proposed for thermal degradation of polysaccharides can account for this phenomenon.

3.2. Degradation experiments in air at 80 °C, 65% RH

For the lifetime prediction of library and archival materials, experiments at lower temperatures (< 100 °C) are especially interesting, as it has been shown that the presence of atmospheric moisture may have a strong effect on the degradation rates (Kleinert & Marraccini, 1963; Zou et al., 1994).

In the presence of moisture, pH of the macromolecular environment is very important. Pullulan standards were therefore applied on glass filter sheets, which were previously immersed into solutions of $Ca(HCO_3)_2$ and dried. The presence of small amounts of $CaCO_3$ should

Table 2
Positions of peaks/shoulders in the mass distribution after 360-min degradation of pullulan samples of different initial peak molecular weight in nitrogen and oxygen atmosphere

M_p (g mol^{-1})	O_2				N_2
	M_1 (g mol^{-1})	M_2 (g mol^{-1})	M_3 (g mol^{-1})	M_4 (g mol^{-1})	M_5 (g mol^{-1})
5800		6500	17,000		16,200
24,000	5200	5500	17,900	200,000	18,100
48,000	4800		17,600	195,000	17,200
100,000	4700		18,400	220,000	18,200
380,000	4560	6800	19,000	215,000	18,500
1,600,000	4870		17,300	240,000	18,000
Average (g mol^{-1})	4830		17,900	214,000	17,700
R.S.D. (g mol^{-1})	240		740	17,800	850

ensure the pH of the environment of ~8, as is usual in experiments with cellulose (Kolar, Strlič, Novak, & Pihlar, 1998). An excess of the alkali further ensures that the acidic components, formed during degradation, are neutralised.

During the 13-day degradation period at 80 °C, the MWDs of pullulan samples remain mono-modal (Fig. 4). Furthermore, there is no evidence of cross-linking so that Ekenstam's model can be used and slopes of the lines DP^{-1} vs. t correspond to first order degradation constants.

However, in the case of pullulans of lower peak molecular weights, the DP^{-1} vs. t plots are composed of two distinct parts; during an initial period the degradation proceeds faster. During later stages of degradation, the rates decrease; this phenomenon may indicate that a steady state concentration of aldehydes has been achieved.

A comparison of initial rates is shown in Fig. 5: they correlate with the concentration of end-groups (i.e. aldehyde groups) in the starting material, in a linear manner: $k = 2.3 \times 10^{-8} + 1.1 \times 10^{-5} \times c$, $R = 0.9821$. The linear fit transforms into the curve in Fig. 5 due to the logarithmic nature of the axes. A similar correlation between the content of carbonyl groups and the rate of oxidative degradation has recently been shown also for cellulose (Malešič et al., 2002). Although pH-dependence was also demonstrated, the actual reaction mechanism in force during oxidative degradation of aldehyde groups affecting the rate of chain scission in polysaccharides remains uncertain.

Simple β-alkoxy elimination, i.e. 'peeling-off' (Nevell & Zeronian, 1985), cannot account for the degradation, as in order for extensive chain cleavage to take place, a reactive chemical species of considerable mobility should be an intermediate. Arts, Mombarg, van Bekkum, and Sheldon (1997) have reviewed several possible mechanisms of oxidation of carbohydrates with atmospheric oxygen. After an initial isomerisation of the aldehyde group into an enediol, the production of both $O_2^{\cdot-}$ and $RO{-}O^{\cdot}$ takes place. Superoxide anion ($O_2^{\cdot-}$) is an oxygen radical of low reactivity and therefore high mobility while disproportionation of its conjugated acid ($HO_2^{\cdot}$) leads to the formation of H_2O_2. In general, peroxides are known to initiate the autoxidative chain reaction route, which may lead to extensive degradation of organic materials, including chain scission (Lazár, Rychlý, Klimo, Pelikan, & Valko, 1989), cf. Fig. 5. Under certain conditions, homolytic scission of the reactive peroxide bond may lead to reactive oxygen radicals, such as hydroxyl radical ($HO^{\cdot}$). Furthermore, the decomposition may be catalysed by transition metals in a Fenton-type reaction. The existence of hydroxyl radicals during oxidative degradation of cellulose was demonstrated recently (Kolar, Strlič, & Pihlar, 2001) and their concentration was shown to correlate with polysaccharide chain-scission.

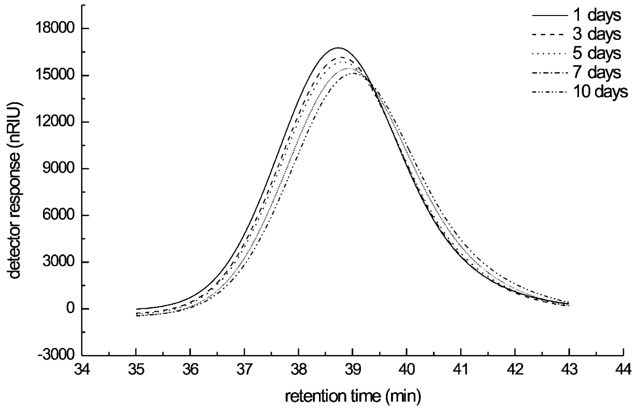

Fig. 4. Chromatograms of a pullulan sample of the initial peak molecular weight 186,000 g mol^{-1} during degradation experiment in air, 80 °C, 65% RH.

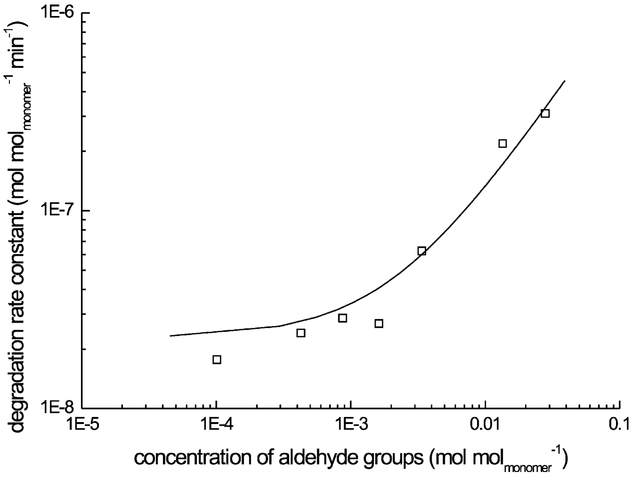

Fig. 5. The first order degradation rate constants (in mol $mol_{monomer}^{-1}$ min^{-1}) for pullulan samples of different initial peak molecular weight calculated for experiments in air, 80 °C, 65% RH. The line represents a linear fit of the experimental data in the log–log scale.

However, we are still lacking a correlation between aldehyde concentration and peroxide formation. Since reactions of peroxides are usually highly energetic, some of the excess energy is released in the form of light, which can be measured.

3.3. Chemiluminescence measurements

Measurements of chemiluminescence have been shown to be particularly convenient in the studies of oxidative degradation, also of cellulosic materials. If a pre-oxidised sample is subjected to a steady increase of temperature in an inert atmosphere, a chemiluminescent peak was shown to correlate with the concentration of peroxide groups in the material (Strlič et al., 2000). A peroxide peak is typical also of pullulan samples, pre-oxidised in oxygen atmosphere at 100 °C for 60 min (Fig. 6). Such a pre-treatment should be sufficient to allow a steady state concentration of peroxides to be built up (Kočar, Strlič, Kolar, & Pihlar, 2002).

The pullulan samples were prepared in the form of thin films (mass of sample 1 mg), cast from aqueous solutions, in order to ensure a homogeneous surface of the material. The signal obtained in experiments similar to the one in Fig. 6 is

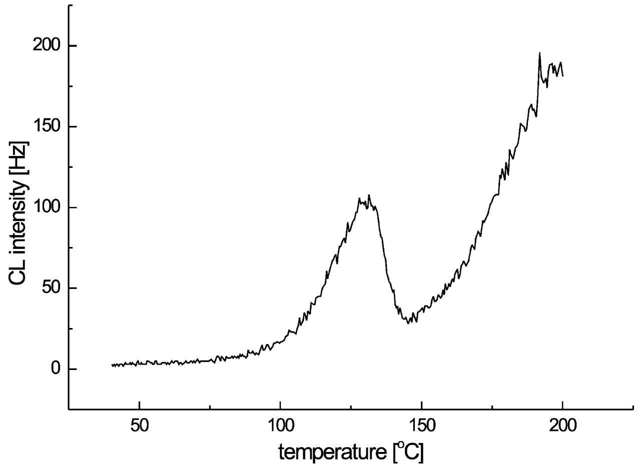

Fig. 6. Dynamic chemiluminescence experiment (rate of temperature increase: 2.5 °C min^{-1}) in nitrogen atmosphere with a pre-oxidised (60 min, O_2, 100 °C) pullulan sample (1 mg) of peak molecular weight 5800 g mol^{-1}.

a consequence of two types of reactions, decomposition of peroxides and transglycosidation (Strlič et al., 2000). Assuming that both processes are mono-molecular and independent, experimental data in Fig. 6 can be modelled and the peroxide peak area may be calculated using non-linear regression (Rychlý, Rychlá, & Strlič, 2000). By performing the experiments with the pullulan samples of different initial peak molecular weights, a correlation with the corresponding peroxide concentrations, as represented by the respective calculated peak areas, can be obtained (Fig. 7). It is evident that a higher initial concentration of aldehyde groups leads to a higher concentration of peroxide groups after the material is exposed to oxidative atmosphere and elevated temperature. The peroxides in question may be either low molecular weight (e.g. H_2O_2) or macromolecular (hydro-)peroxides.

As just demonstrated, the carbonyl-assisted formation and decomposition of peroxides is probably the key mechanism leading to oxidative degradation of polysaccharides in general. Since it is accompanied by intensive chemiluminescence, a deeper knowledge of the mechanism is of high interest in order to further explore the use of chemiluminescence measurements and the possibilities of material stabilisation.

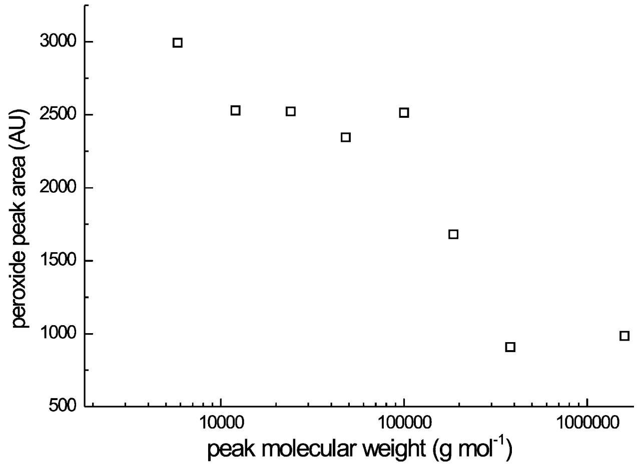

Fig. 7. Peroxide peak areas for pre-oxidised (60 min, O_2, 100 °C) pullulan samples of different initial peak molecular weight.

4. Conclusions

Degradation experiments with pullulan samples of narrow MWD and different initial peak molecular weights revealed several interesting features, some of which may also be applied to the degradation studies of other polysaccharides, in general. At higher temperatures (180 °C):

- the degradation process is not random, as evidenced by the multi-modal distribution of the degraded material and formation of typical fragments after long times of degradation;
- formation of cross-links is related to the content of carbonyl groups. In oxygen atmosphere, it is the most prominent feature after long reaction times, however, during a short initial degradation period the extent of cross-linking depends on the molecular weight of the starting material (content of aldehyde groups);
- a short degradation period was identified, during which the validity of the Ekenstam's model was demonstrated. The obtained rate constants for degradation in oxidative and inert atmosphere were similar;
- the existence of limiting DP of 20-22 was demonstrated, regardless of the atmosphere used.

At lower temperatures (80–100 °C), some additional features were observed:

- the rate of oxidative degradation depends on the initial content of aldehyde groups. Contrary to higher-temperature experiments, no cross-linking was observed and the degradation remains random, as no peaks in the MWDs could be identified;
- a correlation between the concentration of aldehyde end-groups in the pullulan samples and content of peroxides after a pre-oxidation treatment was demonstrated using chemiluminescence measurements.

The relation between the initial content of carbonyl (aldehyde) groups, concentration of peroxides in the polysaccharide material, and its stability towards oxidative degradation is the topic of our future research.

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