Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1209–1216
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Alkaline degradation kinetics and CE-separation of cello- and xylooligomers. Part I

Jürgen Sartori,[a] Antje Potthast,[a] Alois Ecker,[b] Herbert Sixta,[b] Thomas Rosenau,[a] Paul Kosma[a,*]

[a]*Christian-Doppler-Laboratory, Institute of Chemistry, University of Agricultural Sciences, Muthgasse 18, A-1190 Vienna, Austria*
[b]*Research & Development, Lenzing AG, Lenzing, Austria*

Received 9 January 2003; received in revised form 3 March 2003; accepted 6 March 2003

Abstract

The degradation kinetics of cello- and xylooligomers under alkaline conditions has been studied, and kinetic order, kinetic rate constants as well as activation parameters (E_A, $\Delta E^{\#}$, $\Delta S^{\#}$) for the different oligomers have been determined. The results were corroborated by a mathematical model of the degradation kinetics. A reliable and convenient method for the separation and simultaneous quantification of cello- and xylooligomers, based on capillary electrophoresis (CE) with pre-column derivatization, has been established. *p*-Aminobenzonitrile was used as the UV tag, and 550 mM borate buffer containing hexadimethrine bromide was employed as the running electrolyte.

Keywords: Cellooligosaccharides; Xylooligosaccharides; Alkaline degradation; Peeling; Capillary electrophoresis

1. Introduction

Reaction of cellulose with strong alkali is an integral part of the manufacturing process of many cellulosic fibers and cellulose derivatives. Sodium hydroxide is mainly used as the alkali source; it fulfils multiple functions as a reagent, swelling agent, and reaction medium. In most large-scale processes for derivatization of cellulose, for instance etherification, or viscose production, the conversion of native cellulose into 'sodium cellulose I' is the key step. Apart from being used in these derivatization reactions, treatment with strong aqueous alkali is employed also for purification of dissolving pulps: hemicelluloses and low-molecular weight celluloses are extracted into sodium hydroxide solutions at different temperatures (hot or cold caustic extraction).

The interaction of cellulose and alkali generally encompasses both physical and chemical processes. The former relate to the heterogeneous transformation of cellulose with morphological changes, such as strong lateral swelling and a change in lattice dimensions in ordered regions above a specific lye concentration.[1] The chemical transformations can be classified into processes starting from the reducing end ('peeling') and reactions affecting the carbohydrate statistically, i.e., at random locations along the polymer chain.

The 'classical peeling reaction'[2–5] causes a stepwise shortening of the polymer from the reducing end. A polymer chain results, which is shortened by one carbohydrate unit and again carries a reducing end group. 'Non-classical peeling' or 'oxidative peeling' effects essentially the same changes, but without regeneration of reducing ends. Both classical and non-classical peeling cause a relatively slow, i.e., stepwise, decrease in molecular weight.

In the presence of oxygen, a second set of reactions becomes more prominent. A considerable chain degradation of the alkali cellulose occurs, which is used for shaping the molecular weight distribution of the cellulose for subsequent derivatization, e.g., in viscose manufacturing. This degradation comprises a number of homo- and heterolytic steps, which are highly dependent on the structure of the material used (cellulose or hemicellulose). In contrast to peeling, these reactions

* Corresponding author. Tel.: +43-1-360066055; fax: +43-1-360066069
E-mail address: pkosma@edv2.boku.ac.at (P. Kosma).

0008-6215/03/$ - see front matter
doi:10.1016/S0008-6215(03)00115-0

occur randomly along the carbohydrate chain, causing chain cleavage and fast, drastic changes in the degree of polymerization (DP) and thus in the molecular weight distribution.

Alkaline treatment of reducing sugars in aqueous solution causes isomerization known as the Lobry de Bruyn-Alberda van Ekenstein rearrangement.[6] The intermediate formation of an enediol anion species in this process has generally been accepted.[7–9] The elimination of a hydroxyl group—in the case of monomeric reducing sugars—or an alkoxyl group—in the case of cellooligosaccharides or cellulose—from the β-position to the anionic intermediate leads to the formation of an α-dicarbonyl compound, which is extremely unstable under alkaline conditions being subjected to various degradation reactions, such as benzilic acid rearrangements. The enolization, being the rate-determining step, is increased by higher OH^- concentrations and by the addition of calcium.[10,11] Lai proposed a dianionic intermediate that causes accelerated degradation of fructose at higher sodium hydroxide concentrations.[12] Mechanistic aspects of the alkaline degradation of cellulosic materials have mainly been addressed by research groups in the 1950s and 1960s.[13–15] Chain cleavage was shown to be predominantly caused by introduction of keto groups at C-2 followed by β-elimination. Already in 1949, Entwistle et al. proposed a radical chain mechanism for the aging of alkali cellulose,[16,17] while MacDonald put forward a combination of hetero- and homolytic processes as mechanistic explanation.[6] The formation of hydrogen peroxide during aging of alkali cellulose under oxygen was demonstrated,[18] as well as the formation of a number of low-molecular weight acids. However, the direct involvement of radical species has not been definitely proven so far, although Kolar et al.[19] have recently demonstrated the presence of hydroxyl radicals for a very similar system—alkaline paper sheets.

In the present paper, we report a comprehensive kinetic study on the degradation of cello- and xylooligomers under strongly alkaline conditions, such as those relevant for industrial alkalization steps in the derivatization of cellulose or in the viscose process. In contrast to the alkalization of cellulose, which is a heterogeneous reaction, the degradation experiments of the oligosaccharides were performed in homogeneous solution, which is a prerequisite to subsequent capillary electrophoresis (CE) analysis.

2. Experimental

2.1. Chemicals

All reagents used were of analytical grade and were obtained from Sigma–Aldrich (Germany), Fluka (Switzerland) or J.T. Baker (Holland). Xylotriose and xylotetraose were purchased from Megazyme Corp. (Ireland). Cellooligosaccharides of different chain lengths have been synthesized by acetolysis of cellulose followed by preparative column chromatography and Zemplén deprotection of the acetates.[20] Since Zemplén deprotection is carried out in alkaline media, formation of byproducts from alkaline degradation cannot be avoided so that further purification by preparative HPLC (10:1:1 (v/v/v) acetone–$CHCl_3$–water) was necessary. NMR assignments of the purified cellooligosaccharides were in full accordance with the literature.[21]

2.2. Buffer solutions

The running electrolyte was prepared by dissolving 1,5-dimethyl-1,5-diaza-undecamethylene polymethobromide (hexadimethrine bromide (HDB), 0.001% w/v), 550 mM boric acid, 5% MeOH and 5% 1-propanol in purified water. The pH was adjusted to 10.7 with 3 M KOH.

2.3. Capillary electrophoresis

For CE measurements, a HPCE-3D instrument (Agilent Technologies) equipped with a capillary column ($L = 64$ cm; $l = 56$ cm $\times$ 50 μm) and UV–DAD detector was used. The capillary was kept at 15 °C throughout. The run current was set to -100 μA. Preconditioning of a new column was performed by flushing with 1.0 M aq NaOH for 10 min. Between the runs, the capillary was conditioned by flushing with electrolyte for 8 min. The derivatized oligosaccharides were detected at 280 nm, the detector being placed 8 cm from the anodic end of the column. Hydrostatic injection was performed by applying 50 mbar for 8 s.

2.4. Derivatization

The oligosaccharide samples were derivatized by reductive amination with 4-aminobenzonitrile (ABN) under slightly acidic conditions.[22] A fresh stock solution for derivatization was prepared daily by dissolving 200 mg of $NaCNBH_3$ and 1.2 g of ABN in a mixture of MeOH (13 mL), water (10 mL), and acetic acid (2 mL). This solution (400 μL) was added to the respective samples, which contained approx 200 μg of reducing sugars. The mixture was kept at 60 °C for 90 min. After cooling to room temperature the reaction mixture was filtered and analyzed by CE without further purification.

2.5. Quantitation

To achieve optimum reproducibility of the quantification, an internal standard was used. Galactose proved to be advantageous as its peak was clearly separated

from all other cello- and xylooligomer signals. A galactose stock solution was prepared by dissolving 50 mg of D-galactose in 20 mL of water. Prior to derivatization, 21 μL of this solution was added to each sample.

2.6. Kinetic studies

The alkalization mixture was kept at constant temperature in a water bath (20, 30, 43, and 50°, respectively). Thermostated NaOH (18% w/w) was added to approx 3.5 mg of the respective oligosaccharide resulting in a total sugar concentration of 4%.[23] After complete dissolution of the oligosaccharides, samples of 10 μL were taken every 10 min. Addition of internal standard, derivatization and CE analysis were carried out as described above.

Table 1
Limit of detection (LoD) and limit of quantification (LoQ) of different model compounds determined by the CE method

Carbohydrate	LoD [a]	LoQ [a]
Glucose	2.8	9.2
Cellobiose	5.1	17.3
Cellotriose	8	27
Cellotetraose	11	37
Cellopentaose	13	45
Xylose	1.6	5.2
Xylobiose	6.5	21
Xylotriose	7.1	23
Xylotetraose	8.1	26
Galactose	3.1	10.4

[a] In μg mL^{-1}.

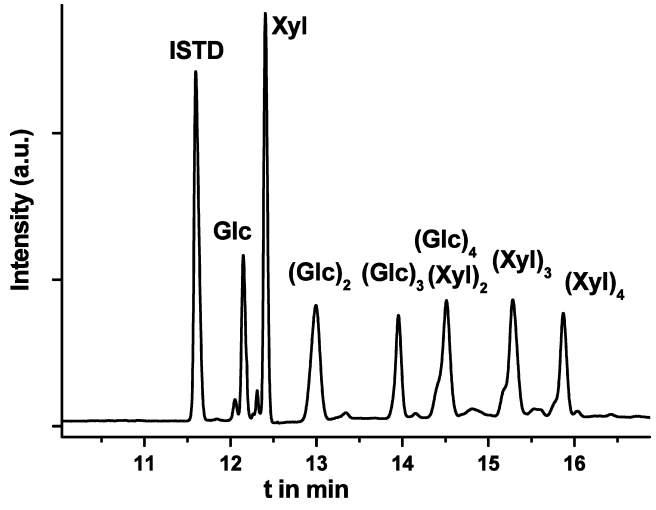

Fig. 1. Electropherogram of a mixture of derivatized cello- and xylooligosaccharides. ISTD, internal standard (D-galactose).

3. Results and discussion

3.1. Separation of oligosaccharides by CE

HDB as electroosmotic flow (EOF) modifier[22] as well as high borate concentrations[24] were employed as these conditions were shown to be highly effective in improving the peak separation and the height-to-width relation of the peaks. Since the electrophoretic mobility increases with improved borate complexation (as the net negative charge increases) and decreases with the size of the oligomers, small oligomers elute first followed by the larger ones. In order to extend the lifetime of the capillary by minimizing Joule heating, CE runs were performed in the constant current mode at -100 μA.

A pre-column derivatization technique selective for reducing end groups was chosen as the reporter method. Ethyl 4-aminobenzoate and 4-aminoquinoline[25] were tested as derivatizing agents in reductive aminations, however, ABN derivatives gave optimum results. Under the optimized conditions as given in the experimental part, the derivatization proceeded reproducibly. Dahlman and coworker[25] have already achieved CE-separation of xylooligosaccharides, but separation of cellooligosaccharides has not been reported so far. The limit of detection (LoD; 3σ) and limit of quantification (LoQ; 10σ) were determined for each cello- and xylooligomer investigated. Both parameters were sufficiently low to guarantee a reliable detection in kinetic studies (Table 1).

With the optimized procedure, neat baseline separation of ABN derivatives of cellooligosaccharides up to celloheptaose (DP 7) and of xylooligosaccharides up to xylotetraose (DP 4) was achieved. Because of the comigration, cellotetraose and xylobiose could not be separated under the conditions used. For oligosaccharides with a higher DP, the solubility in the electrolyte was decreasing drastically, so that mere detection was still feasible, but reliable quantitation was rendered impossible. Therefore, all degradation experiments were carried out with oligomers of a DP 5 or smaller. Fig. 1 gives a representative electropherogram.

3.2. Alkaline degradation of cello- and xylooligomers

The kinetics of the alkaline degradation of cellopentaose and of intermediate smaller cellooligosaccharides at 43 °C is given in Fig. 2. Due to the detection mode, which monitors only intermediates having reducing end groups, neat chromatograms were obtained. Formation of stabilized oligosaccharides, e.g., with carboxylic end groups, contributes also to the consumption of oligosaccharide material, however, as all those electrophoretically mobile compounds without aldehyde groups were blinded out, overcrowding of the electropherogram was avoided, which made recording and evaluating the kinetics very straightforward.

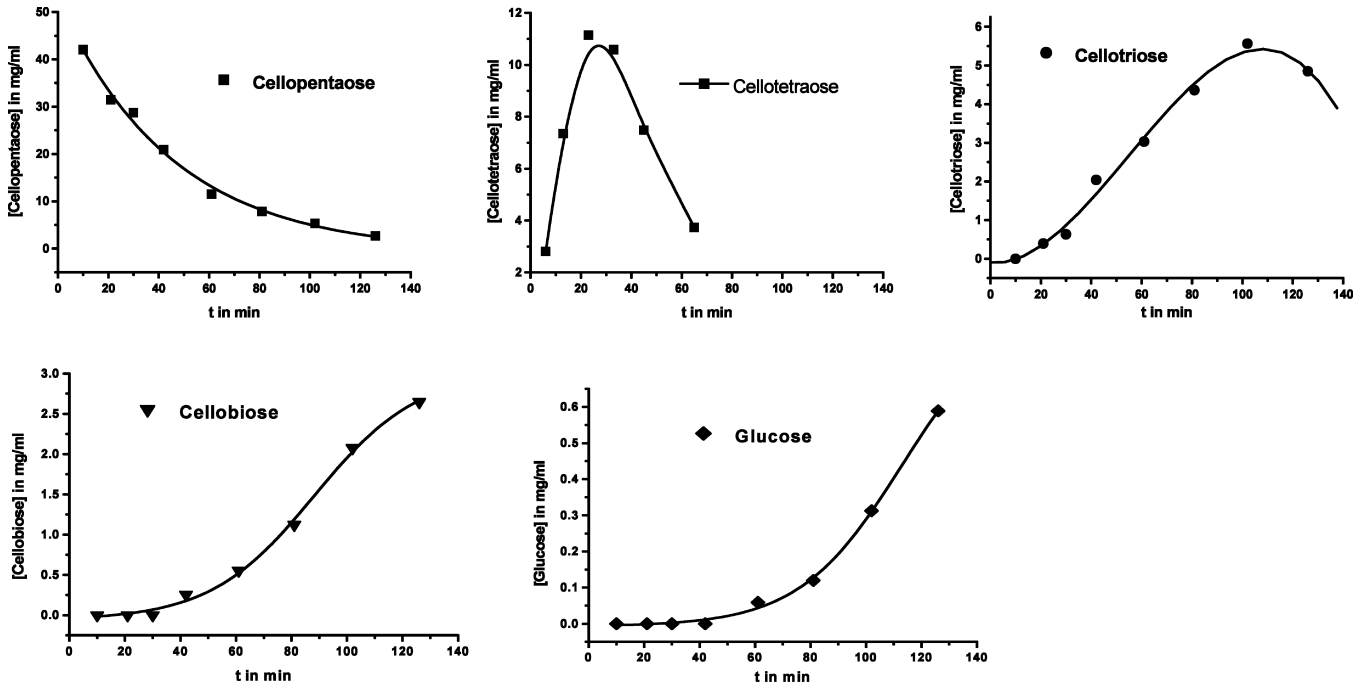

Fig. 2. Kinetics of the alkaline degradation of cellopentaose and -oligosaccharide intermediates.

The degradation of cellopentaose (CP), and analogously degradation of all other oligomers investigated, proceeded according to the general rate law $d[CP]/dt = k[OH^-][CP]$, from which a pseudo-first-order rate law followed under the assumption of a very large OH^- concentration, which was regarded constant over the reaction time: $d[CP]/dt = k'[CP]$, with $k' = k[OH^-]$. This rate law, experimentally found for cellopentaose as well as for all other oligomers employed, was confirmed according to the substitution method. In this approach, the concentration values at specific times were evaluated according to different integrated rate laws, resulting in different plots. Linearization and regression analysis of these graphs allowed evaluating the reaction order. The concentration profiles of smaller oligomers formed during the reaction—cellotetraose down to glucose—gave the typical curve shapes of intermediates in sequential reactions.

The degradation rate for different cello- and xylooligomers increased from the monosaccharide over the biose to the triose, see Fig. 3. From a DP of 3 on, the values of the kinetic rate constant k decreased slightly. The trioses degraded about twice as fast as the respective monomeric sugars. These relations apply to cellooligosaccharides as well as to xylooligosaccharides. For the latter, the absolute k values were slightly higher.

Recording the kinetics at four different temperatures allowed determining the activation parameters of the alkaline degradation. The respective Arrhenius plots are shown in Fig. 4. The course of the activation parameters was similar for cello- and xylooligosaccharides: the activation energies were comparable—slightly increasing with increasing DP—, while the activation entropy became slightly less negative, which was reasonable as the degree of order in the transition state was supposed to decrease with increasing chain length (Table 2).

The kinetics demonstrated unambiguously that the alkaline degradation proceeded through stepwise shortening by one carbohydrate unit. For instance, $(Glc)_3$ was first detected after $(Glc)_4$ had been formed, and $(Glc)_2$ was found only after the formation of $(Glc)_3$, and so on. No statistical chain breakage was detected:

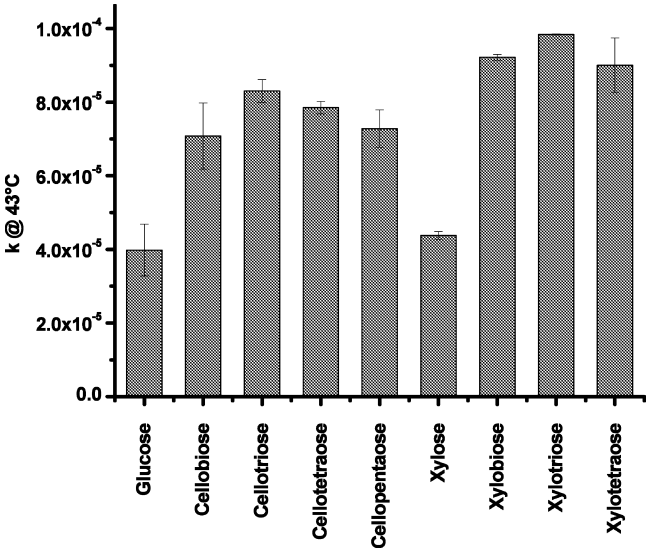

Fig. 3. Kinetic rate constants k in $s^{-1}\ M^{-1}$ for the degradation of cello- and xylooligomers at 43 °C in 18% NaOH.

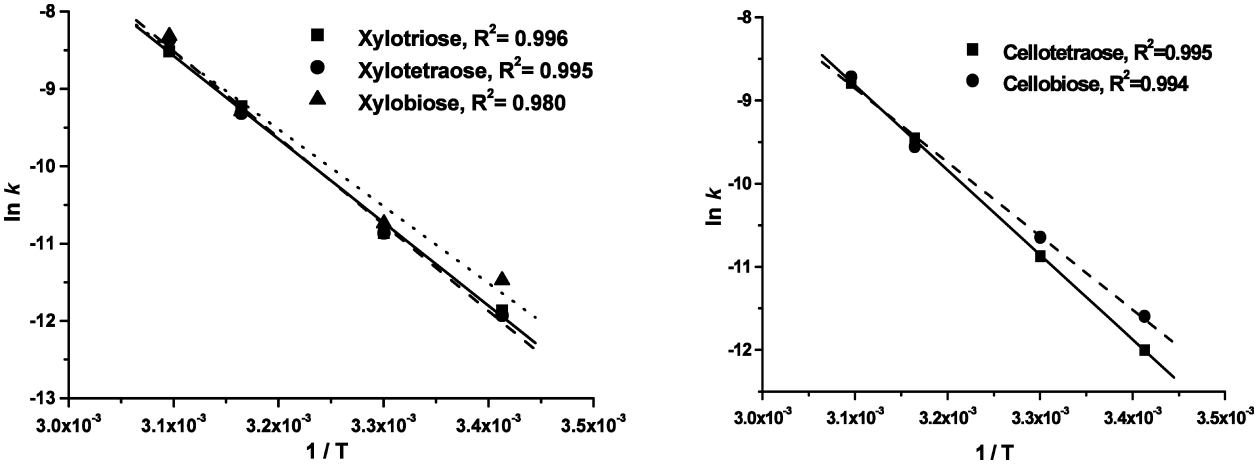

Fig. 4. Arrhenius plots for xylo- and cellooligomers, cf. Table 2.

in this case fragments, such as glucose, cellobiose and cellotriose, were supposed to occur right from the beginning of the reaction, which was not observed.

Apart from the differentiation between endwise peeling reactions and random chain cleavage, also the relation between 'classical' and 'non-classical' peeling can be evaluated because of the detection mode chosen which only registers reducing end groups, see Fig. 5. About 60% of the consumed cellopentaose was degraded according to the classical peeling pathway, which means that the resulting oligosaccharides carry again reducing end groups so that the sequence cellotetraose–cellotriose–cellobiose is passed through along the conversion of cellopentaose to glucose as the final detectable product. The remaining part of starting material was disintegrated by 'non-classical' peeling. Here, the oligosaccharide intermediates have no reducing end groups and are, thus, not detectable. The reducing ends are either converted to carboxylic acid groups and lactone structures by oxidation, or epimerized into ketose moieties. Ketoses are not labeled by the present approach. A complementary method reporting also keto sugars, which is based on oximation with subsequent reduction, is currently being developed.

The ratio of 'classical' to 'non-classical' peeling, slightly increased over time (Fig. 6). While classical peeling proceeds exclusively according to ionic processes, such as tautomerization, rearrangements and eliminations,[†] non-classical peeling comprises two different sets of reactions: first, processes under involvement of molecular oxygen that have at least one homolytic step, such as oxidation of C-2 or carboxylic acid formation from an aldehyde, and second, epimerization of the reducing end to ketose structures. None of the products of the two reaction sets are detected by the reductive amination method chosen. The ratio between classical and non-classical degradation processes was roughly equal to 1 at room temperature and 30 °C,

Table 2
Activation parameters for different cello- and xylooligomers, temperature range from 30 to 70 °C

Carbohydrate	E_A (kJ mol^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (J (mol K)$^{-1}$)
Cellobiose	73.7	71.0	−98.9
Cellotetraose	84.6	82.1	−64.3
Xylobiose	82.4	79.9	−68.6
Xylotriose	89.5	86.9	−47.3
Xylotetraose	93.0	90.5	−35.7

E_A was obtained from a regression analysis according to the Arrhenius equation, $\Delta H^{\#}$ and $\Delta S^{\#}$ according to the Eyring equation.

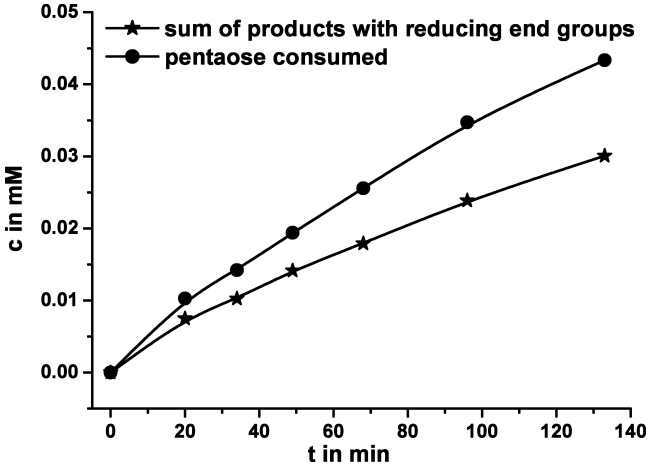

Fig. 5. Alkaline degradation of cellopentaose at 30 °C: comparison of consumed cellopentaose with generated peeling products having reducing end groups.

[†] Also the so-called 'stop reaction', which produces saccharinic acid residues and interrupts the peeling process, is a heterolytic process, although it does not generate new reducing ends.

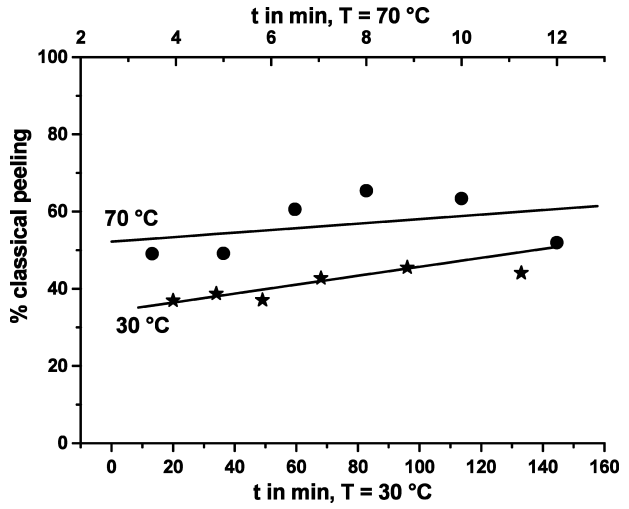

Fig. 6. Ratio between 'classical' and 'non-classical' (oxidative) peeling at two different temperatures.

but increased with increasing reaction temperature (Fig. 6). It is known that alkali treatment of simple monosaccharides at elevated temperatures causes formation of extremely complex mixtures of furanoid, benzoid and anellated aromatics, many of them being activated phenols and thus potent antioxidants.[26,27] These products, progressively formed with increasing temperature, counteract homolytic degradation pathways and thus reduce the non-classical, homolytic peeling processes, which might explain the observed temperature dependence.

3.3. Mathematical model of the alkaline degradation of cellooligomers

For the alkaline degradation of cellopentaose a model was chosen, which would agree with the chemical processes, reflecting the observed concentration profiles as closely as possible.‡ For starting material and intermediates (index N) generally degradation according to first-order kinetics was assumed, with the rate constants k_N:

$$\frac{dc_N}{dt} = -k_N c_N \tag{1}$$

The time course of the concentration is then given by:

$$c_N = c_N(0)\, e^{-k_N t} \tag{2}$$

Now, the first degradation product (index $N-1$) is formed, with only a part g of the starting compound N forming the degradation product $N-1$, from the remaining starting material byproducts are produced. The kinetics of these degradation steps are given by Eq. (3), assuming a first-order course throughout:

$$\frac{dc_{l-1}}{dt} = -k_{l-1}c_{l-1} + g_l k_l c_l \quad l = 2, \ldots, N \tag{3}$$

This system of differential equations can be solved in the case of geminately different constants k_l by the approach:

$$c_l = \sum_{i=l}^{N} \alpha_l^i\, e^{-k_i t} \tag{4}$$

Due to the linearity of the equations follows from Eq. (3):

$$c_{l-1} = e^{-k_{l-1}t}\left(c_{l-1}(0) + \int_0^t g_l k_l c_l(\tau)\, e^{k_{l-1}\tau}\, d\tau\right) \tag{5}$$

which by substitution of Eq. (4) and subsequent integration yields:

$$c_{l-1} = e^{-k_{l-1}t}c_{l-1}(0) + g_l k_l \sum_{i=l}^{N} \frac{\alpha_l^i}{k_{l-1}-k_i}\left(e^{-k_i t} - e^{-k_{l-1}t}\right) \tag{6}$$

Comparing coefficients with Eq. (4) produces equation set (7):

$$\alpha_N^N = c_N(0) \tag{7a}$$

$$\alpha_{l-1}^i = g_l k_l \frac{\alpha_l^i}{k_{l-1}-k_i} \qquad l = N, \ldots, 2, \quad i = l, \ldots, N \tag{7b}$$

$$\alpha_{l-1}^{l-1} = c_{l-1}(0) - \sum_{i=l}^{N} \alpha_{l-1}^i \tag{7c}$$

From these equations, the rate constants were determined by an iteration procedure, so that the calculated concentration profiles gave the closest possible match with the experimental curves. The fit of the concentration profiles for the degradation of cellopentaose and the cellooligosaccharide intermediates is shown in Fig. 7.§

The calculated kinetic rate constants at 43 °C for the oligomeric sugars—cellopentaose down to cellobiose—were found to be nearly equal: $k_5 \cong k_4 \cong k_3 \cong k_2 = (3.83 \pm 0.33) \times 10^{-4}\ s^{-1}$, the rate constant for the degradation of the monomer glucose being half of that for the oligomers: $k_1 = (2.00 \pm 0.05) \times 10^{-4}\ s^{-1}$.

From the mathematical evaluation of the degradation experiments at different temperatures, also the

‡ Mathematical approach and complete data sets can be obtained from the authors upon request.

§ Also another mathematical model, assuming the formation of relatively stable intermediates, such as for instance ketoses, between the different cellooligosaccharide stages was applied. Here, cellooligosaccharide $N-1$ is not directly formed from N, but via an intermediate. Using this model, the amount of 'non-classical peeling' was calculated to be much smaller: 40% at 30 °C and below 10% at 70 °C. The model thus assumes that the major part of compounds, which have no reducing end groups and are therefore not reported by the method used, is still present—as a quasi reservoir—in the intermediate stage, from which they can later form the resulting cellooligosaccharide.

activation energy E_A according to Arrhenius was accessible. The value of $E_A = 85.4 \pm 3.9$ kJ mol^{-1} at a 95% confidence, being the same for cellopentaose, cellotetraose, cellotriose and cellobiose, is in good agreement with the experimental values in Table 2.

In summary, alkaline degradation of cellooligomers dissolved in 18% NaOH proceeded according to pseudo-first-order rate laws. Direct chain cleavage, also in the presence of oxygen, was not at all a dominant process. This is a major difference to the heterogeneous reaction in the case of alkali-treated cellulose, for which direct chain cleavage is a major process. Activation parameters showed that the degradation of xylooligosaccharides proceeded somewhat faster than the degradation of cellooligosaccharides of the same chain lengths. At room temperature, 'classical peeling' was slightly favored over 'non-classical peeling', which is composed of homolytic oxidative side reactions and epimerization to ketoses, having approx a 60-to-40 ratio. This ratio was shifted in favor of the classical peeling processes at elevated degradation temperatures. Mathematical modeling of the chemical system according to a complex of parallel first-order reactions was in good agreement with the experimental data. The kinetic rate constants of oligomeric reactants were found to be nearly equal and about twice as large as that of the monomer glucose.

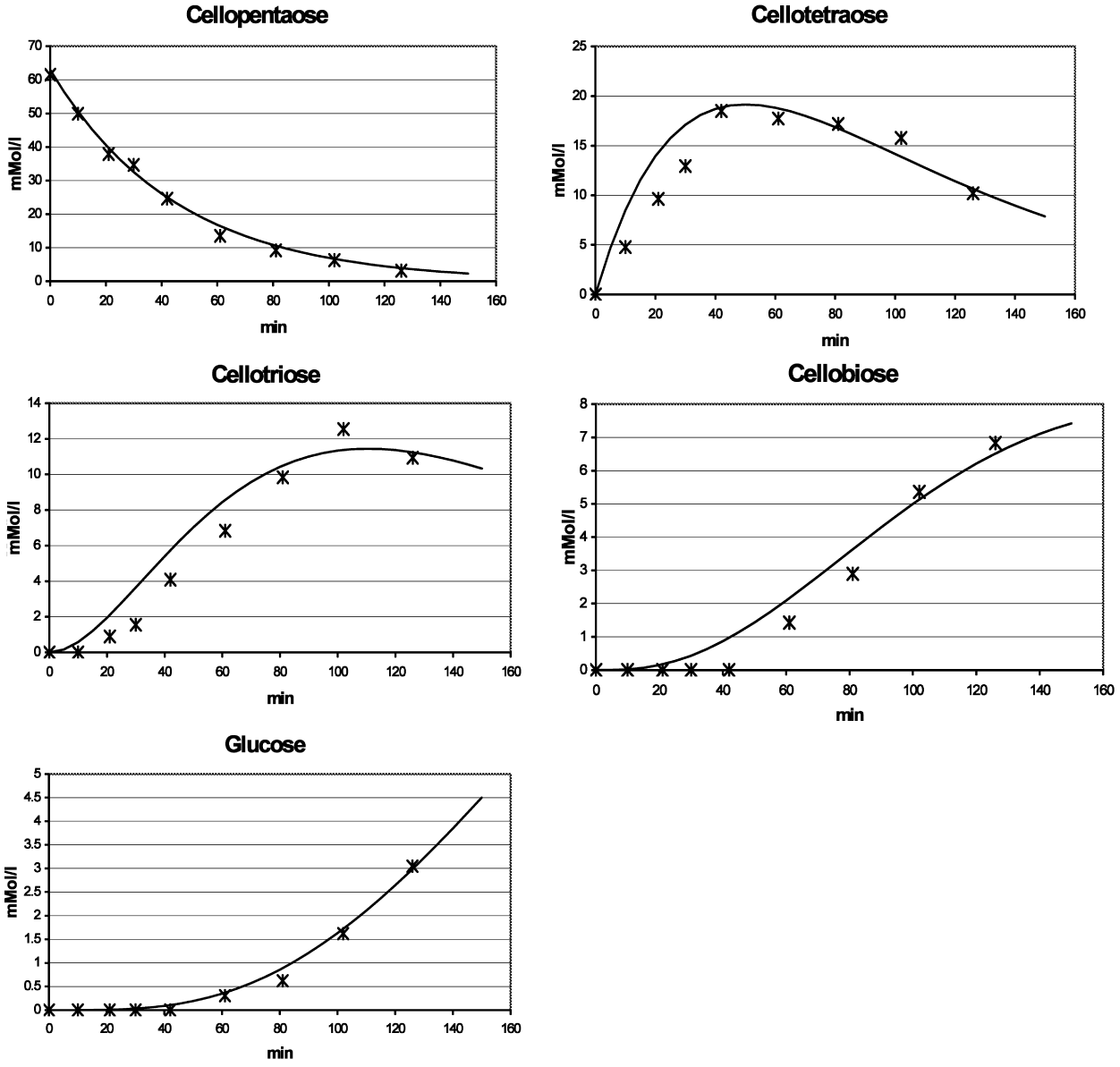

Fig. 7. Mathematical modeling of the kinetics of the alkaline degradation of cellopentaose and -oligosaccharide intermediates, according to Eq. (7a) to (7c).

References

1. Fengel, D. *Papier* **1980**, *34*, 428–433.
2. Malinen, R.; Sjöström, E. *Papper och Trä*. **1973**, *8*, 451–468.
3. Malinen, R.; Sjöström, E. *Papper och Trä*. **1973**, *8*, 547–556.
4. Malinen, R.; Sjöström, E. *Papper och Trä*. **1975**, *3*, 101–114.
5. Ericsson, B.; Lindgren, B. O.; Theander, O. *Cellulose Chem. Technol*. **1973**, *7*, 581–591.
6. Lobry de Bruyn, C. A.; Alberda van Ekenstein, W. *Recl. Trav. Chim. Pays-Bas*. **1895**, *14*, 203.
7. Speck, Jr., J. C. *Adv. Carbohydr. Chem*. **1958**, *13*, 63–103.
8. De Wit, G.; Kieboom, A. P. G.; van Bekkum, H. *Carbohydr. Res*. **1979**, *74*, 157–175.
9. Topper, Y. J.; Stetten, Jr., D. *J. Biol. Chem*. **1951**, *189*, 191–202.
10. De Bruijn, J. M.; Kieboom, A. P. G.; van Bekkum, H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 35–43.
11. Johansson, M. H.; Samuelson, O. *Chem. Scr*. **1976**, *9*, 151–154.
12. Lai, Y. Z. *Carbohydr. Res*. **1973**, *28*, 154–157.
13. Göransson, S. *Svensk Papperstidn*. **1968**, *71*, 131–136.
14. MacDonald, D. M. *Tappi J*. **1965**, *48*, 708–713.
15. Majdanac, L.; Galogaza, V.; Theodorovic, M. *Cellulose Chem. Technol*. **1983**, *17*, 333–340.
16. Entwistle, D.; Cole, E. H.; Wooding, N. S. *Textile Res. J*. **1949**, 527–546.
17. Entwistle, D.; Cole, E. H.; Wooding, N. S. *Textile Res. J*. **1949**, 609–624.
18. Lindgren, B. O.; Sundin, S. *Svensk Papperstidn*. **1978**, *81*, 485–488.
19. Kolar, J.; Strlič, M.; Pihlar, B. *Anal. Chim. Acta* **2001**, *431*, 313–319.
20. Wolfrom, M. L.; Thompson, A. *Methods Carbohydr. Chem*. **1963**, *3*, 143–149.
21. Flugge, L. A.; Blank, J. T.; Petillo, P. A. *J. Am. Chem. Soc*. **1999**, *121*, 7228–7238.
22. Nguyen, D. T.; Lerch, H.; Zemann, A.; Bonn, G. *Chromatographia* **1997**, *46*, 113–121.
23. For alkalization conditions see: Verein der Zellstoff- und Papierchemiker und -ingenieure, Arbeitsblatt A III/1/72, Nov. 27, 1972.
24. Dahlman, O.; Jacobs, A.; Liljenberg, A.; Olsson, A. I. *J. Chromatogr., Sect. A* **2000**, *891*, 157–174.
25. Rydlund, A.; Dahlman, O. *J. Chromatogr., Sect. A* **1996**, *738*, 129–140.
26. Ericsson, B.; Lindgren, B. O.; Theander, O. *Carbohydr. Res*. **1972**, *23*, 323–325.
27. Forsskahl, I. *Carbohydr. Res*. **1976**, *48*, 13–21.