

**The inter- and intramolecular selectivity of the carbonate radical anion in its reactions with lignin and carbohydrates**

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and Engineering**

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## 1 Abstract

In the present thesis, the effects of the carbonate radical anion on lignin and cellulose were investigated.

The carbonate radical has a rather high reactivity towards aromatic lignin constituents. It reacts especially fast with phenolates. All these reactions occur by way of electron transfer. Small carbohydrates react with  $\text{CO}_3^{\cdot-}$  much slower than aromatics. These reactions are hydrogen transfer reactions. However, in very basic media, where the carbohydrates deprotonate to some extent, their anions react with  $\text{CO}_3^{\cdot-}$  by way of electron transfer and the rates approach those of non-phenolic aromatics.

These findings suggest that in neutral or slightly alkaline media  $\text{CO}_3^{\cdot-}$  might serve as an excellent delignifying agent of pulp down to very low lignin contents.

With small carbohydrates possessing one or two glucosidic bonds,  $\text{CO}_3^{\cdot-}$  abstracts hydrogen predominantly from C1 – H bonds, which results in rupture of the glucosidic linkage. Interestingly, however, the glucosidic bonds in cotton linters are rather resistant towards  $\text{CO}_3^{\cdot-}$ . This has probably morphological reasons. These results imply that, even at very low lignin contents, where  $\text{CO}_3^{\cdot-}$  is bound to react with cellulose, the reactions will not lead to substantial decrease in pulp viscosity.

At present the cheapest and most practical way of producing  $\text{CO}_3^{\cdot-}$  radicals in the presence of pulp is to mix the latter with peroxyxynitrite and  $\text{CO}_2$ . We have performed such experiments on pulp with very promising results. The Kappa number decreased substantially, brightness increased, while the viscosity remained high. This confirms the predicted excellent properties of the carbonate radical.

However, before the peroxyxynitrite method can be implemented in the pulp industry, a number of technical problems has to be solved. Chief among them is a slow and steady dosage of peroxyxynitrite to minimise side reactions of the radicals with peroxyxynitrite and the nitrite impurity. The fate of the  $\cdot\text{NO}_2$  radical, the coproduct of  $\text{CO}_3^{\cdot-}$ , has also to be assessed.  $\cdot\text{NO}_2$  will probably have to be removed by vigorous degassing in order to block the possible nitration of cellulose.

## List of Papers:

Stenman D., Carlsson M., Jonsson M. and Reitberger T. **2003** "Reactivity of the carbonate radical anion towards carbohydrate and lignin model compounds." *Journal of Wood Chemistry and Technology*, 23(1), 47-69.

Carlsson M., Stenman D., Merényi G. and Reitberger T. **2005** "The Carbonate Radical as One-Electron Oxidant of Carbohydrates in Alkaline media" *Holzforschung*, Vol. 59, pp. 143–146 •

Carlsson M., Oscarson S., Kenne L., Andersson R. and Reitberger T. "D-*gluco*-Hexodialdose in Aqueous Solution; Determination of the Main Forms by NMR Spectroscopy" Manuscript.

Carlsson M. Lind J. and Merényi G. "A Selectivity Study of the Carbonate Radical Anion Reacting with Methyl- $\beta$ -D-cellobioside and Methyl- $\beta$ -D-glucoside in Oxygenated Aqueous Solutions" Manuscript.

Carlsson M., Stenman D., Merényi G. and Reitberger T. **2005** "A comparative study on the degradation of cotton linters induced by carbonate and hydroxyl radicals from peroxyxynitrite" *Holzforschung*, Vol. 59, pp. 132–142

Stenman D., Carlsson M. and Reitberger T. **2004** "Peroxyxynitrite mediated delignification of pulp. A comparative study on the bleaching properties of the carbonate and hydroxyl radicals." *Journal of Wood Chemistry and Technology*, 24(2), 83-98

## 2 Introduction

Most pulps obtained from wood pulping processes are dark coloured due to the presence of a small fraction of residual lignin. The residual lignin should be completely removed by bleaching processes to produce a high brightness paper, and until recently molecular chlorine, hypochlorite and chlorine dioxide were the main reagents used in pulp bleaching. Growing environmental concerns have evoked interest in using totally chlorine-free (TCF) bleaching sequences. TCF-bleaching normally refers to the use of oxygen, peroxide, ozone and peracid as bleaching agents. However, such sequences are generally less selective than chlorine and result in lower yields and decreased pulp strengths. Therefore, it is important to develop the chemistry of TCF bleaching in such a way that both the efficiency and the selectivity can be improved. This may be accomplished by using enzymes, transition metal complexes, polyoxometalates or photocatalysts (Argyropoulos 2001). However, these “advanced oxidation technologies”, AOT’s, are generally difficult to implement on the industrial level.

An AOT reagent for a new bleaching technology must not only have a benign environmental influence but should also be economical, compatible with existing technology and produce a final product of similar or superior quality. In this context, the carbonate radical anion ( $\text{CO}_3^{\bullet-}$ ) stands out as a possible candidate. It does not produce any hazardous effluents or by-products. Due to its high one-electron reduction potential of 1.59 V vs. NHE at  $\text{pH} > 10.3$ , the carbonate radical can oxidize lignin by way of radical cation formation, the preferred mechanism to achieve lignin fragmentation. Like the hydroxyl radical, the carbonate radical can also attack carbohydrates by H-atom abstraction mechanisms, but at a much lower rate.

Decisive for the use of the carbonate radical in pulp bleaching is the selectivity, *i.e.* the reactivity of the carbonate radical anion towards lignin relative to carbohydrate structures. High *intermolecular* selectivity can be achieved if the carbonate radical reacts much faster with lignin than with cellulose. Another aspect of selectivity, which has not been given much attention to date, is whether an oxidant preferentially attacks certain sites in carbohydrates. This *intramolecular* selectivity may determine the extent of cleavage of inter-unit bonds. The hydroxyl radical, an archetypically non-selective radical, can be used in selectivity investigations as a suitable reference for radical reactions on lignin and cellulose model compounds. Cotton linters were chosen in this investigation because this material represents pure high molecular cellulose.

This thesis also deals with the use of peroxynitrite ( $\text{ONOO}^-$ ) as a chemical precursor for formation of radicals. This compound can generate the carbonate radical in a fast reaction with carbon dioxide. It can also generate hydroxyl radicals upon protonation to form  $\text{ONOOH}$ . A possible draw-back may be the simultaneous production of  $\text{NO}_2$  radicals, although the latter are much less reactive than either the carbonate radical or the hydroxyl radical. Hence both radicals can be formed without involvement of transition metal ions, heat, light or radiation. Therefore, in this way the degradation of cotton linters caused by the carbonate radical and the hydroxyl radical can be compared.

### 3 Composition of wood

#### 3.1 Lignin

Lignin is one of the essential wood components, ranging in amount from 26 to 36 % of native softwood and from 37 to 57 % of native hardwoods. Lignin is a macromolecule/polymer assumed to be formed by enzymatic dehydrogenation of phenylpropanes. This is followed by radical coupling of three different building blocks (monolignols), i.e. p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Softwood lignin is mainly composed of coniferyl alcohol, while hardwood lignin is composed of a mixture of coniferyl and sinapyl alcohols. Grass lignin contains all three of the monolignols. Further reactions in the lignification process leads to a variety of linkages and functional groups, (Sjöström 1993).

#### 3.2 Cellulose

Cellulose is the most abundant organic material on Earth. It is the main component of plant sources, serving as the structural material by which plants, trees, as well as grasses sustain their strength to stay upright. Cellulose is a linear macromolecule composed of (1→4)-β-D-glucopyranose (Figure 1) and only the configuration of the C1 position is different from that of amylose, the latter being made up of (1→4)-α-D-glucopyranose (Krässig 1993). The degree of polymerization (DP) is estimated to be about 10 000 (Sjöström 1993). The strength of wood depends on this linear and moderately crystalline, cellulose structure.

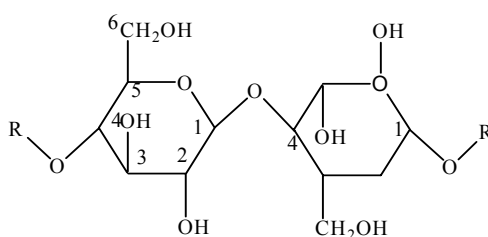


Figure 1. Cellulose.

At the supramolecular (above the molecular) level, the cellulose chains are held together by strong inter- and intramolecular hydrogen bonds. Since every second glucose unit is rotated through 180°, the repeating unit is considered to be cellobiose rather than glucose. The aggregates of cellulose chains may be ordered (crystalline) or unordered (amorphous). The ordered cellulose may crystallize in several forms; cellulose I<sub>β</sub> is the dominant form in higher plants such as cotton and wood. Cellulose I can be transformed to cellulose II by alkali treatment (4 M NaOH), mercerisation, and this process is used to increase the reactivity of the cellulose. Cellulose crystallinity determines the access of solvents and reactants to cellulose; therefore it will also be of great importance for the degradation of cellulose (Krässig 1993).

#### 3.3 Hemicellulose

Hemicellulose is the most abundant organic material, next to cellulose, on the Earth. The hemicellulose content ranges from 16 to 27 % for softwoods and from 20 to 37 % for hardwood. The hemicellulose consists of heteroglycans containing several different types of sugar components. In hardwood species such as birch and aspen, the major hemicellulose is an O-acetyl-4-O-methylglucuronoxylan, whereas the predominant hemicelluloses in softwood such as spruce and pine are O-acetyl-galactoglucomannan and arabino-(4-O-ethylglucurono)xylan (Jacobs 2001).



### **3.4 Pectin**

Pectins, a family of heterogeneous polysaccharides present in primary cell walls, consist primarily of (1→4)-linked  $\beta$ -D-galacturonic acids. In wood tissue, pectin is also found in the middle lamella and in reaction wood. The content of pectin in wood comprises approximately 1 to 4 % of the constituents of wood (Sjöström 1993).

### **3.5 Extractives**

Cellulose, lignin and hemicelluloses are macromolecules and the main components constructing the cell wall of wood. In addition to these main components, wood contains minor components soluble in water or organic solvents that are called "extractives".

The extractives are lower molecular weight compounds, distributed in the lumen or specific tissues such as resin canal, and do not combine with the components constructing the cell wall. The content of extractives in most woods is very low, usually less than 5 % (Sjöström 1993). The wood cells are complex biocomposites that consist mainly of cellulose, lignin and hemicelluloses (glucomannan and glucuronoxylan), lignin and extractives. The proportions and chemical compositions of lignin and hemicelluloses differ in hardwood and softwood, while cellulose is a uniform component present in all types of wood.

## 4 Technology of bleaching

The cellulose used in industries for producing paper, board, fibres etc. is obtained from wood and, to a lesser extent, from cotton. In wood and in many plants, cellulose is ingeniously organised with hemicellulose, extractives and lignin. In order to isolate cellulose from wood, pulping processes using acidic or alkaline liquors for hydrolytic removal of lignin must break up the wood composite. The most widely used chemical pulping process is the "kraft" process. The alkaline pulping liquor used contains sodium hydroxide and sodium sulphide.

Wood chips are impregnated with pulping liquor for 1 to 2 h at 150-180 °C in a batch or in continuous systems. After pulping, most of the lignin has been depolymerised and the soft chips can be fiberised with little mechanical action.

The kraft cooking process cannot completely delignify the pulp without adversely affecting the strength of the pulp, due to low selectivity at the end of the process. Final delignification is therefore performed by bleaching under much milder conditions.

### 4.1 Bleaching of pulp

Bleaching of pulp is a chemical process used to increase the brightness of the pulp. This effect is obtained by removal of chromophores in the pulp. Most of the chromophores are found in the lignin part and the removal of lignin is therefore an efficient way to increase brightness.

The goal with bleaching can be different for different products; for mechanical pulps the purpose is simply to bleach the coloured groups and to leave the lignin in the fibres, *lignin preserving bleaching*, i.e. brightness improvement. For higher quality papers and other products made of chemical pulps, the goal is to more or less completely remove the residual lignin by depolymerisation and introduction of hydrophilic groups, *lignin removing bleaching*.

### 4.2 Chemistry of bleaching

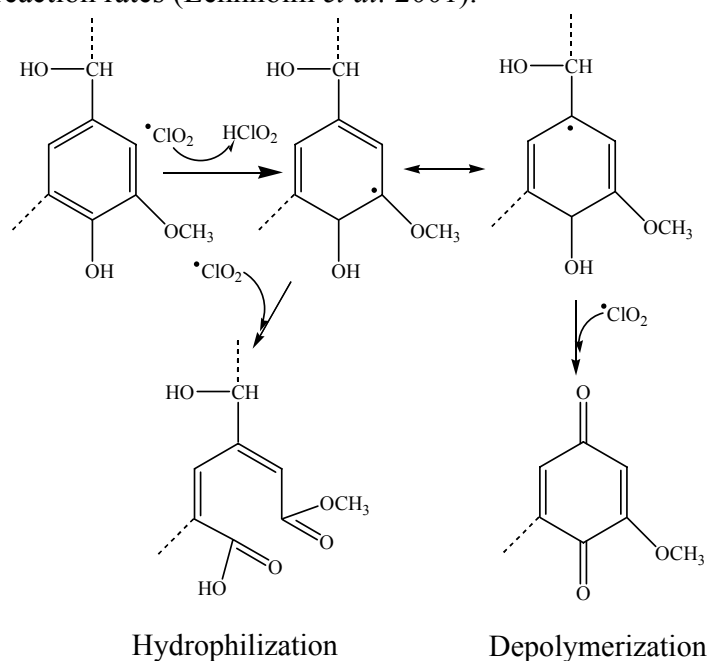
Decisive for the bleaching capability of any chemical species in a bleaching stage is its kinetic selectivity towards lignin vis á vis carbohydrates. Detrimental effects in pulp arise when chemical species exhibiting reactivity towards cellulose are formed. These are often free radicals (Ek *et al.* 1989). The selectivity during bleaching is therefore contingent on the formation of highly reactive free radicals. As a result of the kinetic competition, the selectivity in free radical-chain reactions will become poorer as the delignification proceeds.

The chemicals used for bleaching, are either 2-electron oxidants that participate in addition reactions (e.g. H<sub>2</sub>O<sub>2</sub>, O<sub>3</sub>), substitution reactions (e.g. Cl<sub>2</sub>) or they are 1-electron oxidants, such as O<sub>2</sub> and ClO<sub>2</sub>.

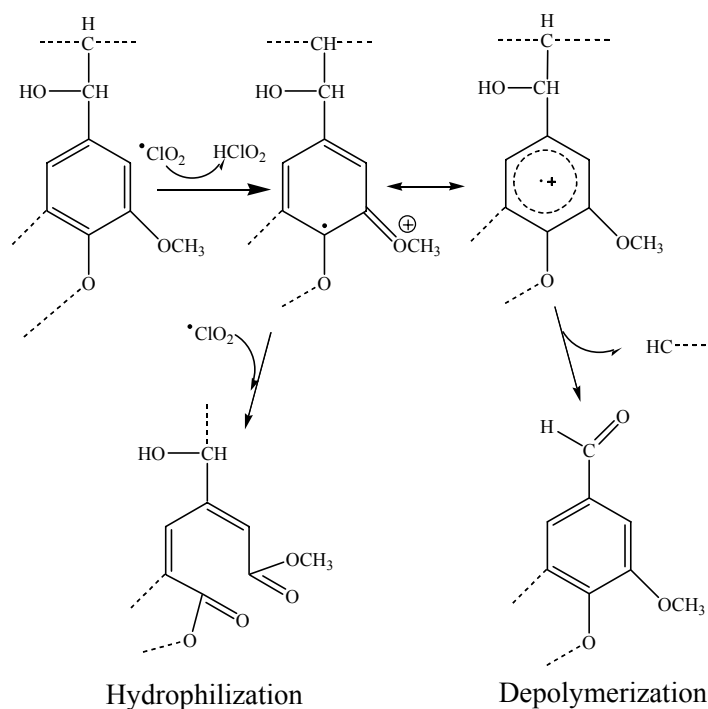
#### 4.2.1 Chlorine dioxide

Chlorine dioxide bleaching is the predominating pulp bleaching method today. It is performed in a weakly acidic medium (around pH 3.5) and at 60<sup>0</sup> C for 30 min. to 4 h. Chlorine dioxide is a poisonous gas, and a relative stable radical. It can both oxidize phenolic groups on the residual lignin, similarly to oxygen delignification, and participate in radical-radical coupling reactions. Chlorine dioxide reactions with phenolic lignin structures result in depolymerizations and hydrophilizations of the phenols, as shown in Figure 2. Chlorine

dioxide may also react with non-phenolic structures with the same results (Figure 3), but at much lower reaction rates (Lennholm *et al.* 2001).



**Figure 2.** Reaction with chlorine dioxide on phenolic lignin.

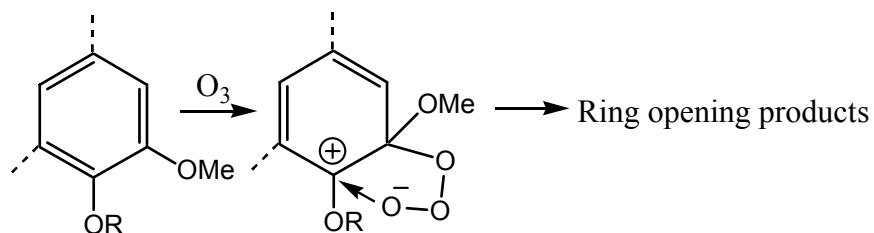


**Figure 3.** Suggested reaction with non-phenolic structures and  $\text{ClO}_2$ .

#### 4.2.2 Ozone

Ozone bleaching is carried out at low pH (2-3) and at 30 °C. The bleaching reaction is very fast and complete within a few minutes.  $\text{O}_3$  is a toxic gas that can react both as a nucleophile and electrophile. In bleaching the functional reactions are considered to be mainly

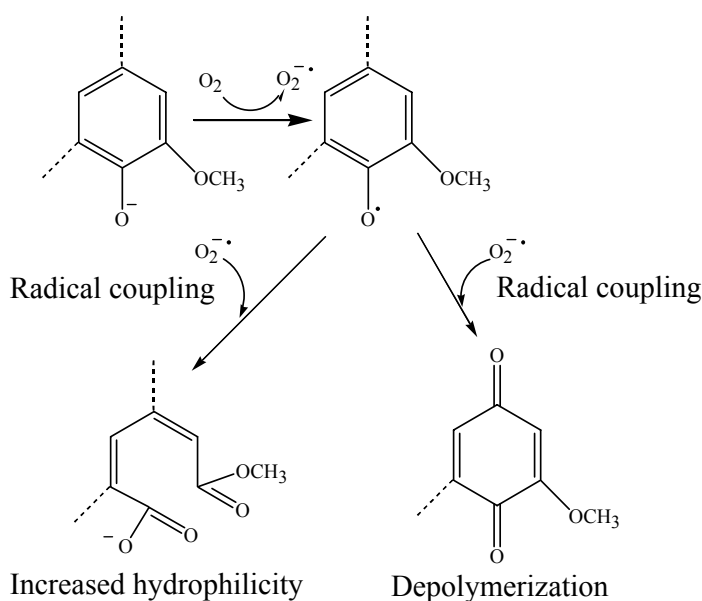
electrophilic, but both reaction types have been suggested. The reaction with non-phenolic structures is complex (Ragnar *et al.* 1999). A suggested reaction leading to ring opening is shown in Figure 4.



**Figure 4.** Suggested reaction of ozone on non-phenolic lignin

#### 4.2.3 Oxygen based bleaching methods

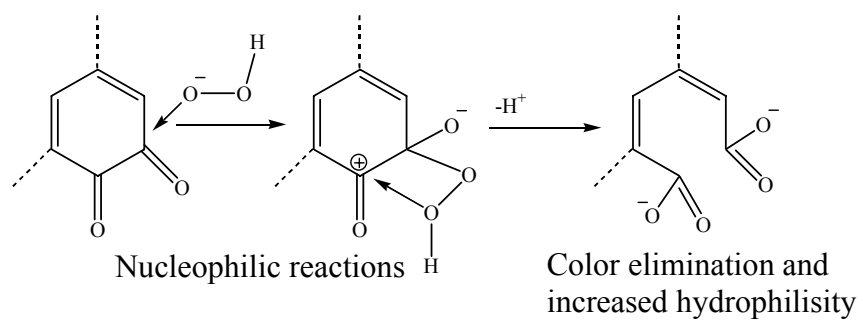
Oxygen delignification is a cheap and environmentally friendly method for oxidizing and solubilizing residual lignin. Oxygen attacks electron rich sites such as phenolate and enolate groups, Figure 5. A 35-50 % decrease in lignin content is typical for oxygen bleaching. The selectivity is, however, a problem and brightness improvement is moderate. Oxygen bleaching is performed in an alkaline medium under oxygen pressure and at temperatures between 85-115 °C (Sjöström 1993).



**Figure 5.** Oxygen reactions with lignin

#### 4.2.4 Hydrogen peroxide

Hydrogen peroxide bleaching is typically performed in alkaline media (pH 11-11.5) for 4 h at 90 °C. Under these conditions, *i.e.* at high pH, the nucleophilic ion  $\text{HOO}^-$  is formed.  $\text{HOO}^-$  attacks coloured lignin structures as shown in Figure 6. Hydrogen peroxide used to be considered as a lignin preserving bleaching agent, but it can depolymerise lignin to some extent, as well as introduce charges (Sjöström 1993).



**Figure 6.** Hydrogen peroxide reactions with lignin in alkali, leading to colour elimination and increased hydrophilicity.

## 5 Free radicals in pulp bleaching and their reactions with cellulose

### 5.1 Free radicals in pulp bleaching

The chemistry of most bleaching technologies is, to a large extent, governed by that of free radicals. One exception to this rule is chlorine, which, under pulp-bleaching conditions acts as an electrophilic substituting agent. The radical reactions occurring in pulp bleaching result in either beneficial or detrimental effects. The positive/negative nature of a radical is linked to its difference in reactivity towards aromatics (lignin) and carbohydrates (cellulose), respectively. Such selectivity has been demonstrated on model compounds (Ek *et al.* 1989).

#### 5.1.1 Chlorine dioxide and chlorine

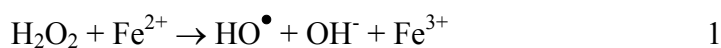
Chlorine dioxide reacts very slowly with polysaccharides and is thus regarded as a highly selective lignin oxidant. Due to the fact that chlorine dioxide also acts as a radical scavenger, oxidation of polysaccharides is prevented in a chlorine dioxide stage. In chlorine based bleaching, chlorine-radicals ( $\text{Cl}^\bullet$ ,  $\text{Cl}_2^\bullet$ ) formed may affect the strength of the pulp. For this reason, bleaching with chlorine is usually carried out in the presence of chlorine dioxide, (Sjöström 1993). In Sweden, bleaching with chlorine is no longer used for environmental reasons. Bleaching with chlorine dioxide on the other hand, is presently the most wide spread pulp bleaching method.

#### 5.1.2 Oxygen and hydrogen peroxide

The reactions that take place during oxygen and hydrogen peroxide bleaching have features in common because in both cases the medium is alkaline, and oxygen and hydrogen peroxide are partly interconverted. Several oxygen species are present during alkaline oxygen and hydrogen peroxide bleaching, including the superoxide anion radical ( $\text{O}_2^{\bullet-}$ ), the hydroperoxyl radical ( $\text{HO}_2^\bullet$ ) and the hydroxyl radical ( $^\bullet\text{OH}$ ).

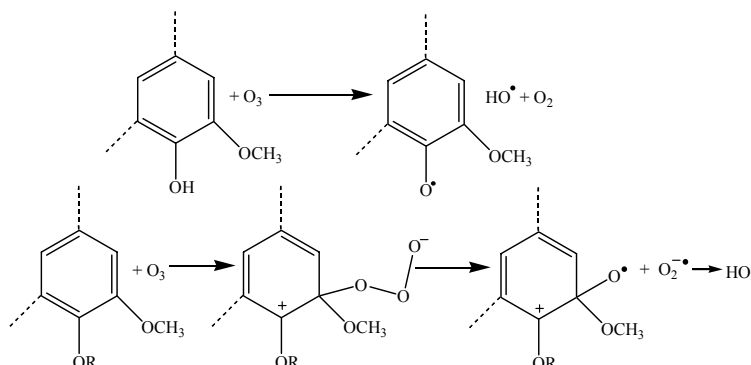
Although  $\text{O}_2^{\bullet-}$  is unreactive compared to many other radicals, different systems can convert it into other more reactive species, such as hydroperoxyl ( $\text{HO}_2^\bullet$ ), peroxy ( $\text{ROO}^\bullet$ ), alkoxy ( $\text{RO}^\bullet$ ) and hydroxyl ( $\text{HO}^\bullet$ ) radicals. Transition metal ions, such as iron and copper, which are present in wood, catalyze this conversion. The hydroxyl radical can originate from the Fenton reaction (1) in which the metal ion participates in redox cycles, with reduction being effected by  $\text{O}_2^{\bullet-}$  and oxidation by its dismutation product, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (2), (Dean *et al.* 1997).

The Fenton reaction



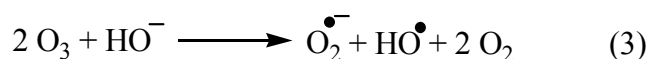
### 5.1.3 Ozone

During ozone bleaching the selectivity can be low due to the formation of hydroxyl radicals. Suggested sources of radical formation are shown in Figure 7.



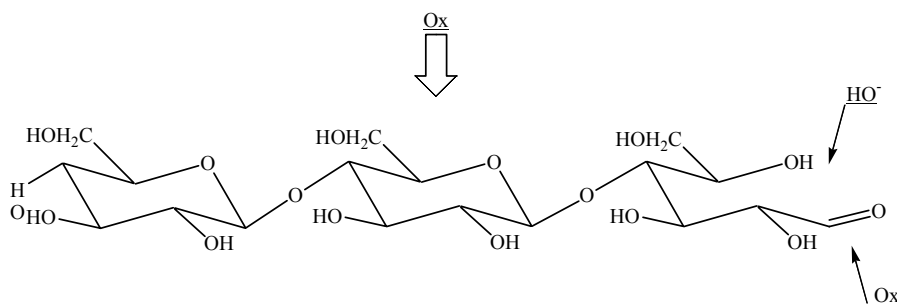
**Figure 7.** Formation of hydroxyl radical during ozone bleaching (Ragnar et al. 1999)

Under alkaline conditions hydroxide ions can react with ozone forming hydroxyl radicals according to (3):



## 5.2 Cellulose reactions

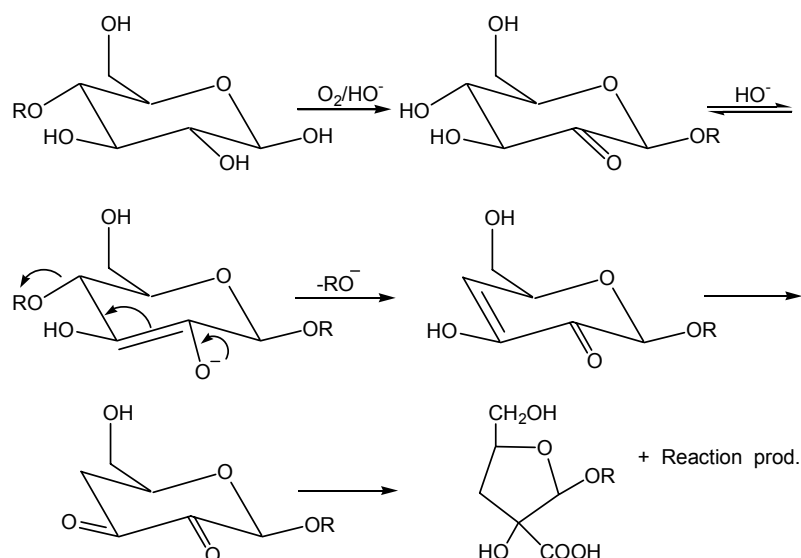
It is generally assumed that the main oxidising attack occurs within the polysaccharide chains, but it can also be directed towards the end groups, Figure 8.



**Figure 8.** Sites in cellulose susceptible to attack of oxidative species (Ox) and alkali (HO<sup>-</sup>).

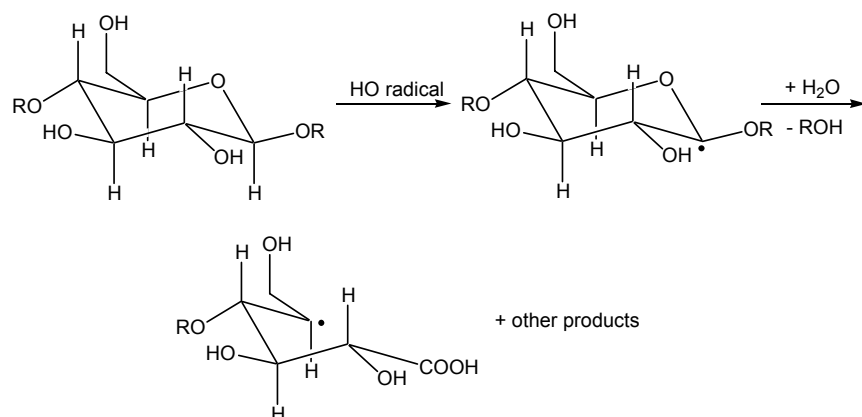
Oxidation at any position within the cellulose chain units (C1, C2 or C6) to carbonyls generates alkali labile glycosidic linkages. Peeling starts from the reducing end group and oxidation of aldehyde end groups to carboxyl groups prevents the peeling reaction.

The presence of carbonyl groups gives rise to alkali instable glycosidic bonds. For example, once carbonyl groups have formed during unfavourable oxidation reactions, the glycosidic bonds of cellulose are easily cleaved in an alkaline treatment, Figure 9 by a  $\beta$ -alkoxy elimination (Sjöström 1993).



**Figure 9.** Cleavage of a glycosidic bond after oxidation and formation of a carbonyl group. R is cellulose.

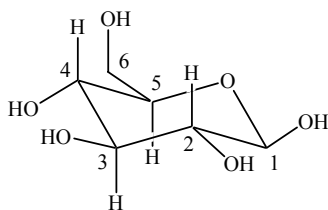
In addition to this type of depolymerization, glycosidic bonds may also be cleaved directly, for example, after a hydroxyl radical attack at the C1 position, which will give rise to an aldonic acid end group, Figure 10.



**Figure 10.** Cleavage of a glycosidic bond in cellulose after attack by a hydroxyl radical. After oxidation of C1, the glycosidic bond is cleaved with formation of an aldonic acid end group. R is cellulose. Radical formation on R (C-4 on R), is of course also conceivable.

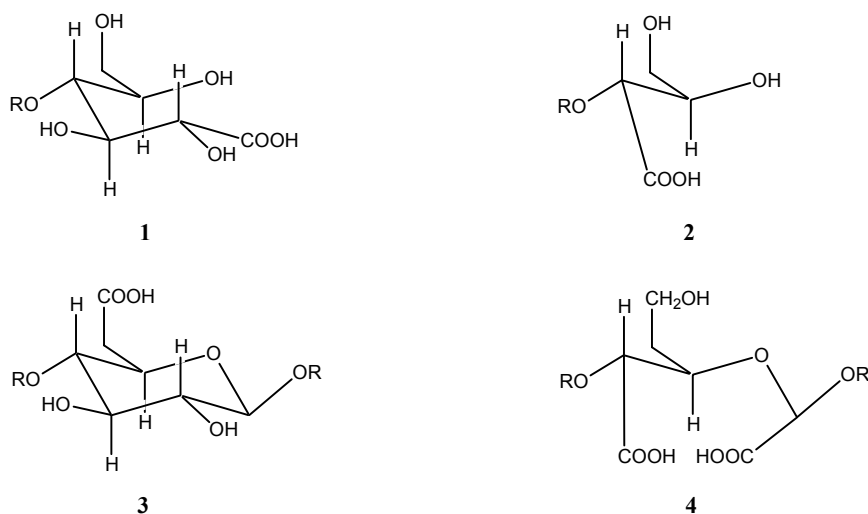
It is suggested that the most common type of depolymerization of cellulose occurs after oxidation at the C-2 position. Oxidation at position C-3 leads to the same result due to migration of the carbonyl group to C-2. The 2-ulose formed is easily degraded by  $\beta$ -alkoxy elimination at C-4, resulting in chain cleavage and formation of a new reducing end group. Although chain cleavage at C-1 is possible (after oxidation at C-3), this does not seem to occur. Oxidation at position C-6 may also occur, giving rise to cleavage of the glycosidic bond at C-4 (Sjöström 1993). Positions C-1 to C-6 is shown in Figure 11.





**Figure 11.** C-1 to C-6 positions in D-glucose

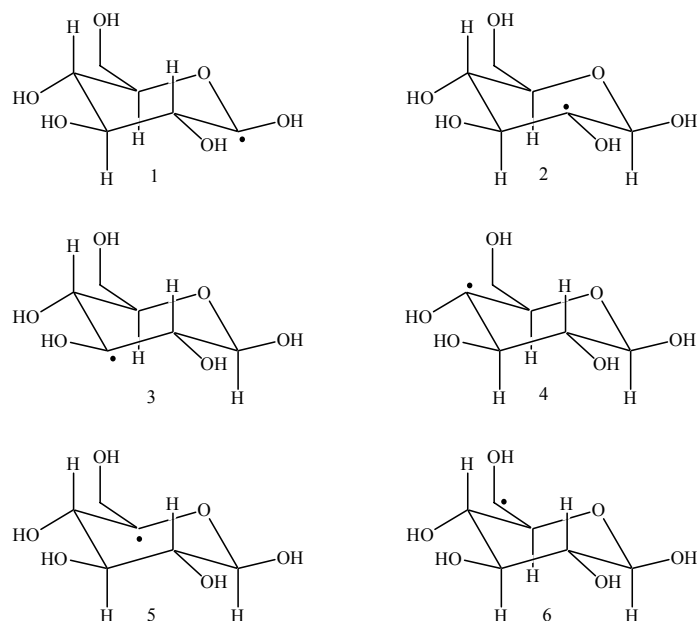
In addition, carboxyl groups can be introduced into cellulose by bleaching agents, Figure 12. Although carboxylic groups, unlike carbonyls, do not render cellulose extremely sensitive towards alkaline degradation, they affect detrimentally the brightness stability of the final bleached pulp. However, carboxylic groups may also have a positive effect since they improve the bonding between fibres in the final paper.



**Figure 12.** Examples of carboxyl structures formed in cellulose by oxidation of bleaching agents. **1:** arabinoic acid, **2:** erythronic acid, **3:** glucuronic acid and **4:** dicarboxylic acid.

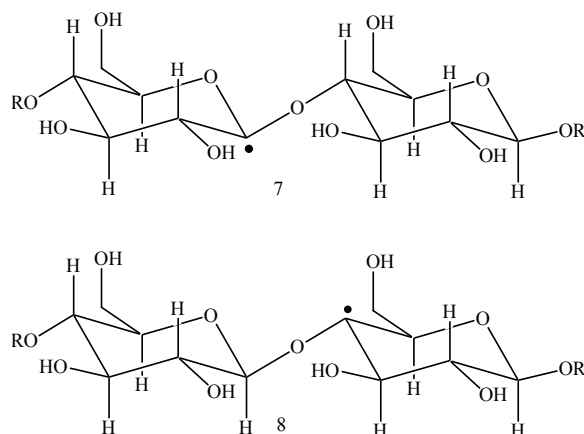
### 5.3 Hydroxyl radical reactions with carbohydrates

The hydroxyl radical reacts with carbohydrates *via* H-atom abstraction. Due to the much lower bond dissociation energy of the C-H bond compared to the O-H bond, only carbon-centred radicals are formed in this reaction. For the OH radical, the reaction rate constants are typically near  $2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  (Buxton *et al.* 1988). In comparison, the carbonate radical is about two to four orders of magnitude slower (Stenman *et al.* 2003). Generally, there is no pronounced regioselectivity (Park *et al.* 1999), *i.e.* the D-glucose radicals 1-6, Figure 13 are formed in comparable yields after a hydroxyl radical attack (Schuchmann & von Sonntag, 1977).



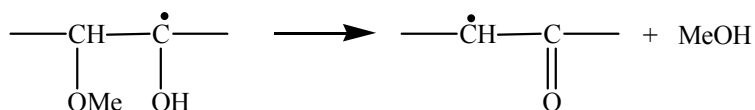
**Figure 13.** Hydroxyl radical reactions with D-glucose

In disaccharides and polymeric carbohydrates, the ether-type radicals such as 7 and 8 in Figure 14, have the radical site proximate to the glycosidic linkage and therefore play a major role in its scission (von Sonntag & Schuchmann, 2001).



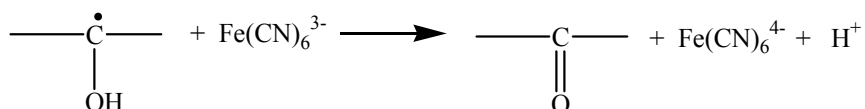
**Figure 14.** Radical sites proximate to the glycosidic linkage which may lead to chain scission.

Many carbohydrates contain the structural element  $-\text{CHOH}-\text{CHOH}-$ . Radicals 1-4 in Figure 7 can eliminate water (Buley *et al.* 1966). This reaction is relatively slow, but becomes faster by acid or base catalysis. Methanol can be similarly eliminated, Figure 15, this reaction also converts the exocyclic glucose-derived radical 6 into a  $\beta$ -ketoalkyl radical (Steenken *et al.* 1986; Karam *et al.* 1986; Petryaev & Shadyro 1986; Shadyro 1987).

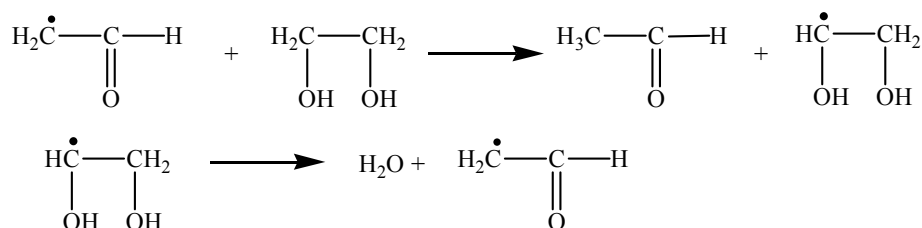


**Figure 15.** Elimination of methanol.

While  $\alpha$ -hydroxyalkyl radicals are of reducing nature, Figure 16, the resulting  $\beta$ -ketoalkyl radicals have oxidizing properties. The latter may cause H-abstraction from the substrate, Figure 17, which in the case of ethylene glycol has been shown to lead to short chain reactions (Adams and Willson 1969; Park *et al.* 1999).



**Figure 16.** Reduction by an  $\alpha$ -hydroxyalkyl radical.

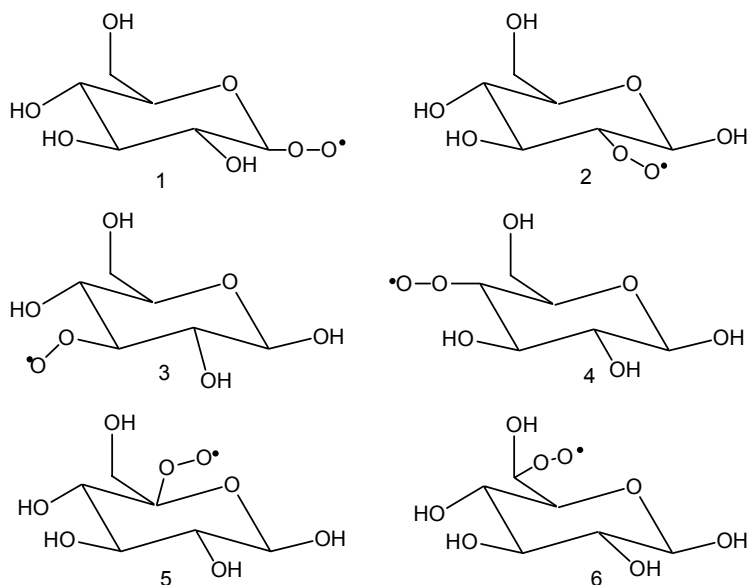


**Figure 17.** Oxidation by a  $\beta$ -ketoalkyl radical.

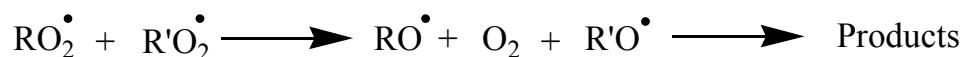
In carbohydrates, the importance of the water elimination reaction is reflected by the mixture of reaction products obtained.

#### 5.4 Reactions with oxygen

Carbon centered radicals generally react very fast with oxygen, giving rise to the corresponding peroxy radicals, Figure 18 (von Sonntag and Schuchmann 1991). The  $\alpha$ -hydroxyalkylperoxy radicals **1-4** and **6** relatively easily eliminate  $\text{HO}_2\cdot$  and are converted to carbonyl compounds. Elimination of  $\text{HO}_2\cdot$  from the peroxy radical **5** is much slower and it is thus likely that this peroxy radical is terminated via a recombination reaction, Figure 19 (von Sonntag and Schuchmann, 2001).

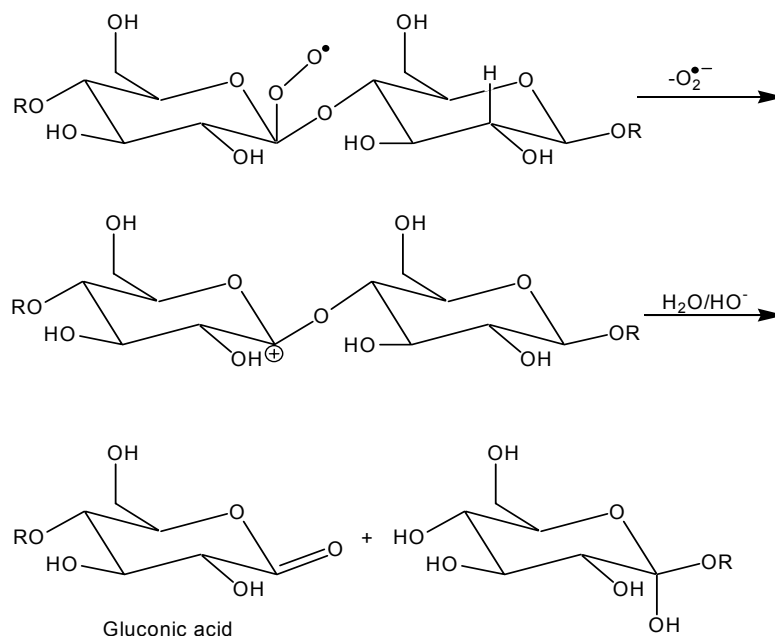


**Figure 18.** Oxygen reaction with carbon centered radicals.



**Figure 19.** Elimination of  $HO_2^\bullet$  from the peroxy radical 5 in Figure 18 via a recombination reaction.

If disaccharides and polymeric carbohydrates have the radical site proximate to the glucosidic linkage, the reaction with oxygen presented in Figure 20 may be envisaged.



**Figure 20.** Reactions of carbohydrates (cellulose) with oxygen, having the radical site proximate to the glucosidic linkage.

## 6 Materials and methods

### 6.1 Chemicals

All reagents employed were of the highest available reagent grades and used without further purification (Apin Chemicals Ltd, Aldrich, Acros Organics, Merck, VWR). Water used for preparation of reagents and solutions was Millipore purified.

For model compound studies, small carbohydrate or lignin-like monomers or dimers were used. The carbohydrates used were glucose, cellobiose, methyl- $\beta$ -D-glucopyranoside and methyl- $\beta$ -D-cellobioside. The lignin model compounds were mono, di or tri-substituted aromatics of various one-electron oxidation potentials.

### 6.2 Cotton linters

The cotton linters were provided by Dr. Gunnar Henriksson at the Department of Pulp and Paper Chemistry and Technology, Royal Institute of Technology, Stockholm, with an average viscosity of 1110 ml/g.

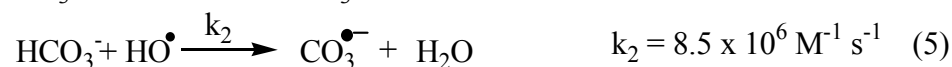
### 6.3 Radiolysis of water

When liquid water is irradiated, the energy deposited commonly produces hydroxyl radicals together with solvated electrons, hydrogen atoms and small amounts of H<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>. (Baxendale and Busi 1982; Buxton *et al.* 1988). The radiolytic yields of hydroxyl radicals and solvated electrons are nearly equal.

#### 6.3.1 Generation of secondary radicals

The radicals initially formed, can be inter-converted or directed towards the formation of new radical species. A convenient and widely used method of improving the hydroxyl radical yield during radiolysis of water solutions is the saturation of such solutions with N<sub>2</sub>O, which leads to the capture of solvated electrons and subsequent OH-radical formation. In such a system the hydroxyl radical yield approaches 90 % of the total radical yield, (Buxton *et al.* 1988).

The hydroxyl radicals thus produced can generate from their corresponding salts different inorganic radicals of lower reactivity and different reduction potentials according to reactions (4-6) (Buxton *et al.* 1988).



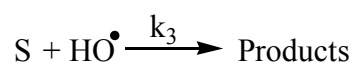
The one-electron reduction-potentials for some radicals which can be generated from the hydroxyl radical can be seen in Table 1.

**Table 1. The one-electron reduction-potentials of some inorganic radicals**

| <i>Radical</i>                   | <i>One electron reduction potential<br/>V vs. NHE</i> |
|----------------------------------|---|
| CO <sub>3</sub> <sup>•-</sup>    | 1.59  |
| Br <sub>2</sub> <sup>•-</sup>    | 1.61  |
| (SCN) <sub>2</sub> <sup>•-</sup> | 1.31  |
| OH <sup>•</sup>                  | 1.91  |
| NO <sub>2</sub>                  | 1.03  |
| ClO <sub>2</sub>                 | 0.936   |
| N <sub>3</sub> <sup>•</sup>      | 1.3   |

Source: NDRL/NIST Solution Kinetic Database on the Web, <http://kinetics.nist.gov/solution/index.php>

In these experiments, the hydroxyl radicals produced will also react with any other substrate present, S, according to:



For both lignin and cellulose structures the above reaction is diffusion controlled, *i.e.* reaction rates fall in the range of  $10^9$ - $10^{10} \text{ M}^{-1}\text{s}^{-1}$ .

Using a sufficient excess of an inorganic salt over the substrate, most of the initial hydroxyl radicals formed can be directed towards the formation of the corresponding inorganic radical. The concentration ratio needed for at least 90% conversion of hydroxyl radical into a secondary radical oxidant can be calculated from equation 7:

$$\frac{k_3[S]}{k_{\text{salt}}[\text{salt}] + k_3[S]} < 0.1 \quad 7$$

Where  $k_{\text{salt}}$  is the rate constant of HO<sup>•</sup> reacting with a salt anion.

With carbonate or bicarbonate in solution the hydroxyl radical will generate the carbonate radical anion. The rate of this reaction is pH controlled as the carbonate (4) and bicarbonate ions (5) show different reactivity towards the hydroxyl radical, equation 8. (Buxton *et al.*1988).

$$\frac{k_3[S]}{k_{r-1}[\text{CO}_3^{2-}] + k_{r-2}[\text{HCO}_3^-] + k_3[S]} < 0.1 \quad 8$$

For practical reasons the carbonate concentration was held at 1 M. Thus, according to equation 8, the substrate concentration must be limited to the mM-range (depending on pH).

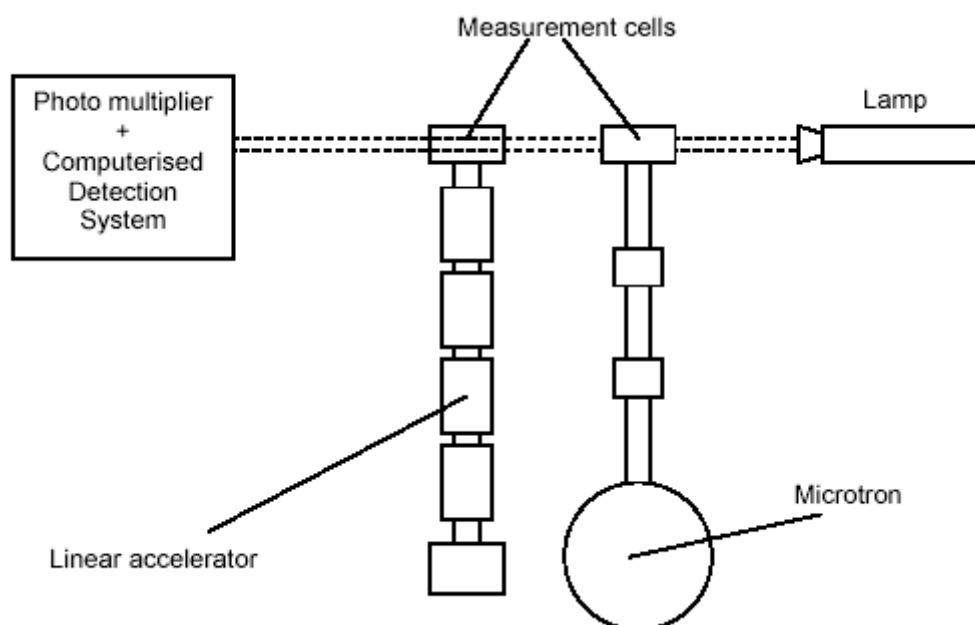
The pH was adjusted by varying the ratio of [CO<sub>3</sub><sup>2-</sup>] to [HCO<sub>3</sub><sup>-</sup>]. For pH > pH 12, NaOH was added. The composition of carbonate buffers is shown in Table 2. All samples were diluted using stock solutions of carbonate to minimize dilution errors.

**Table 2. Composition of carbonate buffers.**

| pH   | Na or K/HCO <sub>3</sub> (M) | Na <sub>2</sub> or K <sub>2</sub> /CO <sub>3</sub> (M) | NaOH (M) |
|------|------------------------------|--|----------|
| 8.3  | Saturated                    | 0  | 0        |
| 9.3  | 0.85                         | 0.15   | 0        |
| 10.3 | 0.5                          | 0.5  | 0        |
| 11   | 0.15                         | 0.85   | 0        |
| 12   | 0                            | 1  | 0        |
| 13   | 0                            | 1  | 0.1      |

#### 6.4 Pulse radiolysis

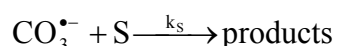
The pulse radiolysis experiments were carried out at room temperature. High-energy electrons were generated by a 3 MeV linear accelerator operating in pulsed mode, Figure 21. The duration of the electron pulse was 5-10 ns delivering doses of ca. 10 Gy/pulse. This corresponds to a radical production of ca. 10<sup>-5</sup> M radicals/pulse. The radical processes were studied exclusively by computerized time-resolved UV-vis spectroscopy. Under ideal conditions the system has a time resolution of approximately 10 ns (Eriksen *et al.* 1976). In some experiments the light source was a 6mW diode laser emitting at 635 nm, a wavelength close to the absorption maxima of CO<sub>3</sub><sup>•-</sup> at 600 nm (Eriksen *et al.* 1985)



**Figure 21.** Experimental set up for pulse radiolysis with UV-VIS detection.

#### 6.5 Kinetic measurements

For the kinetic measurements the decay of the carbonate radical anions formed after irradiation of the sample was measured at ambient conditions, *i.e.* ~22 °C. The decay was the result of the second order reaction between substrate and radicals, equation 9.



$$-\frac{d[\text{CO}_3^{\bullet-}]}{dt} = k_s[\text{CO}_3^{\bullet-}][\text{S}]$$

9

If the amount of substrate present in solution is significantly higher than the amount of radicals formed from the pulse, the change in substrate concentration can be regarded as insignificant, implying that equation 9 can be rewritten as a pseudo-first order decay of the carbonate radical anion as described by equation 10:

$$-\frac{d[\text{CO}_3^{\bullet-}]}{dt} = k_s^*[\text{CO}_3^{\bullet-}] \quad 10$$

$$k_s^* = k_s[\text{S}]_{\text{constant}}$$

Integration of equation 10 results in:

$$\ln(\text{CO}_3^{\bullet-})_t - \ln(\text{CO}_3^{\bullet-})_0 = -k_s^*t \quad 11$$

which can be combined with Beer's law,  $A = C \epsilon l$ , to yield:

$$\ln(A)_t = -k_s^*t + \ln(A)_0 \quad 12$$

where  $A_t$  is the absorbance of the carbonate radical anion, measured at 600 nm (Eriksen *et al.* 1985). Thus, the logarithm of the absorbance is a linear function of time, the slope being the *pseudo*-first order rate constant.

By measuring the *pseudo*-first order rate constant at varying substrate concentrations, the second order rate coefficient of reactions can be obtained. To minimise radical losses in non-desirable termination reactions, the substrate concentration must be high enough to promote a fast substrate-radical reaction. By combining this restriction with that invoked by equation 9 above, one can select for the substrate a suitable concentration range, within which pseudo-first order kinetics prevails.

The substrates on which kinetic measurements were performed were either lignin model compounds or carbohydrates. To elucidate how the reactivity of these substrates varied with pH, measurements were performed over a range of pH values, Table 2.

## 6.6 Radical spectra

Radical spectra were obtained from measurements at different wavelengths of the optical absorbance observed immediately after the electron pulse. The dose per pulse was calibrated against the KSCN-dosimeter. An extinction coefficient of  $7900 \text{ M}^{-1}\text{cm}^{-1}$  at 500 nm was used for the  $(\text{SCN})_2^{\bullet-}$  radical (NATO Advanced Study Institutes Series, D).

## 6.7 $\gamma$ -irradiation

Using a Gammacell 220  $^{60}\text{Co}$   $\gamma$ -source, known amounts of free-radicals were generated and reaction products could be obtained. For dosimetry, a Fricke dosimeter was employed (Choppin *et al.* 1995).

## 6.8 Formation and recovery of formic acid

Aqueous solutions of D-glucose (1mM) were saturated with  $\text{N}_2\text{O}/\text{O}_2$  (80:20 v/v) before and during irradiation. Irradiations were carried out at room temperature and in solutions around pH 8 and pH 12. The pH was maintained by adding to the solutions 1 M  $\text{KHCO}_3$  (pH ~8), 1 M  $\text{K}_2\text{CO}_3$  (pH ~12) or  $10^{-2}$  M sodium hydroxide (pH 12). The concentration of bromide was



0.05 M. The solutions containing the reactants were irradiated at a dose rate of 0.05 Gy/s until approx. 20 % of the glucose substrate was converted.

Perchloric acid (HClO<sub>4</sub>) was added to the irradiated solution. This resulted in the removal of most K<sup>+</sup> ions due to precipitation of KClO<sub>4</sub>(s), as well as the conversion of CO<sub>3</sub><sup>2-</sup> to CO<sub>2</sub> due to the pH being lowered to 2-3. Subsequently, formic acid was extracted into diethyl ether, using a Soxhlet type of extraction equipment designed for lighter solvents. After extraction over-night, the ether phase was made alkaline by addition of 1 M NaOH. Formate ions could then be extracted into the water phase, the latter being the proper solvent for ion-chromatography.

### **6.9 $\gamma$ -irradiation and analysis of products formed by $\gamma$ -irradiation of Methyl- $\beta$ -D-glucoside and Methyl- $\beta$ -D-cellobioside.**

Aqueous solutions of Methyl- $\beta$ -D-glucoside and Methyl- $\beta$ -D-cellobioside (5mM) were saturated with N<sub>2</sub>O or N<sub>2</sub>O/O<sub>2</sub> (80:20 v/v) before and during irradiation. Irradiations were carried out at ~4 °C (ice bath) in solutions around pH 10. The pH was maintained by adding to the solutions 0.5 M KHCO<sub>3</sub> and 0.5 M K<sub>2</sub>CO<sub>3</sub>. The solutions containing the reactants were irradiated at a dose rate of 0.04 Gy/s until approx. 10-15 % of the substrate was converted.

To decrease the ion concentration, perchloric acid (HClO<sub>4</sub>) was added to the irradiated solutions, at 4° C (ice bath). This resulted in the removal of most K<sup>+</sup> ions due to precipitation of KClO<sub>4</sub>(s), as well as the conversion of CO<sub>3</sub><sup>2-</sup> to CO<sub>2</sub> due to the pH being lowered to 2-3. The pH was then immediately adjusted to pH ~7 by adding NaOH, the pH was determined by pH-meter.

GC/MS analyses were conducted using a Finnigan SSQ 7000 GC-MS system including a Varian 3400 GC. EI-mass spectra were obtained at 70 eV with the ion source at 150 °C. Myo-inositol was used as internal standard. The samples were silylated before injection using BSTFA-TMCL (99:1) silylating reagent. The procedure followed was: pyridine (500 ul) and BSTFA-TMCL (500 ul) per 1 mg of freeze-dried residue were added and allowed to react over-night at room temperature. After silylation the samples were dried by evaporation and hexane was added as solvent. Separations were done on a DB-1 capillary column, film thickness 0.25 microns, length 30 m. Myo-inositol was used as internal standard.

Methanol was analysed using a Hewlett and Packard 6890 GC instrument, equipped with a HP-Wax column with a length of 20 m and film thickness of 0.3 microns. Acetone was used as internal standard.

<sup>1</sup>H and <sup>13</sup>C NMR analyses were recorded in D<sub>2</sub>O solution at 25 °C on Bruker 400 or 600 MHz instruments, using acetone ( $\delta_H = 2.225$ ,  $\delta_C = 31.05$ ) as internal reference. From 2-D experiments and 1D experiments, the assignment of the spin-systems was achieved

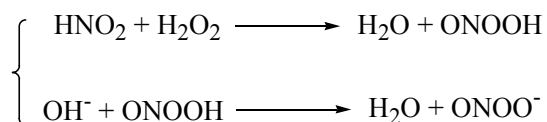
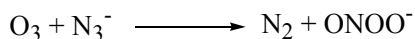
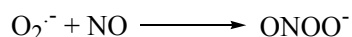
The quantitative determinations of the products were done by GC, using a calibration curve for each compound with myo-inositol as an internal standard, and by NMR.

The hydrogen peroxide and hydroperoxide analysis has been described elsewhere (Patrick *et. al* 1949; Ovenston *et. al* 1950; Nimura *et. al* 1992; Backa *et. al* 1997)

### **6.10 Peroxynitrite**

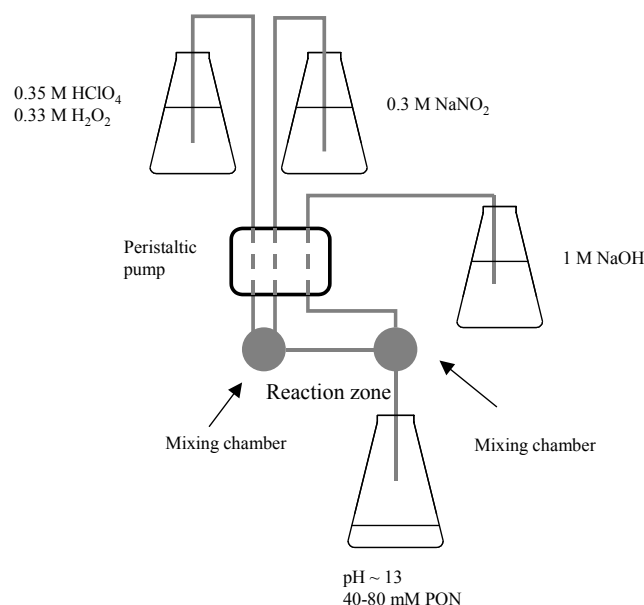
Peroxynitrite, ONOO<sup>-</sup>, was used as a feasible precursor of radicals in pulp and cotton linter suspensions. There are several reported methods for the synthesis of peroxynitrite, such as

photolysis and radiolysis of nitrate, autoxidation of hydroxylamine in alkaline solution, reaction between superoxide and nitrogen monoxide, ozonation of azide and acidic nitrosation of hydrogen peroxide (Uppo *et al.* 1996).



### 6.10.1 Production of peroxyxynitrite

One convenient way of synthesizing peroxyxynitrite in the laboratory is by mixing acidified hydrogen peroxide with nitrite in a flow system and quenching the ONOOH by converting it to ONOO<sup>-</sup> in alkaline solution (Saha *et al.* 1998). For this purpose we assembled the equipment shown in Figure 22.

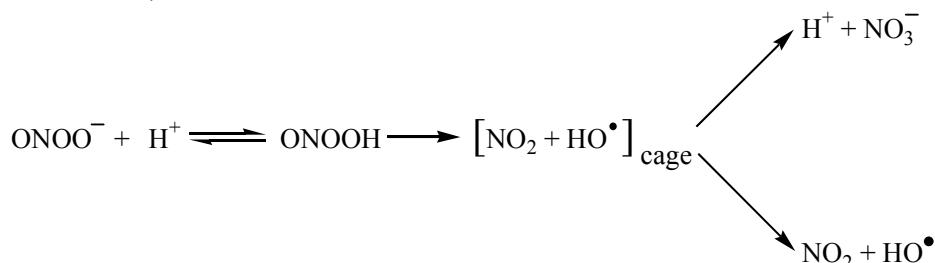


**Figure 22.** Quench- flow reactor for production of peroxyxynitrite (ONOO<sup>-</sup>).

A solution of 0.30 M NaNO<sub>2</sub> was mixed with a solution of 0.35 M H<sub>2</sub>O<sub>2</sub> and 0.3 M HClO<sub>4</sub> to form ONOOH. The reaction mixture was allowed to react for some hundred milliseconds before quenching with 1M NaOH. The ONOO<sup>-</sup> concentration obtained was about 50 mM. In a few preparations the NaOH solution contained 1 mM DTPA to complex metal ions introduced with the salts used. The concentration of ONOO<sup>-</sup> was determined spectrophotometrically at 302 nm using an extinction coefficient of 1670 cm<sup>-1</sup>M<sup>-1</sup> (Goldstein *et al.* 1996). All ONOO<sup>-</sup> solutions produced contained some residual H<sub>2</sub>O<sub>2</sub> (< 10 mM). To further stabilise the solutions, they were stored cold and dark.

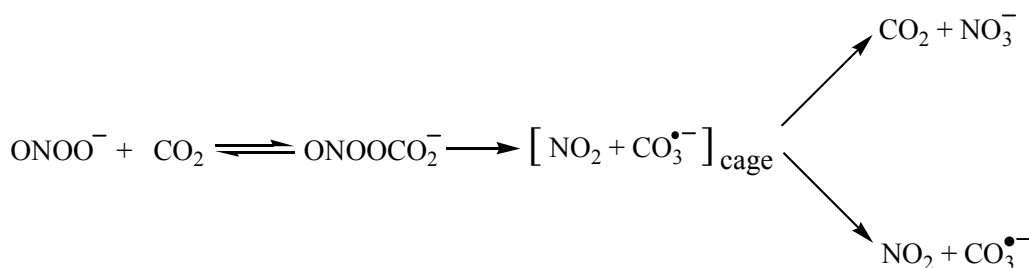
### 6.10.2 Generation of radicals from peroxyxynitrite

The free ion,  $\text{ONOO}^-$  is very stable, and depending on reaction conditions peroxyxynitrite can be used to initiate formation of various reactive free radicals. In acidic solution the conjugate acid,  $\text{ONOOH}$ , (peroxyxynitrous acid,  $\text{pK}_a$  6.5-6.8) decomposes within a second by homolysis of the weak O-O bond (Goldstein *et al.* 1996; Benton *et al.* 1970), yielding about 30 %  $\cdot\text{OH}$  and  $\text{NO}_2$  free radicals, while the remainder recombines to nitric acid in the solvent cage (Goldstein *et al.* 1995):



The lifetime of  $\text{HOONO}$  enables it to diffuse over substantial distances before decomposing into radicals. Therefore this species acts as a vehicle for the hydroxyl radical. This allows more or less homogenous distribution of hydroxyl radicals, which otherwise only would react close to the site of its generation.

The carbonate radical is formed through a rapid reaction ( $k = 3 \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ) between carbon dioxide and  $\text{ONOO}^-$  (Lyman and Hurst 1995):



The intermediate  $\text{ONOOCO}_2^-$  is extremely short lived ( $< 100 \text{ ns}$ ) and has no chemical significance. The yield of  $\text{CO}_3^{\cdot-}$  and  $\text{NO}_2$  has been reported to be about 30%, as calculated per peroxyxynitrite charged (Lyman and Hurst 1995; Hodges and Ingold 1999; Goldstein *et al.* 2001; Goldstein *et al.* 2002).

In the experiments reported here it is assumed that the initial yields of hydroxyl and carbonate radicals from a given peroxyxynitrite are equal.

Using peroxyxynitrite as the radical precursor also involves formation of the nitrogen dioxide. It is also a moderately potent oxidant,  $E_0 = 1.04 \text{ V}$  vs. NHE (Stanbury 1989), capable of one-electron transfer as well as hydrogen abstraction reactions. However, it is not considered to react directly with carbohydrates and cellulose ( $< 0.1 \text{ M}^{-1} \text{ s}^{-1}$ ) (Svetlov *et al.* 1974). In the experiments gas flows were applied to carry away most of the  $\text{NO}_2$  formed, and thus minimize its contribution to the products formed.

### 6.11 Treatments of cotton linters using peroxyxynitrite

Cotton linter treatments were performed in a glass reactor with a metallic stirrer. An even distribution of gas in the fibre suspension was allowed by using a stirrer with built in gas dispenser.

In one series of treatments the cotton linters (5g) were suspended to 2% consistency in 0.25 dm<sup>3</sup> of KHCO<sub>3</sub> (1M) or K<sub>2</sub>HPO<sub>4</sub> (0.1 M) buffer solutions (pH ~8). After 5 minutes, 0.25 dm<sup>3</sup> of alkaline peroxyntirite (pH 13) was pumped into the suspension at a rate of 8 ml/min, generating carbonate or hydroxyl radicals, respectively.

In another series of experiments 5g of cotton linters were suspended to 2% consistency in 0.25 dm<sup>3</sup> of alkaline peroxyntirite solution (pH 13) after which CO<sub>2</sub> (g) or concentrated H<sub>3</sub>PO<sub>4</sub> (aq) was added until all peroxyntirite was consumed (colour change) lowering the pH to ~8. In some treatments, oxygen was removed from the solutions by purging with N<sub>2</sub> (g) prior and during the experiments.

In order to study the accumulated effect of the radicals, the number of treatments with peroxyntirite was repeated. After treatment the samples were washed thoroughly with tap water and stored in the cold and dark until they were analysed.

**Table 3. Treatment of the cotton linters.**

| <i>Sample ID</i> | <i>Initial solution</i>                 | <i>Initial pH</i> | <i>Treatment</i>   | <i>End pH</i> |
|------------------|---|-------------------|--|---------------|
| R1               | Aqueous                                 | 7                 | Washing effect on cotton linters   | 7             |
| R2               | 0.1 M HO <sup>-</sup>                   | 13                | Alkaline washing effect on cotton linters  | 13            |
| R3               | 50 mM ONOO <sup>-</sup>                 | ~13               | Direct effect of ONOO <sup>-</sup> on cotton linters   | 13            |
| E1               | ONOO <sup>-</sup>                       | ~13               | Purging with CO <sub>2</sub> until all ONOO <sup>-</sup> was consumed (5 min)  | ~11           |
| E2               | ONOO <sup>-</sup> N <sub>2</sub> purged | ~13               | Purging with CO <sub>2</sub> until all ONOO <sup>-</sup> was consumed (5 min)  | ~11           |
| E3               | 1 M HCO <sub>3</sub> <sup>-</sup>       | 8.3               | 0.25 dm <sup>3</sup> of ONOO <sup>-</sup> was slowly pumped into the solution  | ~8.5          |
| E4               | 1 M HCO <sub>3</sub> <sup>-</sup>       | ~8.3              | 0.25 dm <sup>3</sup> of ONOO <sup>-</sup> was slowly pumped into the solution, continuous N <sub>2</sub> purging           | ~8.5          |
| E5               | ONOO <sup>-</sup>                       | ~13               | Dropwise addition of 0.1 M H <sub>3</sub> PO <sub>4</sub> until all color disappeared                                      | ~6            |
| E6               | ONOO <sup>-</sup>                       | ~13               | Dropwise addition of 0.1 M H <sub>3</sub> PO <sub>4</sub> until all color disappeared, , continuous N <sub>2</sub> purging | ~6            |
| E7               | 0.1 M KH <sub>2</sub> PO <sub>4</sub>   | ~4                | 0.25 dm <sup>3</sup> of ONOO <sup>-</sup> was slowly pumped into the solution  | ~9            |

In some cases the fully treated cotton linters were subjected to a reducing step before the viscosity was measured.

### 6.12 Sodium borohydride reduction

At 1 % consistency, sodium borohydride, 0.1 g per gram oven dry cotton linters, in aqueous solution was added and left overnight at room temperature.

### 6.13 Cotton linter and pulp characterization

Cotton linters viscosity was determined according to SCAN-CM 15:88 and ISO 5351. Kappa measurements were performed using a standardised method, as described by SCAN C1 and ISO 302. Brightness was determined according to SCAN 11 and ISO 3688. The measurements were performed at STFI and KCL.

### 6.14 Size-exclusion chromatography

In a first step, the cotton linters samples were pre-swelled in water followed by solvent exchange using methanol and N,N-Dimethyl acetamide, DMAC. In the second step, the cotton linters samples were derivatized using ethylisocyanate and dissolved in DMAC/0.8 %LiCl, the treatment being completed after five days at +23 °C (Berthold *et al.* 2001).

The HPSEC system is equipped with a prefilter, precolumn and two PLgel Mixed B columns, the column temperature was 80 °C and DMAc/0.8 % LiCl was used as eluent. The elution curves were monitored with a RI-detector, Erma ERC-7515 A. The columns were calibrated with monodisperse polysaccharides (pullulans) and the molar masses and molar mass distributions were calculated by PL Caliber™, LC/GC Software, version 7. All measurements were performed by Dr Bo Hortling at KCL (Keskuslaboratorio- Centrallaboratorium AB) Finland.

## 6.15 Synthesis of D-*gluco*-hexodialdose and determination of its main forms by NMR.

### 6.15.1 General methods

For the synthetic products NMR spectra were recorded for samples in CDCl<sub>3</sub> at 25 °C on Varian 300 or 400 MHz instruments, using TMS ( $\delta_{\text{H}} = 0.00$ ) as internal reference. Column chromatography was performed on silica gel (0.040-0.063 mm, Amicon) and TLC on Merck precoated 60 F<sub>254</sub> plates with detection by UV light and/or by charring with 8% sulphuric acid.

### 6.15.2 Synthesis of D-*gluco*-hexodialdose

Pyridine (60  $\mu\text{L}$ , 0.74 mmol), trifluoroacetic acid (57, 0.74 mmol) and DCC (0.6 g, 2.6 mmol) were added at rt to a solution of 1,2,3,4-tetra-*O*-benzoyl-D-glucopyranose (**1**, 0.5 g, 0.84 mmol) in DMSO (25 mL) and the solution was stirred overnight. Oxalic acid (1.75 g, 2.94 mmol), dissolved in MeOH (25 mL), was added to the reaction mixture in order to precipitate formed *N,N*-dicyclohexylurea, and after 30 min the mixture was filtered. Toluene and brine were added to the filtrate, and the phases were separated. The organic phase was washed with water, aqueous NaHCO<sub>3</sub> and water. The organic phase was separated, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to dryness. Silica gel chromatography (toluene-EtOAc, 9:1) gave 1,2,3,4-tetra-*O*-benzoyl-D-*gluco*-hexodialdo-1,5-pyranose (**2**, 0.32 g, 65%); <sup>13</sup>C-NMR:  $\delta$  68.4, 69.1, 70.1, 70.4, 70.8, 72.6, 72.7, 87.2 (C-2-5), 89.7, 92.8 (C-1 $\alpha,\beta$ ), 125.6-138.1 (aromatic C), 164.6-166.1 (PhCO), 195.2 (CHO).

Compound **2** (0.2 g, 0.34 mmol) was dissolved in MeOH (10 mL) and the pH was adjusted to 1 by addition of *p*-toluenesulfonic acid. To the solution trimethylorthoformate (0.1 mL, 0.91 mmol) was added and the solution was stirred for 72 h at rt. Pyridine was added to neutralize the solution, and the solution concentrated to dryness. Silica gel column chromatography (toluene-EtOAc, 9:1) of the residue gave 1,2,3,4-tetra-*O*-benzoyl-D-*gluco*-hexodialdo-1,5-pyranose 6-dimethyl acetal (**3**, 0.18 g, 82%); <sup>13</sup>C-NMR:  $\delta$  55.0, 55.7, 68.4, 69.1, 70.1, 70.6, 70.9, 71.2, 72.6, 72.7 (C-2-5), 87.0, 90.1 (C-1 $\alpha,\beta$ ), 102.5, 102.7 (C(OMe)<sub>2</sub>), 125.5-137.8 (aromatic C), 164.2-165.6 (PhCO).

NaOMe (1 mM in MeOH) was added to a solution of **3** (0.18 g, 0.195 mmol) in MeOH (10 mL) until pH 11, and the mixture stirred until the reaction was complete according to TLC (EtOAc-MeOH-HOAc-H<sub>2</sub>O, 12:3:3:1; ~5 h). The solution was neutralized with Dowex 50 (H<sup>+</sup>) ion exchange resin, filtered, and concentrated to yield D-*gluco*-hexodialdo-1,5-pyranose 6-dimethyl acetal (**4**, 55 mg, (81%)), which was used in the next step without further purification. <sup>13</sup>C-NMR:  $\delta$  55.8, 56.1, 71.4, 71.9, 72.2, 73.4, 74.6, 75.9, 76.5, 77.8 (C-2-5), 93.8, 98.4 (C-1 $\alpha,\beta$ ), 103.1, 103.6 (C(OMe)<sub>2</sub>).

An equivalent amount (w/w) of Dowex 50 (H<sup>+</sup>) ion exchange resin was added to a solution of **4** (55 mg, 0.24 mmol) in MeOH/H<sub>2</sub>O (3:2, 5 mL). The mixture was stirred for 24 h, then filtered and evaporated. Silica gel column chromatography (CHCl<sub>3</sub>-MeOH 5:1) of the residue yielded the aldehyde, D-*gluco*-hexodialdose **5** (70%, 30 mg).

### 6.15.3 NMR spectroscopy

NMR spectra were recorded for sample in D<sub>2</sub>O solution at 30 °C on Bruker DRX-400 or DRX-600 instruments, using acetone ( $\delta_{\text{H}} = 2.225$ ,  $\delta_{\text{C}} = 31.05$ ) as internal reference. One- and two-dimensional spectra (COSY, TOCSY, ROESY, HSQC-DEPT and HMBC) were acquired and processed using standard Bruker software. In the TOCSY experiments the mixing times were 60 and 150 ms. The delay time in HMBC was 65 ms and the mixing time in the ROESY experiment 150 ms.

## 7 Results and discussion

The objective of this thesis was to elucidate the effects of the carbonate radical anion on lignin and especially, on carbohydrate structures. In this context we determined the kinetic selectivity (*intermolecular* selectivity) of the carbonate radical anion towards certain lignin and carbohydrate model compounds. Furthermore, the mechanisms by which the carbonate radical reacts with certain carbohydrates were scrutinized.

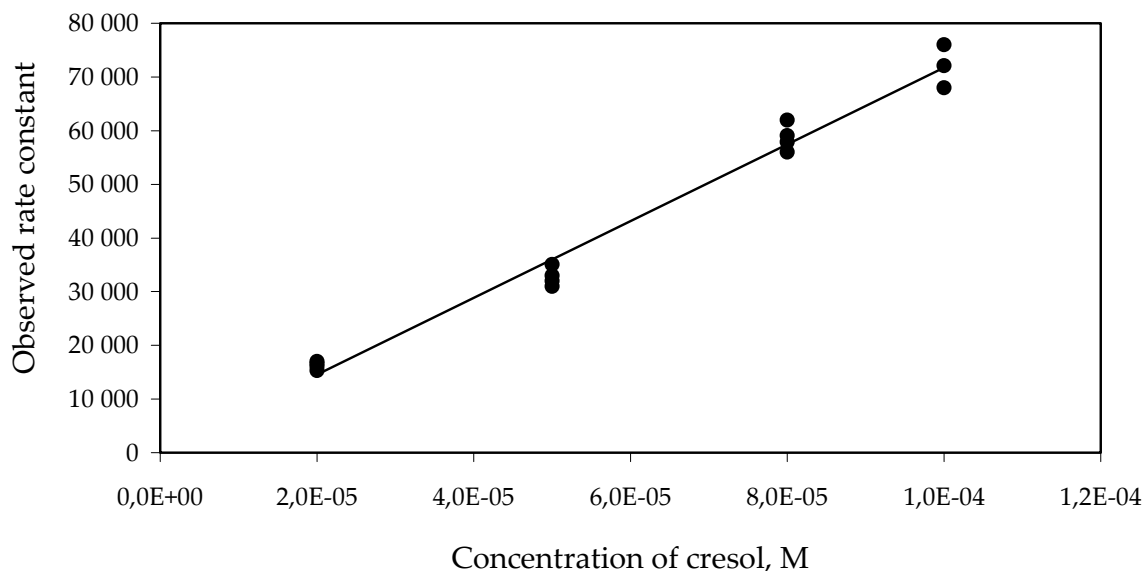
The results from the kinetic experiments prompted us to investigate the pH-dependent mechanism of the carbonate radical anion reacting with glucose.

The degradation of cotton linters by the carbonate radical on the one hand and the hydroxyl radical on the other hand was compared to study whether the carbonate radical is an oxidant that preferentially attacks certain specific sites in carbohydrates. This *intramolecular* selectivity may determine the extent of cleavage of inter-unit bonds. Cotton linters were chosen because this material represents pure high molecular cellulose. The hydroxyl radical is the archetype of a non-selective species in its reactions and would thus be expected to abstract H-atoms statistically, creating all the possible carbon centred radicals.

In part of this study the radicals form during the decomposition of peroxyxynitrite. This compound can generate the carbonate radical in a fast reaction with carbon dioxide or it can produce the hydroxyl radical upon protonation. Thus, depending on conditions, both radicals can be formed without the involvement of transition metal ions, heat, light or radiation.

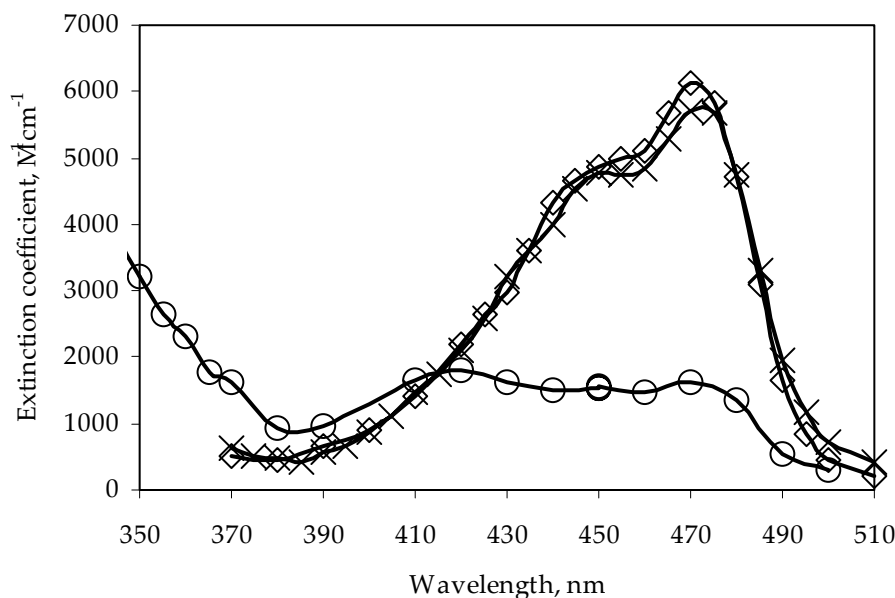
### 7.1 Kinetic reactivity towards lignin model compounds

For the lignin model compounds investigated, kinetic measurements were performed by means of pulse radiolysis. Pseudo-first order reaction rate constants, for the reactions between the carbonate radicals and lignin model compounds, were obtained as function of substrate concentration and pH. When the *pseudo*-first order rate constants were plotted against the substrate concentrations, a straight line was obtained. The slope of the linear plot gives the rate constant of the second order reaction, as described by equations 9 and 12. A typical plot is shown in Figure 23.



**Figure 23.** Pseudo-first order rate constants of  $\text{CO}_3^{\bullet-}$  reacting with cresol (4-methylphenol) at pH 10 versus the cresol concentration

To clarify the mechanism by which the carbonate radical anion reacts with aromatic structures to generate aromatic radical cations, a transient spectrum of 1,2,4-*tri*-methoxybenzene after reactions with different radical oxidants was measured. The transient spectra obtained after irradiation of the solutions are presented in Figure 24.



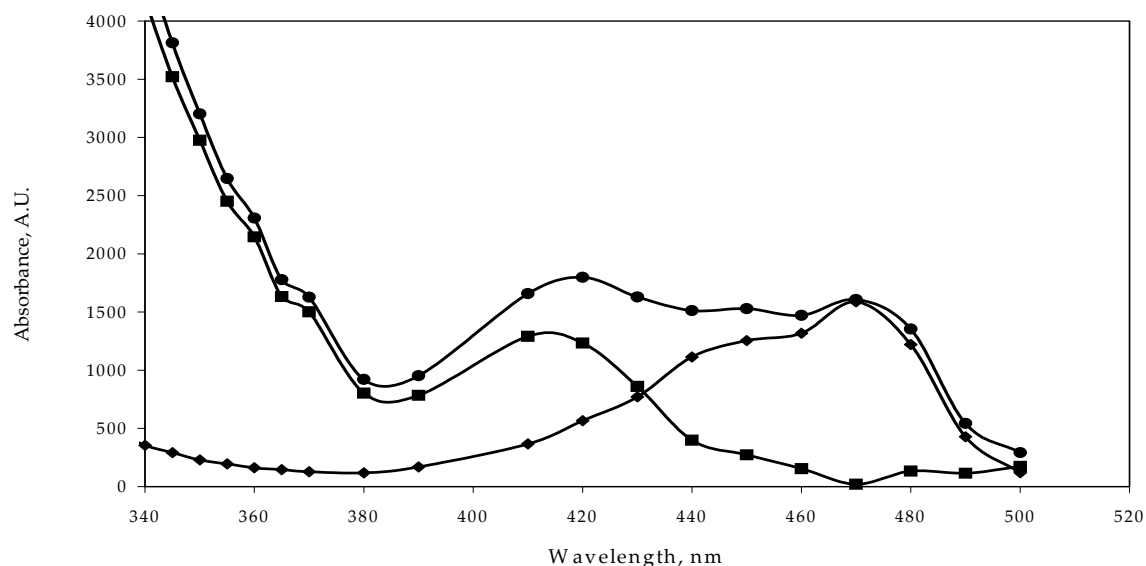
**Figure 24.** Transient spectra after the respective reaction of the carbonate (X), azidyl ( $\diamond$ ) and OH-radicals (O) respectively with 1,2,4-*tri*-methoxybenzene.

The reaction of the carbonate radical anion ( $\text{CO}_3^{\bullet-}$ ) towards 1,2,4-*tri*-methoxybenzene results in a transient species with virtually identical spectral properties to the one formed by the azidyl radical ( $\text{N}_3^{\bullet}$ ) and is distinctly different from that obtained by the hydroxyl radical product, Figure 25. The azidyl radical has been shown to oxidise 1,2,4-*tri*-methoxybenzene quantitatively to the corresponding radical cation (Jonsson *et al.* 1993). Hence it was



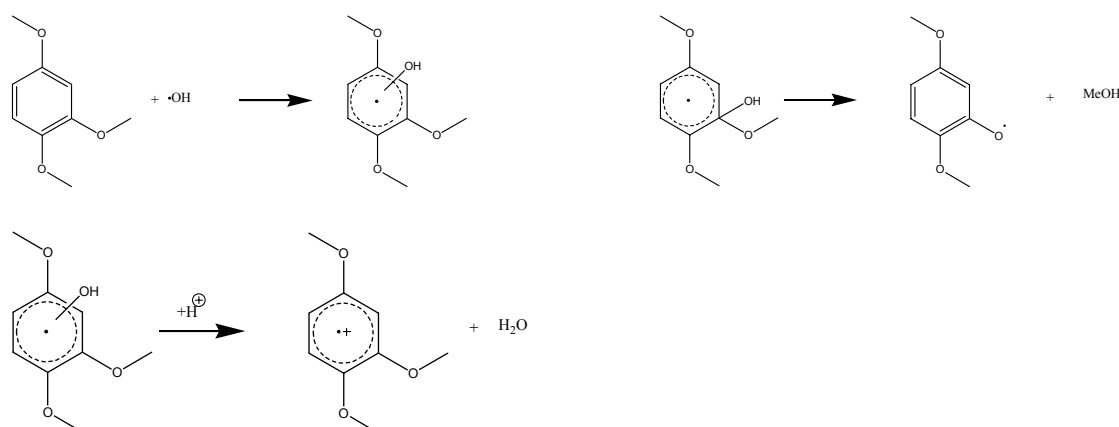
concluded that the reaction of  $\text{CO}_3^{\bullet-}$  with 1,2,4-*tri*-methoxybenzene must proceed *via* electron transfer resulting in the formation of a radical cation. It has been shown that such radical can undergo different transformations and degradations (Russo-Caia and Steenken 2002).

It was noted that the transient spectrum after OH-radical reaction shows some absorption above 380 nm with peaks at 420 and 470 nm. The peak at 470 nm coincides with that of the aromatic radical cation. After deconvolution of the transient OH-radical reaction spectrum with the absorption spectrum of the aromatic radical cation the peak at 420 nm was enhanced, Figure 25.



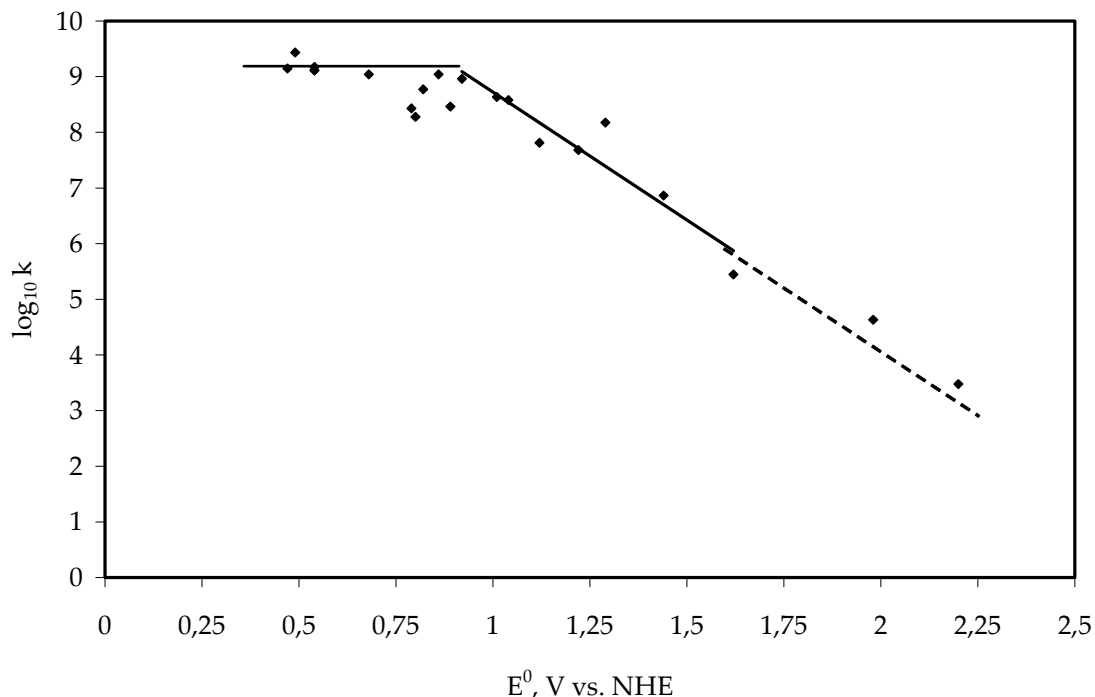
**Figure 25.** Deconvoluted spectrum of 1,2,4-*tri*-methoxybenzene OH-radical adduct (■), original transient spectrum (●) and scaled transient aromatic radical cation spectrum (◆).

The absorption at 420 nm can be explained by the formation of phenoxyl radicals (Moore *et al.* 1977; Lind *et al.* 1990). Reaction of the hydroxyl radical with 1,2,4-*tri*-methoxybenzene thus yields the hydroxyl radical adduct as well as phenoxyl radicals and radical cations according to Figure 26:



**Figure 26.** Reaction of the hydroxyl radical with 1,2,4-*tri*-methoxybenzene.

As can be seen in Figure 27, there is a strong correlation between the one-electron oxidation potential of the substituted benzene and  $\log_{10} k$  for its reactions with the carbonate radical anion. Oxidation potentials below approximately 0.9 V vs. NHE result in diffusion controlled reaction rates, while the reactivity of substrates with potentials above 0.9 V vs. NHE follow a linear free relationship.



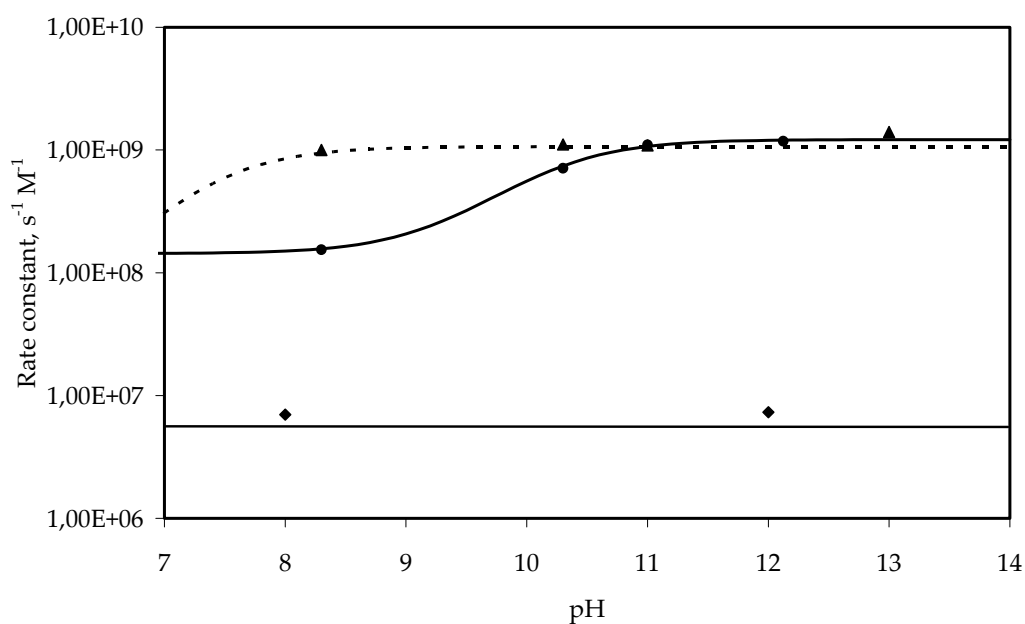
**Figure 27.** The rate constant of reaction of  $\text{CO}_3^{\cdot-}$  reacting with different substituted aromatics ( $\log k$ ) versus their oxidation potentials ( $E_0$  vs NHE).

Using the plot in Figure 27 and existing free energy relationships correlating the oxidation potential of substituted aromatics to their structural properties (Jonsson *et al.* 1993; Jonsson *et al.* 1995), it is possible to evaluate the kinetics of the carbonate radical anion towards most lignin structures.

Since the phenolates ( $A^-$ ) are much easier to oxidize than their corresponding phenols ( $HA$ ), such compounds will show a pH-dependence of their reaction rate with  $\text{CO}_3^{\cdot-}$ . This pH-dependence is described by equation 13:

$$k_{\text{obs}} = \frac{k_{\text{HA}} + k_{\text{A}^-} \cdot 10^{\text{pH}-\text{pK}_\text{A}}}{1 + 10^{\text{pH}-\text{pK}_\text{A}}} \quad 13$$

Using equation 13 the pH behaviour of cresol was calculated, Figure 28. It is further possible to evaluate the kinetics of the pure phenol/phenolate forms using the measured values over a pH-interval. This has been shown for vanillin, Figure 28, where measurements were only possible on the vanillate form. For non-phenolic substances we did not observe any pH-dependence (e.g. *o*-methoxyphenoxyacetic acid).



**Figure 28.** Reaction rates of three substituted benzenes vs pH, Cresol (●), Vanillin (▲) and o-methoxyphenoxyacetic acid (◆). The calculated lines were evaluated using measured values and equation 12.

## 7.2 Reactivity of the carbonate radical anion towards carbohydrates

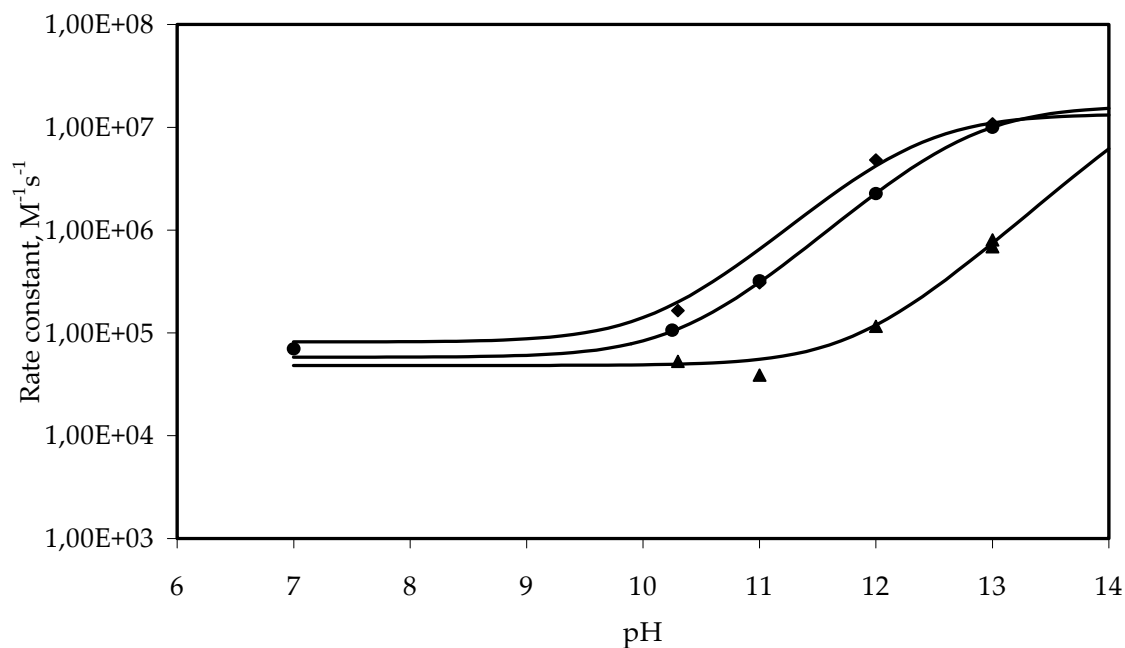
### 7.2.1 Kinetic reactivity of the carbonate radical towards carbohydrate model compounds.

The results of the pulse radiolysis measurements in Figure 29 show that carbohydrates react with the carbonate radical anion with rate constants covering a range of a few times  $10^4$  to ca.  $10^7 \text{ M}^{-1}\text{s}^{-1}$ . Now, since at the doses pertaining to the pulse experiments, the reaction rate of  $\text{CO}_3^{\bullet-}$  with the carbohydrates is rather low compared to the rate of  $\text{CO}_3^{\bullet-}$  reacting with the generated carbohydrate radicals, ca. 2  $\text{CO}_3^{\bullet-}$  will be consumed for every reacting carbohydrate. Given that the rate is monitored by way of the unique 600 nm carbonate radical absorbance, this implies a stoichiometric factor of 2 and hence the true second order rate constants have half the value of the experimentally determined ones.

Interestingly, the kinetics show a pH-dependence and the rate of reaction increases above pH 10. In a series of experiments run at varied temperatures (10-80 °C) there was no observable change in the reaction kinetics of  $\text{CO}_3^{\bullet-}$  towards the carbohydrates, indicating a low activation energy for the reaction.

As the carbonate radical is a monoanion (pH invariant) at all pH-values the observed increase in reactivity must be attributed to the deprotonation of the carbohydrates themselves. Using equation 13, the measured rate constants and a reported  $\text{pK}_A$ -value of 12.4 for glucose (Serjeant and Dempsey 1979), the rate constants can be calculated for the neutral and deprotonated forms. It can be seen that the calculated line correlates well both with reported  $\text{pK}_A$ -values and measured data, Figure 29. Cellobiose, having similar structure and  $\text{pK}_A$  at its reducing end group, shows the same reactivity versus pH as that observed for glucose.

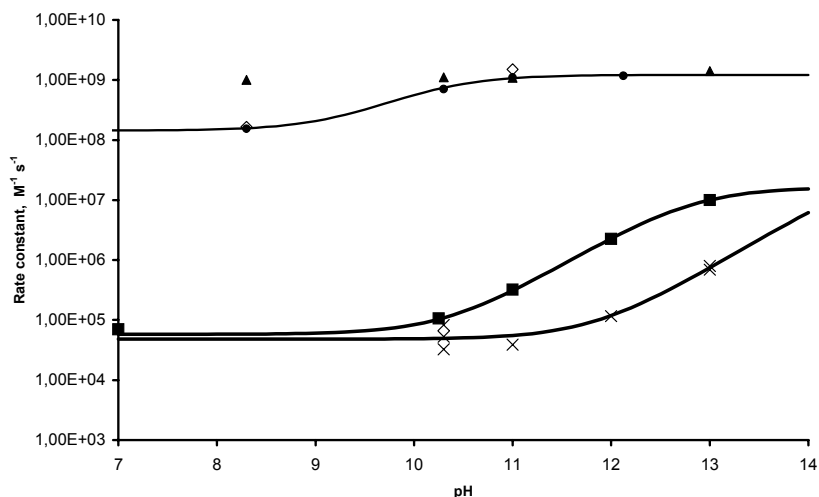
For Methyl- $\beta$ -D-glucopyranoside increased reactivity due to deprotonation was observed at pH above 11. Using equation 13 and the measured rate constants, and assuming that the fully deprotonated form of glucopyranoside reacts as rapidly as fully deprotonated glucose we can estimate the  $pK_A$ -value of Methyl- $\beta$ -D-glucopyranoside to approximately 14.4.



**Figure 29.** Reaction constants of  $\text{CO}_3^{\bullet-}$  towards Glucose (●), Cellobiose (◆) and Methyl- $\beta$ -D-glucopyranoside (▲) as function of pH as measured in  $\text{N}_2\text{O}$ -saturated solutions.

### 7.2.2 The kinetic selectivity of the carbonate radical anion

The kinetic data obtained show that the carbonate radical has a higher reactivity towards lignin-like structures than carbohydrates. This kinetic selectivity varies with pH due to pH dependent activation of phenolic lignin and carbohydrate model compounds. In pulp, the maximum selectivity should therefore occur when most phenols are dissociated and the cellulose (carbohydrates) is undissociated, *i.e.* at pH between 10 to 11, Figure 30.



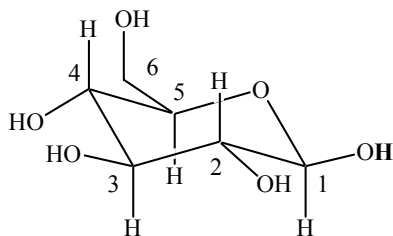
**Figure 30.** Reactivity of  $\text{CO}_3^{\bullet-}$  towards different lignin and carbohydrate model compounds versus pH. Cellobiose (X), glucose (●), vanillin (▲), cresol (●) and creosol (◇).

The increased reactivity observed, as well as the  $\text{pK}_A$ -value obtained for methyl- $\beta$ -D-glucopyranoside, indicates that intra-molecular glucose units in the cellulose polymer should be activated towards oxidative reactions by deprotonation as the pH is raised. The extent of such activation will depend on the oxidant and may be of importance in technical processes running at high pH, *i.e.* pulping and oxygen bleaching.

Regarding selectivity it should be noted that the carbonate radical can attack carbohydrates and would therefore only be beneficial in systems still containing some lignin structures. In such systems the carbonate radical can be said to have a kinetic selectivity by a factor of approximately  $10^3$ - $10^4$  depending on pH and the particular compounds discussed.

### 7.3 The carbonate radical as one-electron oxidant

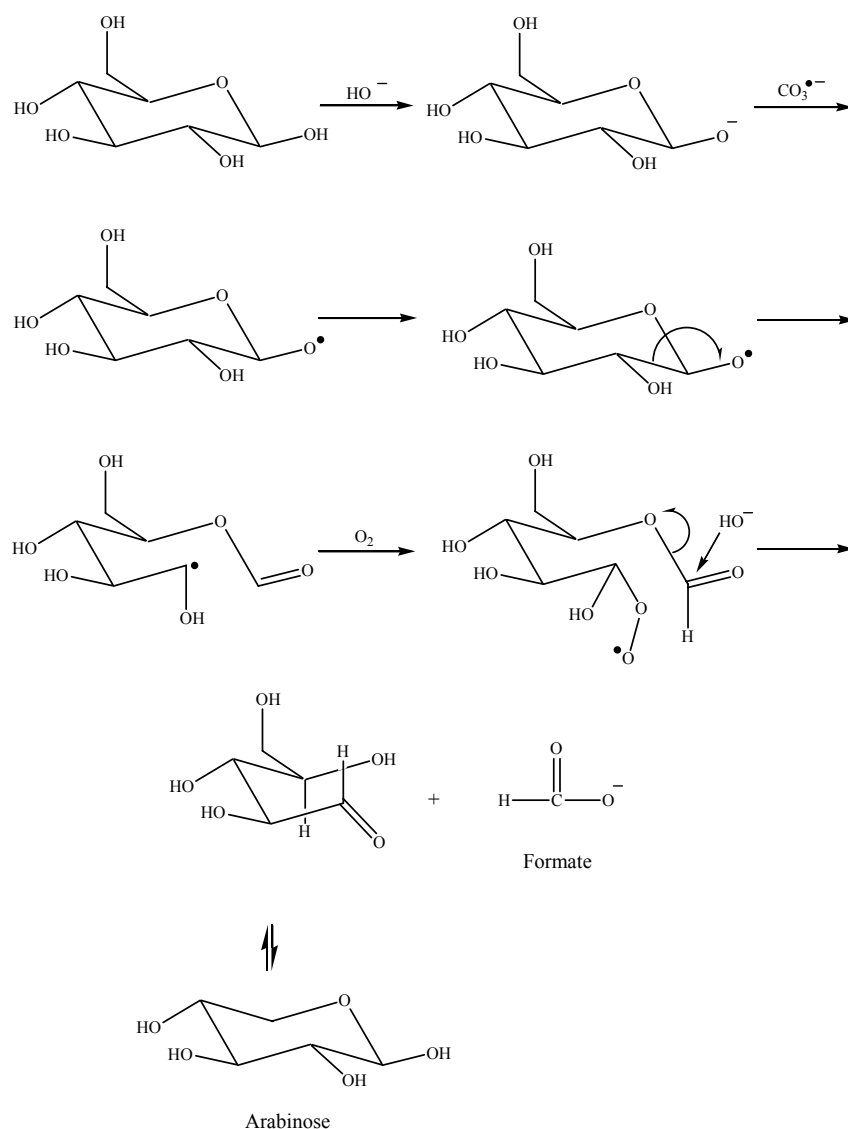
The observed increase in reaction rates of  $\text{CO}_3^{\bullet-}$  towards carbohydrates indicates that the reaction may proceed via an electron transfer mechanism when the carbohydrates are deprotonated. Experiments run at different temperatures (60 to 90  $^{\circ}\text{C}$ ) did not reveal a significant change in activation energy with pH. The most acidic hydrogen in glucose is believed to be the one attached to an oxygen atom on the C-1 carbon, Figure 31.



**Figure 31.**  $\beta$ -D-glucopyranoside

This is because this moiety has a gem-diolic structure and the  $\text{pK}_a$  of the latter is known to be lower by ca. 2 units than that of a corresponding normal alcohol (cf.  $\text{pK}_a(\text{CH}_3\text{OH}) = 15.7$  with  $\text{pK}_a(\text{CH}_2(\text{OH})_2) = 13.3$ ) (Serjeant and Dempsey 1979). For glucose the  $\text{pK}_a$ -value was determined to be 12.2 (Stenman *et al.* 2003). It occurred to us that abstraction of an electron

from the deprotonated oxygen at C-1 in glucose should eventually result in the formation an alkoxyl radical, which, by a  $\beta$ -scission between C-1 and C-2, eventually ends in the formation of formic acid as a unique product. After the  $\beta$ -scission the remaining part may, in the presence of oxygen, give rise to arabinose, Figure 32.



**Figure 32.** A proposed one-electron reaction mechanism between the carbonate radical and D-glucose in alkaline media and formation of formic acid and arabinose.

In the absence of oxygen the formic acid yield should be unaffected, whereas the arabinose yield should decrease, at the expense of other possible products.

The formation of formic acid would thus be a proof for one-electron oxidation of glucose. To investigate such an electron-transfer reaction experiments were performed using the carbonate as well as the dibromidyl ( $\text{Br}_2^{\bullet-}$ ) and the azidyl ( $\text{N}_3^{\bullet}$ ) radicals under similar conditions. The latter radicals are well known one-electron oxidants with no tendency to abstract hydrogen atoms from C-H bonds (Stanbury 1989; Alfassi and Schuler 1985). The yields of formic acid were determined as described in the methods section of this thesis and are summarized in Table 4 including the maximum yields possible under prevailing conditions.

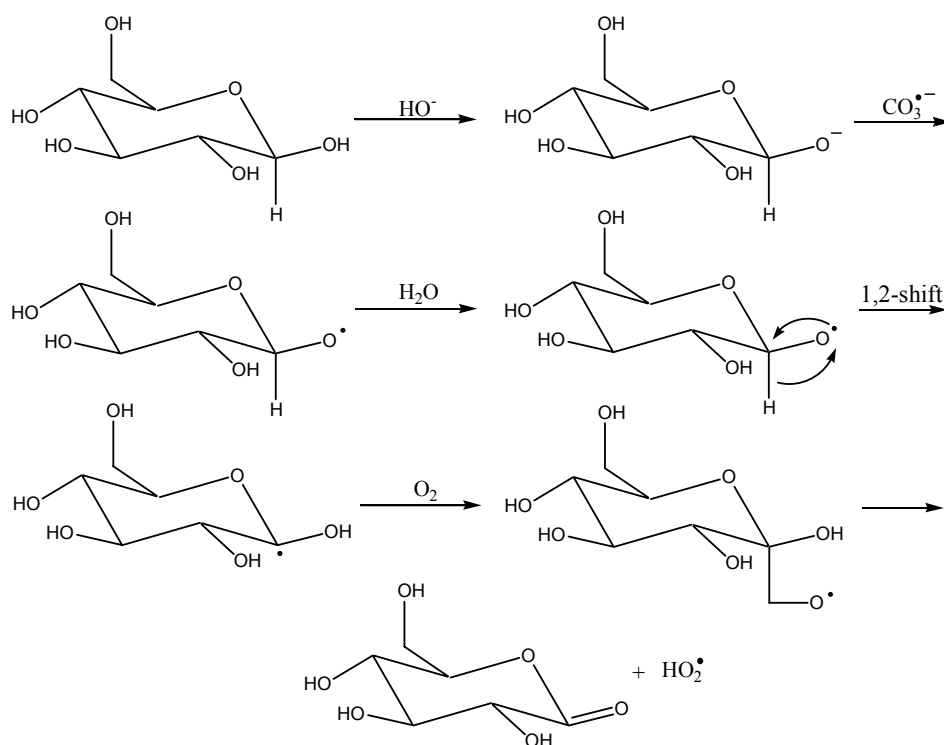
**Table 4.** Yields of formic acid due to oxidation of D-Glucose by  $\text{CO}_3^{\cdot-}$ ,  $\text{Br}_2^{\cdot-}$  and  $\text{N}_3^{\cdot}$  radicals in the presence and absence of oxygen.

| Radical                        | Measured yields of Formic acid<br>mM |        | Maximum<br>possible yields<br>mM | Procentual yields of formic acid |        |
|--------------------------------|--------------------------------------|--------|----------------------------------|----------------------------------|--------|
|                                | Oxygenated                           | Anoxic |                                  | Oxygenated                       | Anoxic |
| $\text{CO}_3^{\cdot-}$ , pH 12 | 0.066                                | 0.064  | 0.12                             | 55                               | 53     |
| $\text{CO}_3^{\cdot-}$ , pH 8  | trace                                | Trace  | 0.11                             | -                                | -      |
| $\text{Br}_2^{\cdot-}$ , pH 12 | 0.057                                | 0.055  | 0.11                             | 52                               | 50     |
| $\text{N}_3^{\cdot}$ , pH 12   | 0.056                                | 0.060  | 0.11                             | 52                               | 55     |

The reaction of  $\text{CO}_3^{\cdot-}$  with glucose in alkaline (pH 12) solution resulted in the formation of formic acid, while in a solution at pH 8 only traces of formic acid were observed. The yield of formic acid is also, within experimental error, independent of the presence of oxygen. Most importantly, the yields with the typical one-electron oxidants,  $\text{Br}_2^{\cdot-}$  and  $\text{N}_3^{\cdot}$  are, within experimental uncertainty, identical to the  $\text{CO}_3^{\cdot-}$  induced yield. These results lend strong support to the reaction mechanism depicted in Figure 24, *i.e.* the carbonate radical anion reacts with deprotonated glucose (pH 12) exclusively by way of a one-electron transfer which yields formic acid *via* a rapid  $\beta$ -scission of the alkoxy radical. Since alkoxy radicals are generally unreactive towards  $\text{O}_2$  and since the  $\beta$ -scission will be rapid in water (Avila *et al.* 1993), one can readily understand why  $\text{O}_2$  does not influence the formic acid yield.

The measured yield of arabinose was found to be 0.043 mM. While not exactly equal, the difference in yields between formic acid and arabinose can be explained by the losses during the derivatisation of arabinose prior to analysis.

At high pH all the oxidants gave rise to ~50% formic acid based on the glucose consumed. Clearly, the alkoxy radical formed must also undergo another mode of reaction than that depicted in Figure 33. According to literature alkoxy radicals bearing  $\text{C}_\alpha$ -hydrogens can undergo a 1,2-hydrogen shift (Gilbert *et al.* 1976). In the presence of oxygen the resulting carbon-centered radical reacts rapidly with oxygen. For glucose this reaction would then give rise to gluconic acid by elimination of superoxide:



**Figure 33.** Formation of gluconic acid from the alkoxy radical by a 1,2-hydrogen shift.

The efficiency of the 1,2-hydrogen shift as compared to  $\beta$ -scission varies with the structure of the alkoxy radical (Konya *et al.* 2000). It has recently been shown (Nakao *et al.* 1999; Hodges and Ingold 1999; Merényi *et al.* 2002) that the alkoxy  $\text{CH}_3\text{CH}(\text{OH})\text{O}^\bullet$  which is a  $\alpha$ -hydroxyl alkoxy radical, undergoes approximately 50% scission and 50% 1,2-hydrogen shift. The measured yield of gluconic acid was found to be 0.07 mM, which is nearly equal to the yield of formic acid. Obviously, the alkoxy radical of glucose has a close structural relationship to  $\text{CH}_3\text{CH}(\text{OH})\text{O}^\bullet$ . It can thus be expected to undergo  $\sim 50\%$  of each reaction mode.

#### 7.4 Synthesis and determination of the main forms of D-gluco-Hexodialdose dialdehyde in aqueous solution.

In addition to several other degradation products, D-gluco-hexosialdose is also expected to be found due to the radical oxidation of carbohydrates, *i.e.* radical oxidation at C-6 of D-glucose (Schuchmann M.N. and von Sonntag C 1977). In order to analyse the products caused by radical oxidation of carbohydrate model compounds D-gluco-hexosialdose was needed as a reference compound.

Although several methods to convert D-glucose into a dialdehyde have been published (Fischer F. G. and Schmidt, H. 1960; Dahlhoff W. V. *et al.* 1980), the final product, the D-gluco-hexosialdose, has never been characterised by NMR spectroscopy. Hence, the different forms of glucose dialdehyde in aqueous solution are largely unknown. The objectives of this work were therefore two: i) to synthesise D-gluco-hexosialdose and ii) to establish the forms that D-gluco-hexosialdose obtains in an aqueous solution. With the aid of (1- $^{13}\text{C}$ )-D-glucose and by recording  $^1\text{H}$  and  $^{13}\text{C}$  1-D NMR together with 2-D experiments, it was possible to assign the spin systems the main components of D-gluco-hexosialdose in aqueous solution.

The synthesis of D-gluco-hexosialdose is given in the experimental section.



#### 7.4.1 *NMR-assignments and structures of the main components*

The NMR parameters of the most abundant species, structures 1-7, are given in Table 5 ( $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts,  $^1\text{H}$ - $^1\text{H}$  coupling constants, HMBC, ROE correlations), and the suggested structures of the main components are given in Figure 34.

A sample was prepared by dissolving *D-gluco*-hexodialdose in  $\text{D}_2\text{O}$  and this sample was analysed by NMR spectroscopy. The  $^1\text{H}$  spectrum displayed a number of overlapping peaks, *inter alia*, signals corresponding to six anomeric protons at  $\delta$  4.63, 5.26, 5.38, 5.43, 5.48, and 5.65. The  $^{13}\text{C}$  spectrum showed signals from seven anomeric carbons at  $\delta$  92.9, 96.9, 99.1, 99.8, 102.7, 104.7 and 106.0. The assignment of each spin-system started with the anomeric proton signal and then the other ring and exocyclic proton signals were assigned by different 2D experiments. The  $^{13}\text{C}$  signals were then assigned by HSQC and HMBC experiments. From the results of the 1-D and the different 2-D experiments together with observed coupling constants, assignment of the different spin-systems to specific components was allowed.

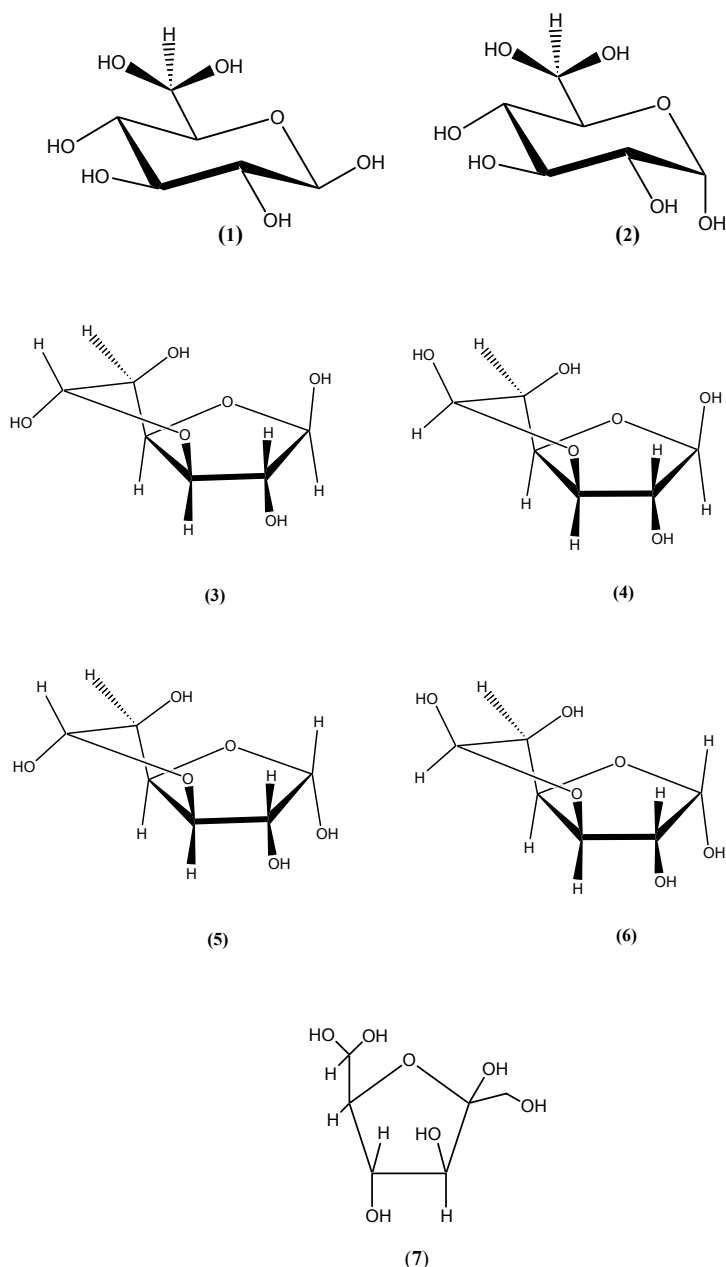
The anomeric resonances at  $\delta$  5.26 and 4.63 in the  $^1\text{H}$  spectrum, and  $\delta$  92.9 and 96.9 in the  $^{13}\text{C}$  NMR spectrum are assigned to the  $\alpha$ - and  $\beta$ -pyranose forms of *gluco*-hexodialdose and the resonance at  $\delta$  88.6 and 88.8 are assigned to the hydrate form of C-6. Both  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts as well as coupling constants are in good agreement with respective glucopyranose, see Tables 5. This information confirmed by H-C correlations, revealed that the components **(1)** – **(2)** attributed to these resonances are  $\alpha$ - and  $\beta$ -*gluco*-1,5-pyranose-6-hydrate.

From the  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts, coupling constants, HMBC and ROE correlations, Table 1 components **(3)** - **(6)** could be assigned to  $\alpha$ - and  $\beta$ -bicyclic *gluco*-3,6-furanoside-hydrates, and component **(7)** to fructo-2,5-furanoside hydrates. From the NMR data it was also possible to determine the position ( $\alpha$  or  $\beta$ ) of the hydroxyl groups on C-1 and C-6. For components **(3)** and **(4)** the hydroxyl group is in a  $\beta$ -position at C-1, trans to the hydroxyl group on C-2, for conformers **5** and **6** in an  $\alpha$ -position, cis to the hydroxyl group on C-2. The hydrate on C-6 is  $\beta$ , trans to the hydroxyl group on C-5, for conformations **(4)** and **(5)**, and in an  $\alpha$ -position for **(3)** and **(6)**, cis to the hydroxyl group on C-5.

From the measurements of chemical shifts, only small amounts of free aldehyde could be detected (<1%). The majority of the conformers found exist as hydrated aldehydes, in monocyclic or bicyclic forms in aqueous solution.

**Table 5.**  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts for the components of *gluco*-hexodialdose.

| Comp.  | H/C-          | H/C-         | H/C-         | H/C-         | H/C-         | H/C-          | Couplings   | HMBC corr.   | ROE   |
|--|---------------|--------------|--------------|--------------|--------------|---------------|---|--|---|
| <b>1. 20%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift: | 4.63<br>96.9  | 3.24<br>74.8 | 3.48<br>76.4 | 3.50<br>71.2 | 3.40<br>77.3 | 5.23<br>88.6  | $J_{1,2}=8.1$ ,<br>$J_{2,3}=8.1$ ,<br>$J_{4,5}=9.5$ ,<br>$J_{5,6}=2.1$ ,  | C-1:H-2,5<br>C-3:H-1,2,4,5<br>C-4:H-5, C-5:H-1<br>C-6:H-5                            | H-1:H-2<br>H-3:H-5                                    |
| <b>2. 16%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift: | 5.26<br>92.9  | 3.53<br>72.2 | 3.71<br>73.4 | 3.49<br>70.8 | 3.77<br>73.0 | 5.25<br>88.8  | $J_{1,2}=3.8$ ,<br>$J_{2,3}=9.5$ ,<br>$J_{3,4}=9.5$ ,<br>$J_{4,5}=10.0$ ,<br>$J_{5,6}=2$ ,<br>$J_1(\text{C,H})=170.4$ | C-1:H-2,5<br>C-2:H-1,3<br>C-3:H-1,2,5<br>C-4:H-5<br>C-5:H-1,4,6<br>C-6:H-4,5         |   |
| <b>3. 6%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift:  | 5.38<br>106.0 | 4.27<br>81.0 | 4.53<br>86.5 | 4.79<br>82.6 | 4.31<br>72.9 | 5.30<br>97.5  | $J_{1,2}<1$ ,<br>$J_{2,3}=1.8$ ,<br>$J_{3,4}=5.1$ ,<br>$J_{4,5}=5.4$ ,  | C-1:H-3,4<br>C-3:H-1,6<br>C-4:H-1,3,6<br>C-5:H-4, C-6:H-3,4                          | H-2:H-(3),6<br>H-3:H-4,5,6<br>H-4:H-5<br>H-5:H-6      |
| <b>4. 18%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift: | 5.43<br>104.7 | 4.17<br>79.8 | 4.67<br>85.6 | 4.85<br>83.0 | 4.03<br>76.7 | 5.35<br>102.7 | $J_{1,2}<1$ , $J_{2,3}<1$ ,<br>$J_{3,4}=5.0$ , $J_{4,5}=5$<br>$J_{5,6}=4.7$ ,<br>$J_1(\text{C,H})=173.2$              | C-1:H-3,4<br>C-2:H-3<br>C-3:H-1,2,4,5,6<br>C-4:H-1,2,3,6                             | H-1:H-5<br>H-2:H-3<br>H-3:H-4,5<br>H-4:H-6            |
| <b>5. 15%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift: | 5.48<br>99.8  | 4.16<br>76.1 | 4.68<br>85.3 | 4.81<br>79.2 | 3.99<br>76.2 | 5.21<br>102.5 | $J_{1,2}=3.9$ ,<br>$J_{2,3}=2.0$<br>$J_{3,4}=5.1$ ,<br>$J_{4,5}=5.1$<br>$J_{5,6}=4.1$<br>$J_1(\text{C,H})=173.0$      | C-1:H-2,4<br>C-2:H-1,3<br>C-3:H-1,4,5,6<br>C-4:H-1,2,3,5,6<br>C-5:H-4<br>C-6:H-3,4,5 | H-1:H-2<br>H-2:H-3<br>H-3:H-4<br>H-4:H-5,6<br>H-5:H-6 |
| <b>6. 6%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift:  | 5.65<br>99.1  | 4.27<br>77.2 | 4.53<br>86.1 | 4.73<br>78.5 | 4.15<br>72.5 | 5.32<br>96.9  | $J_{1,2}=3.9$ , $J_{2,3}<2$<br>$J_{3,4}=5.3$ ,<br>$J_{4,5}=5.3$<br>$J_{5,6}=5.3$                                      | C-2:H-1<br>C-3:H-1,2,6<br>C-4:H-1,6<br>C-6:H-1                                       | H-2:(H-3)<br>H-3:H-4<br>H-4:H-5<br>H-5:H-6            |
| <b>7. 9%</b><br>$^1\text{H}$ shift:<br>$^{13}\text{C}$ shift:  | 3.57<br>63.2  | 102.7        | 4.11<br>76.3 | 4.21<br>76.4 | 3.65<br>83.1 | 5.02<br>91.7  | $J_{3,4}=7.9$<br>$J_{4,5}=7$<br>$J_{5,6}=6.1$   | C-1:H-3, C-2:H-1,5<br>C-3:H-1,4<br>C-4:H-3,6<br>C-6:H-4,5                            | H-4:H-6<br>H-5:H-1                                    |



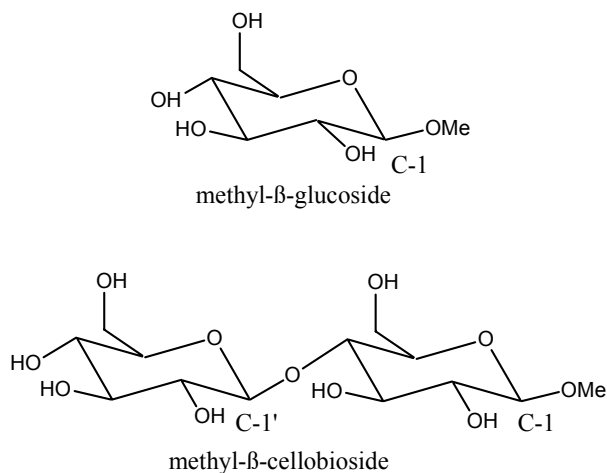
**Figure 34.** Structures of the main components.

## 7.5 The intramolecular selectivity of the carbonate radical anion in its reactions with methyl-β-D-cellobioside and methyl-β-D-glucoside.

### 7.5.1 Why study the title compounds?

At pH-values below ca. 11 the  $\text{CO}_3^{\cdot-}$  radical reacts with carbohydrates with pH-independent rate constants, whose values are well below ca.  $10^5 \text{ M}^{-1}\text{s}^{-1}$ . Previously we have determined this rate constant to be  $2.3 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$  for methyl-β-D-glucoside. In the present study this rate constant was measured at pH 10.3 with methyl-β-D-cellobioside to be  $3.4 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ , i.e., almost the same as with methyl-β-D-glucoside. These rate constants are significantly lower than those between  $\text{CO}_3^{\cdot-}$  and aromatic lignin constituents, the latter having rate constant of  $10^7 \text{ M}^{-1}\text{s}^{-1}$  or higher. This comparison implies that lignin would protect cellulose quite efficiently. However, once the lignin content has decreased to very low values,  $\text{CO}_3^{\cdot-}$  would

eventually react with cellulose. It is therefore important to find out, what sites on cellulose the  $\text{CO}_3^{\cdot-}$  radical would attack. To simplify matters we have chosen the two simplest cellulose mimics for study. These are methyl- $\beta$ -D-glucoside with one and methyl- $\beta$ -D-cellobioside with two glucosidic linkages Figure 35.

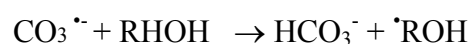


**Figure 35.** methyl- $\beta$ -D-glucoside and methyl- $\beta$ -D-cellobioside

$\text{CO}_3^{\cdot-}$  was generated radiolytically in the presence of oxygen and both compounds were brought to react with  $\text{CO}_3^{\cdot-}$ . Afterwards the products were analysed. The results reveal that the preferred reaction of  $\text{CO}_3^{\cdot-}$  with methyl- $\beta$ -D-glucoside is hydrogen abstraction from the C1-H bond. In the case of methyl- $\beta$ -D-cellobioside, more than  $\frac{3}{4}$  of the  $\text{CO}_3^{\cdot-}$  radicals attack the C1-H and the C1'-H bonds, respectively. These reaction modes give rise to a rupture of the glucosidic linkage.

#### 7.5.2 Why does $\text{CO}_3^{\cdot-}$ preferentially attack glucosidic C-H bonds?

The high selectivity of  $\text{CO}_3^{\cdot-}$  attack at glucosidic C-H bonds may have thermodynamic or kinetic reasons. One might assume that the C1-H (C1'-H) bond is significantly weaker than other C-H bonds in carbohydrates. There are no reliable thermodynamic data to establish this point. However, we can assume that all the C-H bonds in the carbohydrate are as weak or weaker than the C-H bond in isopropanol,  $(\text{CH}_3)_2(\text{OH})\text{C-H}$ , the latter having a bond strength of approximately 92 - 94 kcal/mol. From known thermodynamic data we calculate that, irrespective of C-H bond, the exergonicity of the hydrogen abstraction reaction is larger than 13 kcal/mol.



Consequently, one would not expect thermodynamics to be the decisive factor. This view is supported by the finding that, the sulfate radical anion,  $\text{SO}_4^{\cdot-}$ , also displays significant selectivity towards small carbohydrates, even though its hydrogen abstraction reactions are much more exergonic than those of  $\text{CO}_3^{\cdot-}$ . It would then seem that the effect is main kinetic. This implies that the transition state of C1-H hydrogen abstraction is stabilized as compared to those from other C-H bonds. This comes about from the presence of a relatively low-lying charge transfer state, consisting of carbohydrate radical cation and  $\text{CO}_3^{2-}$ , whose energy is close to the transition state of the pure C-H homolysis. If so, there is an efficient interaction

between the two “pure” states, resulting in the lowering of the energy of the overall transition state. It is easy to understand why a charge transfer state would have lower energy for the C1-radical as compared to the others. Clearly, the positive charge on the C-1 atom will be stabilized much more than that on any other carbon atom, C1 being flanked by two oxygens rather than by one or none.

## **7.6 Cotton linters experiments**

The reaction of the carbonate radical with the simple model compounds resulted gave mainly rise to the rupture of the glucosidic bonds. From a practical point of view this result is rather disappointing. It points namely to the possibility that the carbonate radical might depolymerise cellulose when reacting with lignin-deficient pulp. However, it is well to remember that simple carbohydrates may not present a true picture of a polymeric system as complex as cellulose. With this point in mind we have undertaken a study of the reactivity of the carbonate radical with authentic cotton linters. For comparative purposes the cotton linters were also reacted with the hydroxyl radical. Peroxynitrite with or without added carbon dioxide was utilized as the chemical reservoir of both of these radicals.

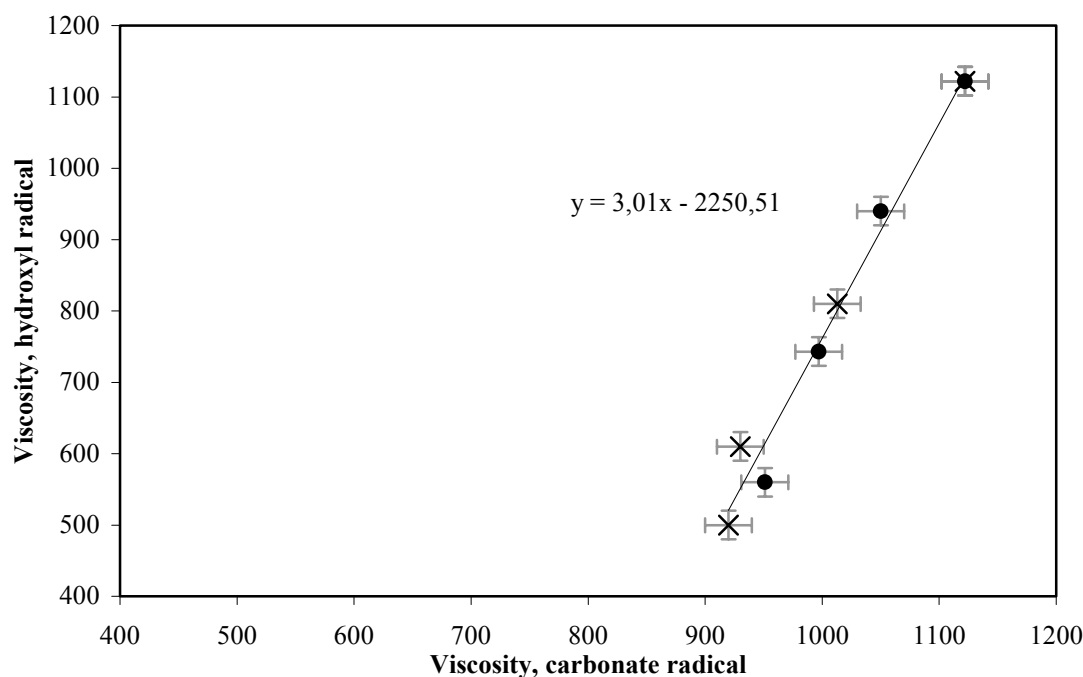
### *7.6.1 References and direct reactions between peroxynitrite and cotton linters*

When cotton linters were treated only with peroxynitrite at high pH, where the latter is reasonably stable, no significant consumption of peroxynitrite was observed over the hour scale. Thus, under the reaction conditions applied, the observed consumption of peroxynitrite must be due to radical formation.

### *7.6.2 Radical reactions on cotton linters induced by peroxynitrite*

From the experimental data, the viscosity changes in the cotton linters caused by carbonate and hydroxyl radicals can be compared.

In Figure 36, degradations of cotton linters by hydroxyl and carbonate radicals are compared at the same charge of peroxyntirite.

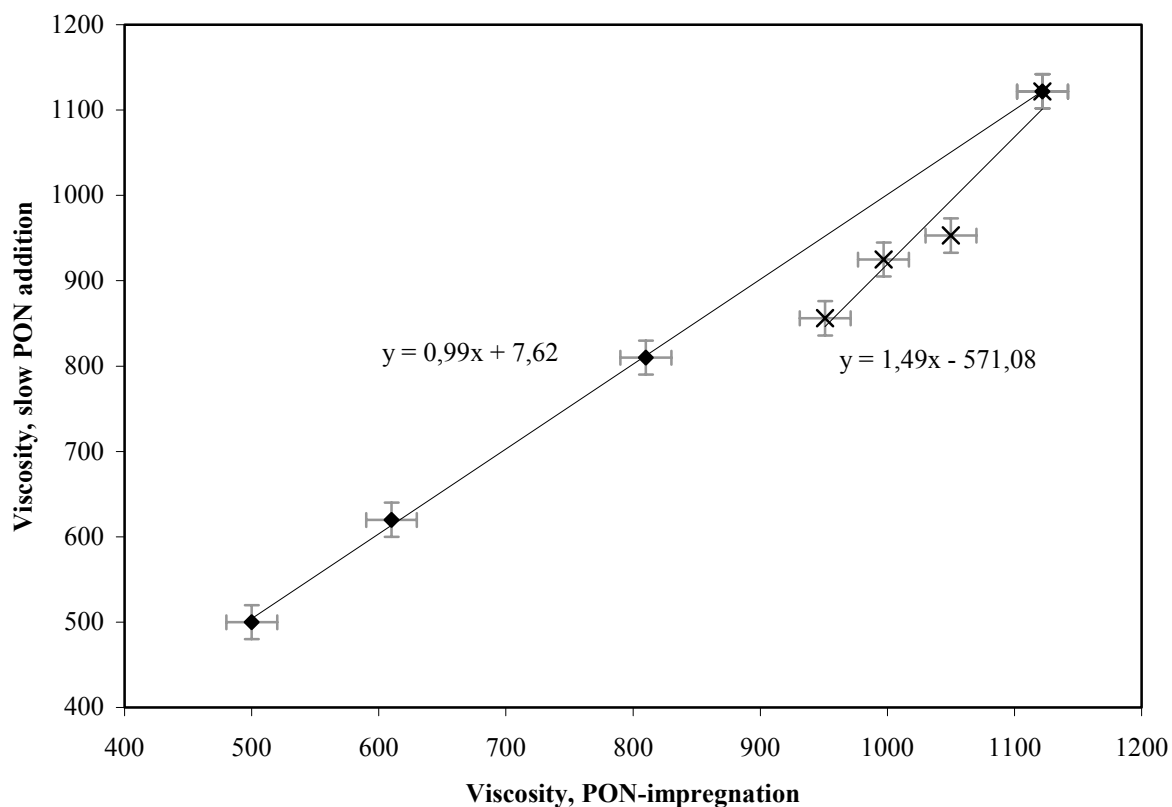


**Figure 36.** Viscosities of hydroxyl and carbonate radical degraded cotton linters in the presence (×) and absence (•) of oxygen

As shown in Figure 36, hydroxyl radical degradation is more extensive compared to the degradation caused by the carbonate radical. As the hydroxyl radical reacts with carbohydrates about  $10^4$  times faster than the carbonate radical (Stenman *et al.* 2003), we can expect the carbonate radicals to be consumed to a larger extent in competing side reactions, *i.e.* some carbonate radicals react with other species than cellulose. Possible reactions for the carbonate radical in the  $\text{ONOO}^-$  solution are:



To test the importance of side reactions, the same amount of peroxyntirite was added slowly to cotton linters, Figure 37.

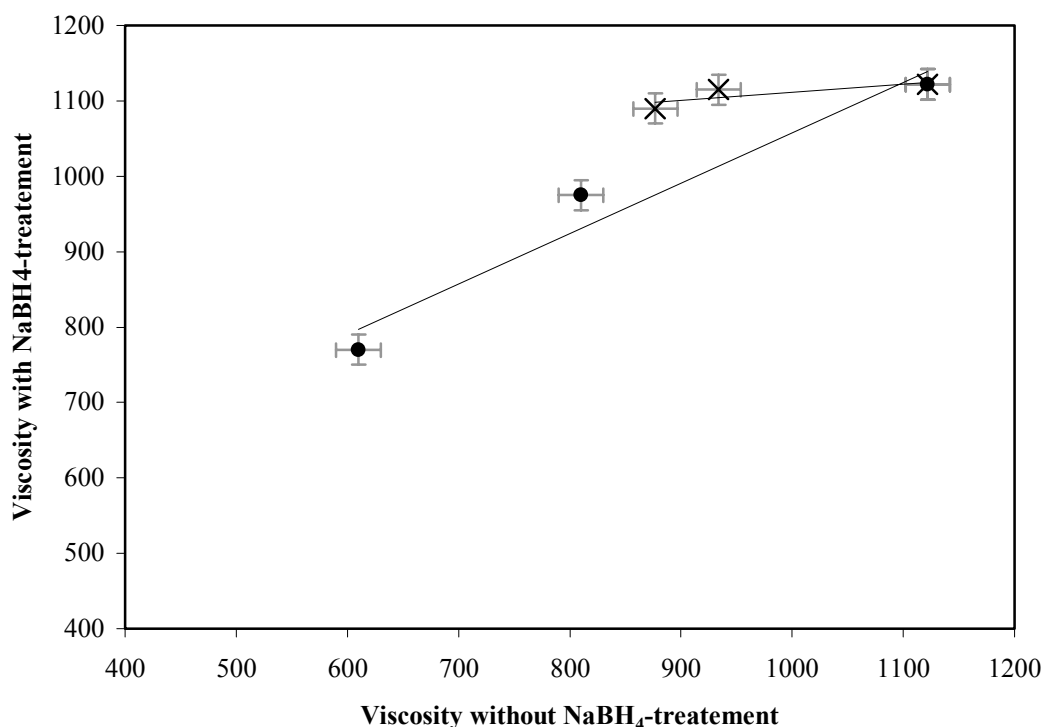


**Figure 37.** Effect of mixing peroxyxynitrite with cotton linters. A slow addition (8 mL/min) of peroxyxynitrite (PON) to a buffer solution compared to the original impregnation method. Hydroxyl(•) and carbonate radical(×).

As can be seen in Figure 37, the degradation of cotton linters by hydroxyl radicals is virtually unaffected by the mixing procedure. This is in keeping with the high reactivity of the hydroxyl radical, which under prevailing conditions reacts entirely with cotton linters. In contrast, the degradation by the carbonate radical is more extensive when cotton linters were first suspended in a bicarbonate buffer followed by a slow addition of peroxyxynitrite. Evidently, due to the relatively low reaction rate between the carbonate radical and cellulose, the mode of mixing affects the relative contribution of non-productive side-reactions

### 7.6.3 $\text{NaBH}_4$ Treatments of Radical Degraded Cotton Linters.

To gain insight into differences in *intra-molecular selectivities* of the two radicals some of the samples treated at intermediate pH were subjected to a reducing step ( $\text{NaBH}_4$ ) before the viscosity was measured. This reduction would allow the reformation of alcohols from alkali-labile groups (aldehydes and ketones) before the dissolution of the fibres under the strongly alkaline conditions prevailing in the viscosity measurements.



**Figure 38.** Viscosities for hydroxyl (•) and carbonate radical (×) degraded cotton linters with and without reducing (NaBH<sub>4</sub>) treatment.

Figure 38 shows the respective viscosity changes of carbonate and hydroxyl radical treated cotton linters with and without a subsequent NaBH<sub>4</sub> reduction. Evidently the viscosity losses caused by the CO<sub>3</sub><sup>•-</sup> radical could be recovered almost completely (> 90%) by treatment with NaBH<sub>4</sub> before the viscosity measurement. In contrast, the hydroxyl radical seems to degrade cellulose in a more fundamental way, as only about 40% of the viscosity could be recovered in this case.

This all the more surprising as the hydroxyl radical is known to be unselective, whereas we have shown in a previous work that the carbonate radical has a pronounced preference for the C-1 hydrogen in carbohydrate model compounds. Hence, this observation cannot be given a simple chemical explanation. We believe that the inertness of the glucosidic bond towards the carbonate radical has morphological reasons, *i.e.*, the carbonate radical has no easy access to these positions in cotton linters. Possibly, the negative charge on the carbonate radical may have something to do with this phenomenon. Clearly, considerably more work is required before this interesting dilemma can be resolved.

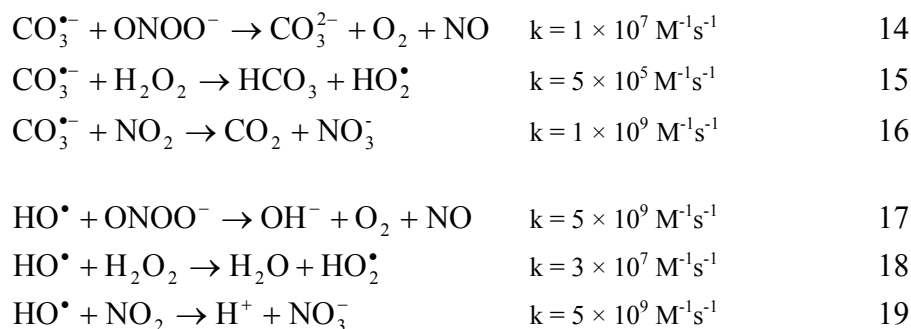
## 7.7 Bleaching of pulp

### 7.7.1 Pulp experiments using peroxyntirite as radical precursor

Using peroxyntirite as the radical precursor, it has been possible to compare the reaction effects of the carbonate and hydroxyl radicals on pulps under controlled conditions.

In the pulp experiments, non-bleaching side-reactions of the radicals produced may also occur with dissolved lignin and carbohydrates, reactions 14-19. These side-reactions represent losses of the bleaching agent and affect the efficiency negatively.



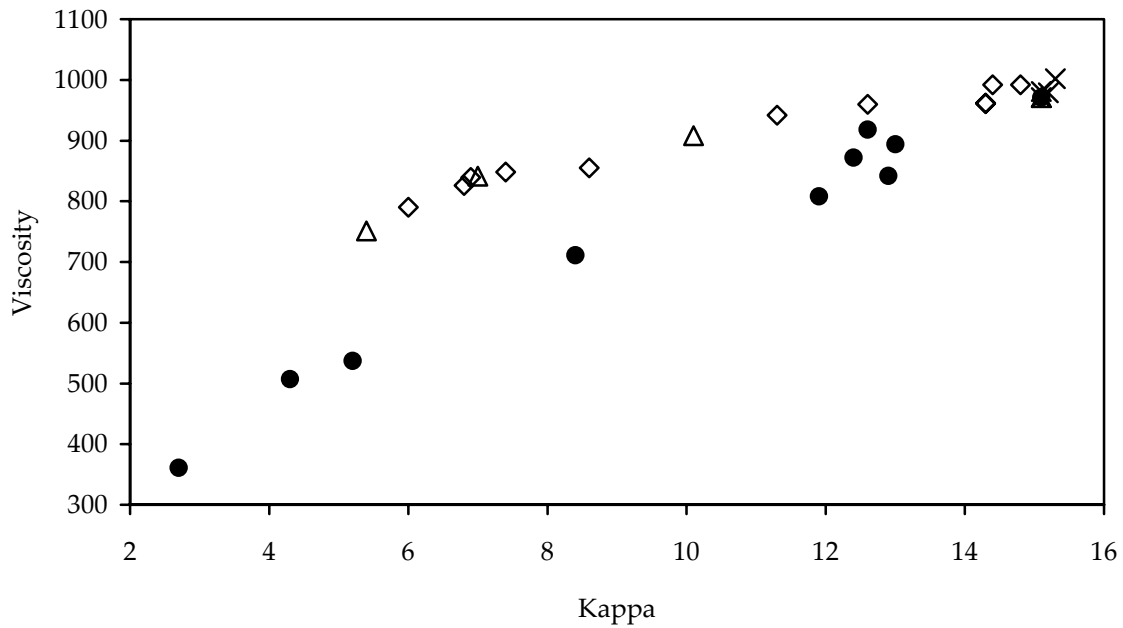


(Goldstein *et al.* 1998; NDRL/NIST Solution Kinetics Database on the Web; Bielski *et al.* 1985; Alfassi *et al.* 1999)

The way peroxyxynitrite is charged to the pulp can govern the extent of these side-reactions. The experimental procedure adopted in the study on pulp, with a high initial peroxyxynitrite concentration, probably boosted the radical attacks on the peroxyxynitrite itself, thus significantly lowering the delignification efficiency. In addition, the unavoidable nitrite contamination may give rise to considerable  $\text{ONOO}^-$  losses as well. It is therefore difficult to make comparisons of delignification responses (kappa, viscosity, brightness) based on the peroxyxynitrite charged. Instead, comparisons invariant to the reacted amount of peroxyxynitrite are the most elucidative, *i.e.* kappa- viscosity, kappa-brightness and viscosity-brightness relationships.

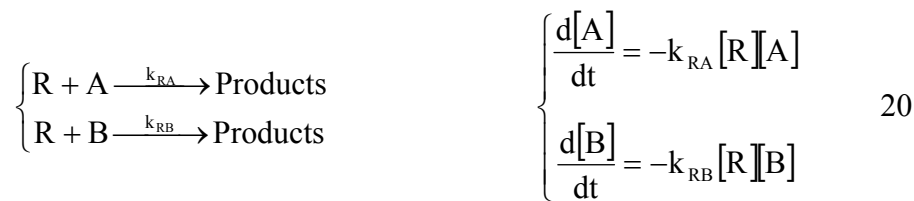
#### 7.7.2 Selectivity (kappa-viscosity)

A plot of the kappa-viscosity relationships for the different treatments clearly shows the higher selectivity of the carbonate radical anion compared to that of the hydroxyl radical, Figure 39. This selectivity difference in pulp reflects the higher *kinetic* selectivity of the carbonate radical towards lignin model compounds over carbohydrates, than that exhibited by the hydroxyl radical.



**Figure 39.** Viscosity versus kappa. Hydroxyl radical(●), carbonate radical(◇), carbonate radical with DTPA (△) and reference samples (x).

If a radical, R, is produced in the presence of two substances, A and B, the extent of the reaction of R with each substance under homogeneous conditions can be described by competition kinetics as:



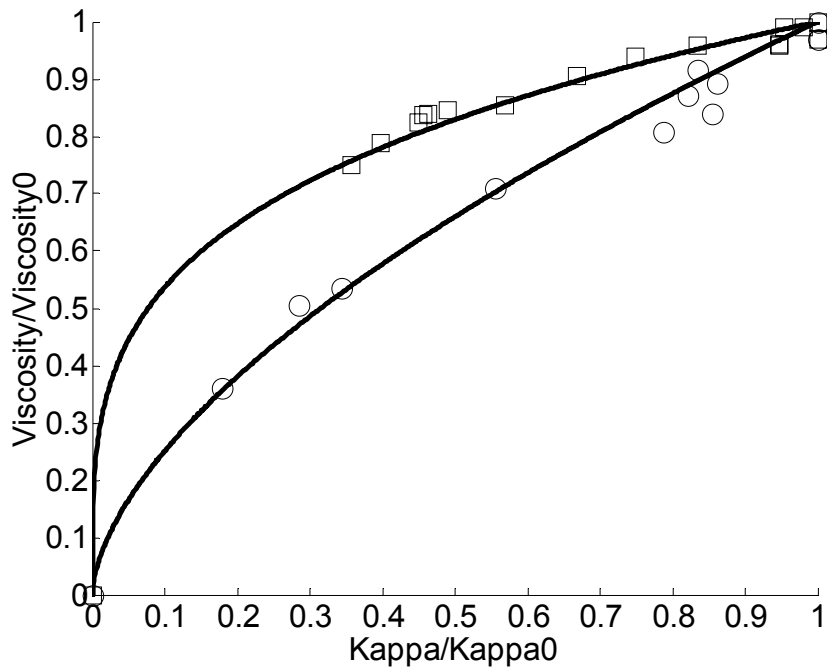
At any time or radical charge, the relationship between the remaining concentrations of A and B can be described as the following solution of equation 20

$$\frac{[\text{A}]}{[\text{A}]_0} = \left( \frac{[\text{B}]}{[\text{B}]_0} \right)^{\frac{k_{\text{RA}}}{k_{\text{RB}}}} \quad 21$$

The power term is the kinetic selectivity of the radical towards reaction with A. Each side of the equation is dimensionless. Thus, as the simplest approximation, we assume that the same type of *dimensionless* relationship can be used to describe the changes in kappa and viscosity, equation 22

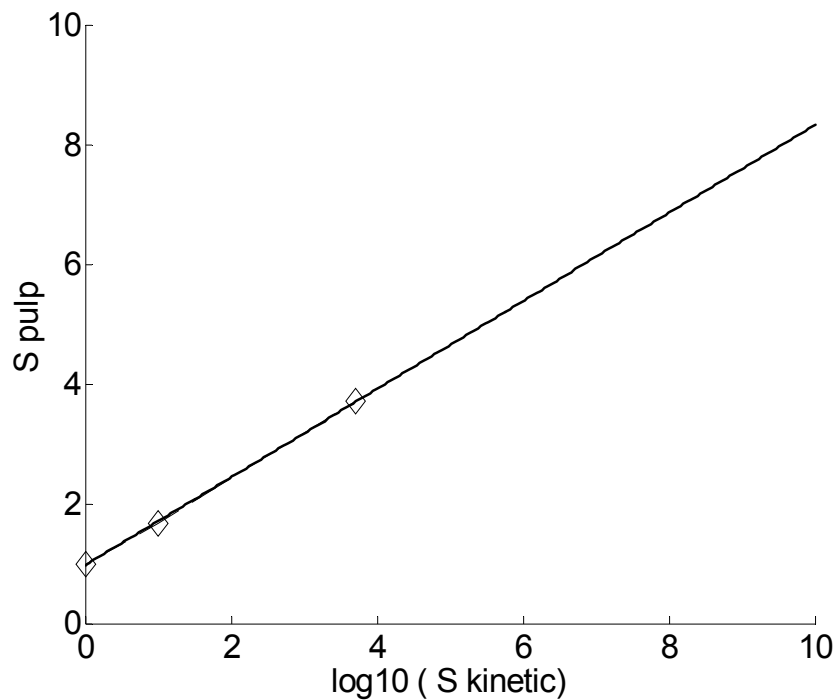
$$\frac{[\text{Viscosity}]}{[\text{Viscosity}]_0} = \left( \frac{[\text{Kappa}]}{[\text{Kappa}]_0} \right)^{\frac{1}{S_{\text{pulp}}}} \quad 22$$

Using numerical methods, the selectivity constant,  $S_{\text{pulp}}$ , in equation 22 as calculated for each radical to yield the best possible fit to the experimental data, Figure 40.



**Figure 40.** Relative changes in viscosity versus changes in kappa. Lines numerically fitted to data points according to equation 21. Hydroxyl radical (o) and carbonate radical (□).

It seems as the experimental data can be described by this simple kinetic approach. The  $S_{pulp}$ -values obtained were 1.8 and 4.3 for the hydroxyl and carbonate radicals, respectively. In Figure 41 these  $S_{pulp}$ -values are plotted versus the logarithmic values of the kinetic selectivities, as determined in previous works (Ek *et al.* 1989).

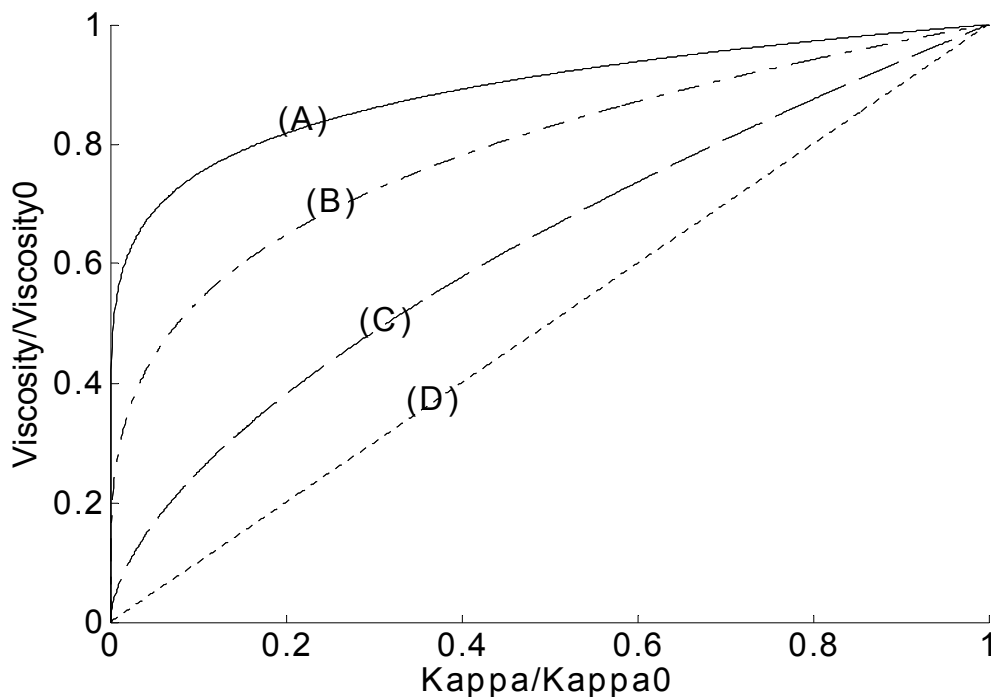


**Figure 41.** The  $S_{pulp}$ -values obtained plotted versus  $\log(\text{kinetic selectivity})$  for the hydroxyl and carbonate radicals as well as for the theoretical completely unselective system with  $S_{pulp} = S_{kinetic} = 1$ .

The use of a logarithm was applied to bring the kinetic selectivities down to the same scale as the  $S_{pulp}$ -values. For the completely unselective system  $S_{pulp} = S_{kinetic} = 1$ . Interestingly, a linear trend was observed corresponding to equation 23:

$$S_{pulp} = 0.83 \log_{10}(S_{kinetic}) + 1 \quad 23$$

From reported kinetic data on radical reactivity, the maximum kinetic selectivity of a radical oxidant should be of the order of  $10^{10}$ . This corresponds to  $S_{pulp} = 9.3$ . For this selectivity, as well as for the completely unselective system with  $S_{pulp} = 1$ , we have calculated the kappa-viscosity relationships for the pulp investigated, shown in Figure 42.



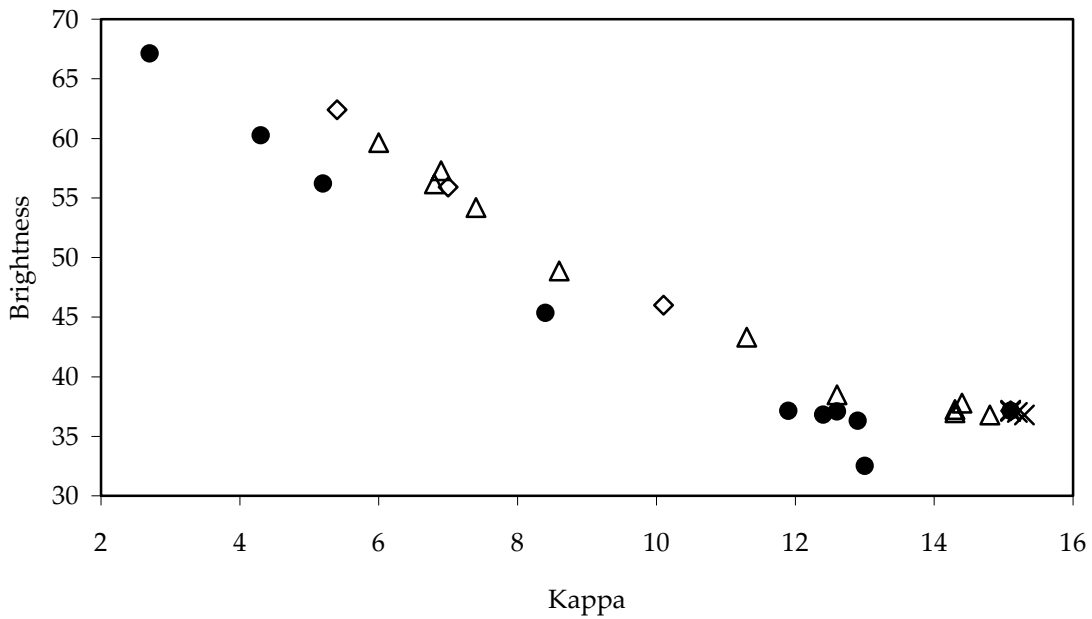
**Figure 42.** Relative changes in viscosity versus the corresponding relative changes in kappa at different  $S_{\text{pulp}}$ -values. (A) :  $S_{\text{pulp}}=9.4$  ; (B) :  $S_{\text{pulp}}=4.3$  ; (C) :  $S_{\text{pulp}}=1.8$  ; (D) :  $S_{\text{pulp}}=1$

This kinetic approach indicates how even very selective reagents will produce low-viscosity pulps at extensively driven delignifications. Even though it has not been tested here, it could be of value to compare this approach on delignification of different pulps, as the relative scale might enable comparisons of bleaching stages with different starting points.

### 7.7.3 Brightness effects and mechanisms of delignification

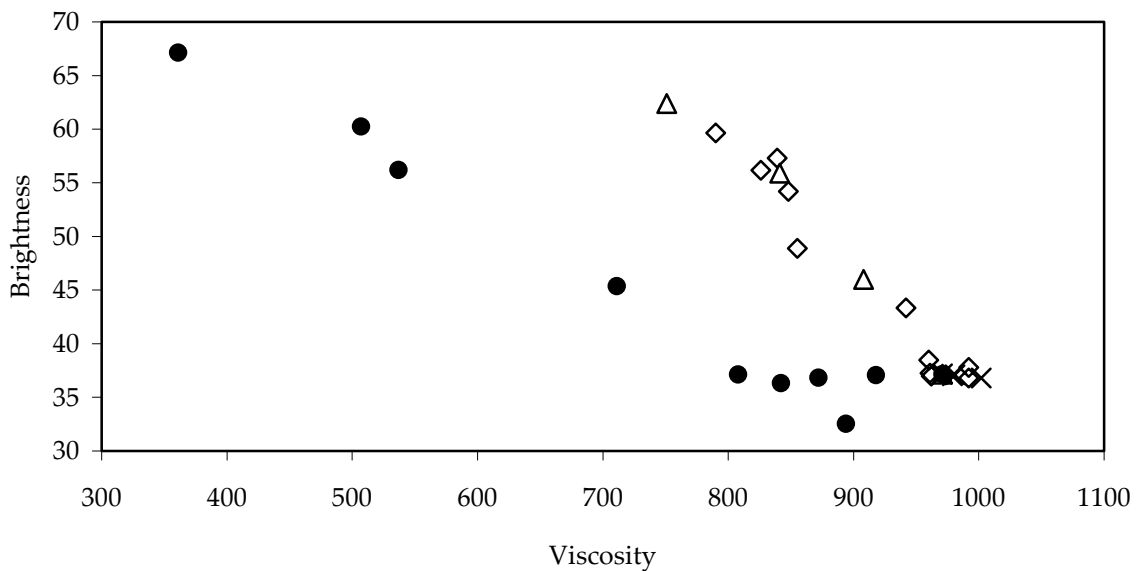
The brightness of pulp has been shown to be proportional to its lignin content, measured as kappa. In Figure 43 we can see that at a given kappa-number the pulp, after treatment with carbonate radicals, is brighter than after treatment with hydroxyl radicals. The bleaching technique used affects the brightness of the residual lignin in different ways depending on the chemistries involved.

*Hydroxyl radicals* readily oxidize phenolic structures to phenoxy radicals. They also react with non-phenolic substrates by electrophilic addition to aromatic rings forming isomeric hydroxycyclohexadienyl radicals, or by hydrogen abstraction from aliphatic residues affording carbon-centered radicals. These radical intermediates will eventually result in hydroxylation-, dealkoxylation- or carbonyl-containing structures (Gierer *et al.* 2001). Thus, the hydroxyl radical does not directly cause significant lignin fragmentation and may even increase the chromophore content. *Carbonate radicals*, on the other hand, react by radical cation formation, a direct route to lignin fragmentation.



**Figure 43.** Hydroxyl radical (●), carbonate radical (◇), carbonate radical with DTPA (△) and reference samples (x).

These effects of different chemistries stand out even better in the brightness-viscosity plot, Figure 44. Apparently, for the hydroxyl radical the brightness starts to increase only after a pronounced viscosity loss of 200 units, whereas the carbonate radical immediately gives a brightness increase at a lower rate of viscosity loss.



**Figure 44.** Brightness versus viscosity. Hydroxyl radical (●), carbonate radical (◇), carbonate radical with DTPA (△) and reference samples (x).

During the pulp experiments a set time of reaction was used to standardise the treatments. It may be worthwhile to note that all peroxyxynitrite was consumed rapidly when CO<sub>2</sub> was introduced or pH was lowered. Thus the combined time of the actual bleaching reactions for any treatment took place in less than 5 minutes. Using peroxyxynitrite it should be possible to

delignify pulps industrially by at least 10 kappa-units with decent selectivity on the minute time-scale at room temperature. Similarly, the hydroxyl radical was shown to delignify pulp, but low selectivity and brightness increase was observed.

## 8 Conclusions

The carbonate radical has a rather high reactivity towards aromatic lignin constituents. It reacts especially fast with phenolates. All these reactions occur by way of electron transfer. Small carbohydrates react with  $\text{CO}_3^{\cdot-}$  much slower than aromatics. These reactions are hydrogen transfer reactions. However, in very basic media, where the carbohydrates deprotonate to some extent, their anions react with  $\text{CO}_3^{\cdot-}$  by way of electron transfer and the rates approach those of non-phenolic aromatics.

These findings suggest that in neutral or slightly alkaline media  $\text{CO}_3^{\cdot-}$  might serve as an excellent delignifying agent of pulp down to very low lignin contents.

With small carbohydrates possessing one or two glucosidic bonds,  $\text{CO}_3^{\cdot-}$  abstracts hydrogen predominantly from C1-H bonds, which results in rupture of the glucosidic linkage. Interestingly, however, the glucosidic bonds in cotton linters are rather resistant towards  $\text{CO}_3^{\cdot-}$ . This has probably morphological reasons. These results imply that, even at very low lignin contents, where  $\text{CO}_3^{\cdot-}$  is bound to react with cellulose, the reactions will not lead to substantial decrease in pulp viscosity.

At present the cheapest and most practical way of producing  $\text{CO}_3^{\cdot-}$  radicals in the presence of pulp is to mix the latter with peroxyxynitrite and  $\text{CO}_2$ . We have performed such experiments on pulp with very promising results. The Kappa number decreased substantially, brightness increased, while the viscosity remained high. This confirms the predicted excellent properties of the carbonate radical.

However, before the peroxyxynitrite method can be implemented in the pulp industry, a number of technical problems has to be solved. Chief among them is a slow and steady dosage of peroxyxynitrite to minimise side reactions of the radicals with peroxyxynitrite and the nitrite impurity. The fate of the  $\cdot\text{NO}_2$  radical, the coproduct of  $\text{CO}_3^{\cdot-}$ , has also to be assessed.  $\cdot\text{NO}_2$  will probably have to be removed by vigorous degassing in order to block the possible nitration of cellulose.



## 9 References

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