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Analysis of cellulose and kraft pulp ozonolysis products by anion-exchange chromatography with pulsed amperometric detection

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ABSTRACT

Efforts to reduce the level of chlorinated organics discharged into the environment have led to interest in ozone bleaching of kraft pulp. In the course of this operation the transformation of cellulose has to be reduced to the minimum. With this aim, the investigation of the cellulose–ozone reaction was carried out from a mechanistic point of view. The initial interest was in the composition of kraft pulp and cellulose ozonation effluents. High-performance anion-exchange chromatography with pulsed amperometric detection was used to separate and partially identify the soluble modified or unmodified carbohydrates of kraft pulp and cellulose ozonolysis. The main results indicate that after ozonation at 0, 25 and 65°C, cellulose gives oligosaccharides, monosaccharides and probably oxidized forms of those products. Formaldehyde was also directly determined under the same conditions as for carbohydrates.

INTRODUCTION

The traditional methods of bleaching involving chlorine and chlorine dioxide followed by an alkaline extraction stage result in excessive waste water and pollution. The worldwide concern over environmental control has motivated a search for new processes of bleaching that will eliminate the problem of pollution caused by chlorinated organic materials [1,2]. One attractive candidate in the development stage is ozone because of its delignifying and brightening abilities [3]. However, while lignin, a phenolic-type high-molecular-mass material, is readily attacked by ozone [4,5], the cellulose is simultaneously modified, which more or less leads to a loss of pulp properties. This problem still awaits solutions in terms of selectivity and remains a great challenge in the pulp and paper industry. Thus, the aim of this work was the elucidation of the action of ozone on cellulose and the reduction of its effect.

Although there is considerable information regarding the by-products from chlorinating pulp [6,7], much less is known about the by-products from ozone bleaching, especially the carbohydrates. Sonnenberg *et al.* [8] recently determined the identities of low-molecular mass pulp ozonolysis products from lignin degradation by gas chromatography coupled with mass spectrometry but they did not consider possible cellulose degradation products. The ozonation of glucose and cellobiose has been reported and some ozonation by-products have been identified [9]. However, so far no analysis of carbohydrates from ozonation effluents of cellulose or kraft pulp has appeared in the literature.

Hence we decided to analyse the soluble ozona-

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tion products of cellulose and kraft pulp. A preliminary study on the liquid part of poplar sawdust ozonation showed [10] that carbohydrate analysis was possible by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection. Carbohydrates, whether present as monosaccharides, disaccharides or oligosaccharides, can be directly injected in aqueous solution without derivatization and pretreatment [11–13]. HPAEC–pulsed amperometric detection provides a simple, selective and sensitive method [14]. For these reasons, this method was applied here to separate and partially identify the soluble, modified or unmodified carbohydrates that originate from the cellulose, transformation of cellulose and kraft pulp ozonation.

Further identification work by nuclear magnetic resonance (NMR) spectrometry, on separated products with preparative chromatography could help to elucidate the prevalent reaction pathways for the modification of the cellulose by ozone. In addition, the knowledge of ozonation by-products is also of interest from an environmental point of view.

EXPERIMENTAL

Ozonation system

Ozone for the experiments was produced from pure oxygen in a laboratory cold plasma ozone generator. The gas flow was 60 l/h (under normal conditions) and the concentration of ozone in the gas mixture leaving the generator was about 30 mg/l (under normal conditions), which was measured with a BMT 961 ozone analyser (BMT Messtechnik). The residual ozone leaving the reactor was measured by the iodimetric method [15]. The carbohydrate–ozone reactions were carried out in a constantly stirred and thermostated glass reactor.

Substrates

Cellulose and softwood kraft pulp were used for the experiments. Cellulose of analytical-reagent grade was obtained from Merck (Darmstadt, Germany) and softwood kraft pulp was provided by the Centre Technique du Papier (Grenoble, France). A 12-g amount of oven-dry kraft pulp or 12 g of oven-dry cellulose were ozonated at a consistency of 1.2% (on oven-dry pulp or cellulose). Kraft pulp was also ozonated at a consistency of 3% (on oven-dry pulp) in the case of ozonation at 65C and ozone charge 4%. The charges of ozone were calculated in relation to the oven-dry substrates.

Instrumentation

The HPLC system was a Dionex (Sunnyvale, CA, USA) 4500i series chromatograph equipped with a pulsed electrochemical detector. Injection was performed using a Dionex inert high-pressure valve. Data acquisition from the detector and determinations of retention times and peak heights and areas were performed on a Compaq IBM-compatible computer using a Model 1 ACI interface and AI-450 software version 3.2 (Dionex).

Column

A (Dionex) CarboPac PA1 column (250 mm × 4 mm I.D.) with polystyrene–divinylbenzene pellicular resin (particle size 10 μm) and a 1700 p.s.i. pressure limit was used (1 p.s.i. = 6894.76 Pa). The degree of cross-linking of the resin was 5%. A Dionex CarboPac PA1 guard column was used.

Reagents

Purified water (18 MΩ) obtained with a Millipore (Bedford, MA, USA) Milli-Q Plus water purification system was used for preparing all solutions (ozonation solutions, mobile phases and standard solutions).

All the chemicals used for standards were of analytical-reagent grade. L-Fucose, D-glucose, cellobiose and D-glucuronic acid were obtained from Fluka (Buchs, Switzerland), L-rhamnose, L-arabinose, gluconic acid, D-xylose, D-mannose, 5-(hydroxymethyl)-2-furaldehyde (HMF), 1,6-anhydro-D-glucose and cellodextrins from Merck, glucaric acid from Sigma (St. Louis, MO, USA), formaldehyde as a 35% aqueous solution and. Anhydrous sodium acetate from Merck and sodium hydroxide solution (50%, w/w) from J.T. Baker (Deventer, Netherlands).

Operating conditions for oligosaccharide analysis

Eluent 1 was 150 m*M* NaOH, eluent 2 was 500 m*M* CH_3COONa–150 m*M* NaOH and eluent 3 was deionized water. The eluent flow-rate was 1.0 ml/min.

A 50-μl sample loop was used. The detector was

operated in the amperometric mode using a gold working electrode and an Ag/AgCl pH-reference electrode. The applied potentials were as follows: E_1 = 0.40 V, t_1 = 500 ms; E_2 = 0.90 V, t_2 = 80 ms; and E_3 = −0.30 V, t_3 = 50 ms.

The gradient programme was as follows:

Time (min)	1 (%)	2 (%)	3 (%)	
0.00	70	0	30	Equilibration
10.00	70	0	30	Injection of sample, start of gradient ramp 1
20.00	60	10	30	Start of gradient ramp 2
35.00	40	30	30	
50.00	40	30	30	End of Analysis

Operating conditions of monosaccharides and formaldehyde analysis

The analysis was performed with the same analytical column. Eluent 1 was 150 m*M* NaOH and Eluent 2 was deionized water. The eluent flow-rate was 1.0 ml/min.

A 100-μl sample loop was used. The detector was operated in the amperometric mode using a gold working electrode and an Ag/AgCl, pH-reference electrode with post-column addition of 500 m*M* NaOH (flow-rate 0.6 ml/min). The applied potentials were as follows: E_1 = 0.40 V, t_1 = 500 ms; E_2 = 0.90 V, t_2 = 80 ms; and E_3 = −0.30 V, t_3 = 50 ms.

The gradient programme was as follows:

Time (min)	1 (%)	2 (%)	
0.00	6	94	Equilibration, start of isocratic elution 1
15.00	6	94	Injection of sample
33.00	6	94	Step gradient
34.00	0	100	Start of isocratic elution 2
45.00	0	100	End of analysis

Sample preparation

Before injection, the samples were passed through a polyvinylpyrrolidone (PVP) filter cartridge (Dionex On-Guard P cartridge), in order to eliminate the phenolic fraction originating from the lignin in the pulp.

Identification of peaks

All peaks were identified by systematic addition of standard solutions to the samples.

RESULTS AND DISCUSSION

Preliminary investigations

Earlier studies [9] have shown that ozonation of D-glucose produced gluconic and D-glucuronic acids, which were confirmed by both amperometric and conductimetric detection (chromatograms and data are not shown here) [16]. In addition, glucaric acid was also produced after a heavy ozone charge (26%). It was not detected by amperometry but was found with conductimetric detection [16].

Ozonation of cellobiose afforded mainly D-glucose together with the same oxidized products such as gluconic and D-glucuronic acids [9]. Moreover, formaldehyde has been identified in both instances *i.e.*, ozonation of D-glucose and cellobiose by HPAEC-pulsed amperometric detection [16].

Also, the ozonation of MαG (methyl α-D-glucopyranoside) has been studied [17]. The results indicated D-glucose to be the major ozonation by-product.

Ozonation of cellulose and softwood kraft pulp

Figs. 1 and 2 illustrate the gradient elution (see Experimental) of ozonation products of cellulose and softwood kraft pulp, respectively, at 0, 25 and 65°C. All chromatograms were allowed to run for over 40 min and no peaks appeared at later times. A chromatogram of a standard solution of a mixture of cellodextrins in water is shown in Fig. 3. Cellotriose is the oligosaccharide found with the highest concentration in the case of ozonation of cellulose at 0°C (about 0.01% of initial cellulose).

It is found that the chromatograms of each substrate have a similar pattern of oligosaccharides but different degrees of intensity, which might indicate the dependence of cellulose degradation on temperature. Apparently the degradation of cellulose is minimized at 0°C. The reason might be the lower decomposition rate of ozone and consequently less attack on cellulose by hydroxyl radicals. The differences between the chromatograms of cellulose and those of kraft pulp can be explained by the presence of hemicelluloses and lignin in the kraft pulp (limited accessibility of cellulose).

Figs. 4 and 5 show the separation by a step gradient of ionic strength (see Experimental) of monosaccharides respectively after ozonation of cellulose and softwood kraft pulp, respectively, (ozone

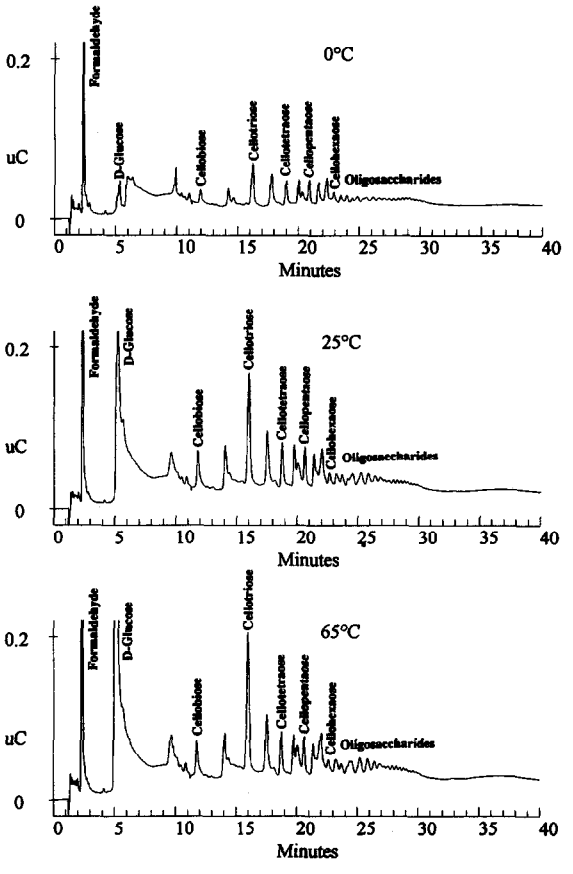

Fig. 1. Separation of ozonation products on an anion-exchange resin using gradient elution and pulsed amperometric detection. Influence of temperature on the degradation of cellulose by ozone (ozone charge 0.92%).

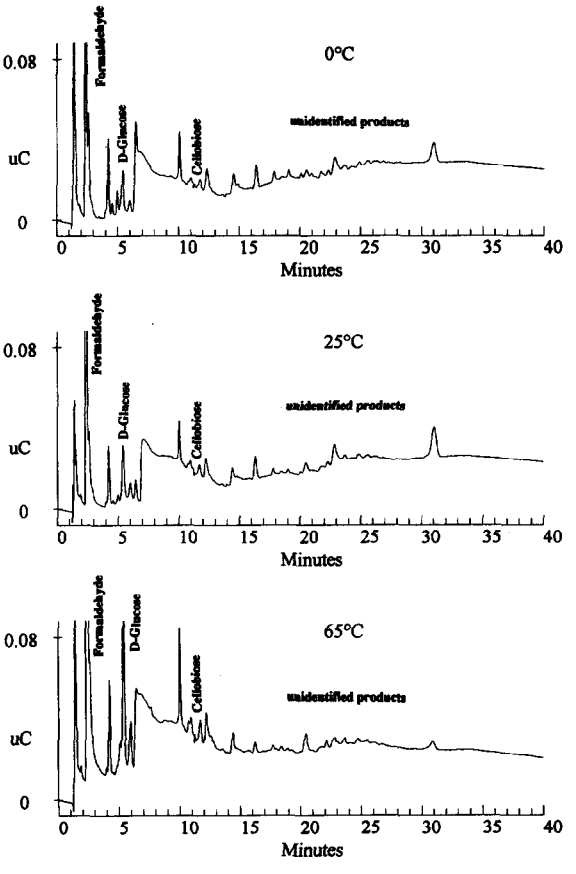

Fig. 2. Separation of ozonation products on an anion-exchange resin using gradient elution and pulsed amperometric detection. Influence of temperature on the degradation of softwood kraft pulp by ozone (ozone charge 0.92%).

charge 2% and 4%, respectively; temperature 65°C). Fig. 6 shows a standard chromatogram of a mixture of monosaccharides with formaldehyde and HMF at ppm (w/w) levels under the same conditions. The chromatograms obtained after ozonation of cellulose indicate the presence of D-glucose after splitting of glycosidic linkages and of L-arabinose in the ozonation solution, probably produced via Ruff degradation [18]. Traces of HMF (very unstable) are obtained at very high charge in ozone, *e.g.* 26% (chromatogram not shown). D-Xylose is the most monosaccharide obtained (about 0.01% of initial cellulose). However, an explanation for why ozonation of cellulose gives relatively more D-xylose than D-glucose remains to be found.

Similar chromatograms are obtained for softwood kraft pulp, where L-arabinose, D-galactose, D-xylose could be contributed to by the cleavage of hemicelluloses contained in pulp. The low concentration of D-glucose relative to the other monosaccharides remains to be explained.

Formaldehyde is obtained in all chromatograms. This presence was predictable, because ozonation of D-glucose [16] and phenol [9] leads to the formation of formaldehyde by oxidative cleavage. Formaldehyde can be detected in HPAEC–pulsed amperometric detection because it is a *gem*-diol in aqueous solution and contains two hydroxyl groups [19]. The analyte has a very high sensitivity for pulsed amperometric detection. The minimum detection

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code
1	2.32	Formaldehyde	0.528	203532	1421367	1
2	5.08	Glucose	0.033	2265	21343	1
3	11.27	Cellobiose	0.528	23929	276046	1
4	14.95	Cellotriose	0.942	37366	488028	1
5	18.15	Cellotetraose	1.115	32546	419327	1
6	20.60	Cellopentaose	0.602	14599	186591	1
7	22.62	Cellohexaose	0.144	2524	32842	1
		Totals	3.892	316761	2845545	

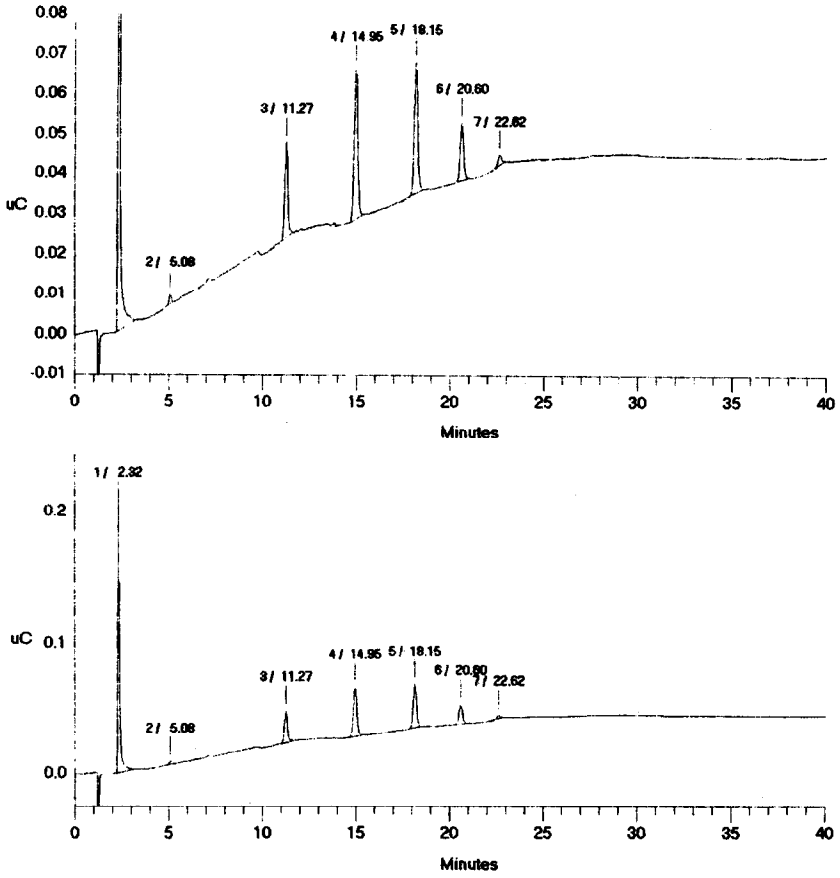

Fig. 3. Typical chromatogram of a standard mixture of formaldehyde and cellodextrines on an anion-exchange resin using gradient elution and pulsed amperometric detection.

limit is about 10 ppb. A better separation of formaldehyde is obtained at lower sodium hydroxide concentrations, *e.g.*, 3 m*M* NaOH, where the retention time was 3.5 min in isocratic elution with post-column addition of strong base (500 m*M* NaOH) in order to optimize the pulsed amperometric detector sensitivity with a Dionex postcolumn pneumatic controller. However, this postcolumn addition can lead to an increase the baseline drift. In the future, the new (Dionex) CarboPac MA1 high-efficiency anion-exchange column will be particularly suited to the analysis and separation of weakly ionizable

********** Component Report: Components Found **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code
2	3.47	Formaldehyde	0.718	242015	3129429	3
5	13.85	Arabinose	0.084	4046	173374	1
6	20.62	Glucose	0.171	9758	419355	1
7	23.78	Xylose	1.350	64176	3737340	1
		Totals	2.323	319995	7459497	

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code
1	1.97		0.000	169305	4194483	2
3	4.30		0.000	3037	38621	4
4	4.73		0.000	5926	68876	2
		Totals	0.000	178268	4301980	

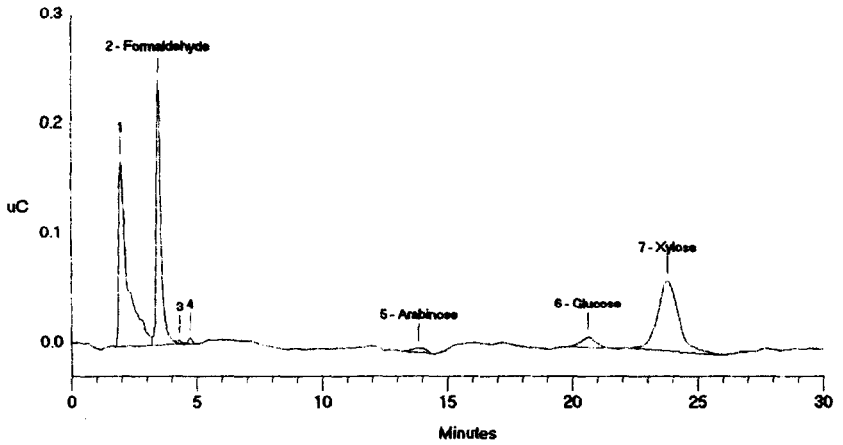

Fig. 4. Separation of monosaccharides on an anion-exchange resin using a step gradient of ionic strength and pulsed amperometric detection after ozonation of cellulose (ozone charge 2%, temperature 65C).

carbohydrates such as the peaks at the beginning of all chromatograms and specifically formaldehyde.

In all the chromatograms obtained by application of a step gradient of ionic strength, an oxygen dip in the baseline with a retention time of 14 min is observed [20]. This dip only appears on the higher sensitivity current scales, and it effectively limits the detection limit by obscuring the arabinose peak when low concentrations are determined.

To give a clearer view of ozone–carbohydrate reactions, Table I summarizes the identified soluble by-products after ozonation of cellulose and softwood kraft pulp using HPAEC–pulsed amperometric detection.

Reactions of ozone with carbohydrates

Based on the chromatograms from ozonation of cellulose and kraft pulp, the increase in the oligosaccharides is evidence for cellulose degradation. To account for this observation, *i.e.*, the splitting of glycosidic linkages, a free-radical chain mechanism involving oxygen in the propagation step was pro-

********** Component Report: Components Found **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code
3	3.42	Formaldehyde	1.401	547405	6106486	2
5	13.82	Arabinose	1.454	99655	2998262	1
6	18.25	Galactose	0.054	3486	137240	1
7	19.87	Glucose	0.032	1924	79213	1
8	23.28	Xylose	0.153	7548	422343	1
		Totals	3.094	660018	9743544	

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code
1	1.93		0.000	28516	393874	2
2	2.50		0.000	24887	435043	2
4	4.65		0.000	6575	77502	2
		Totals	0.000	59978	906419	

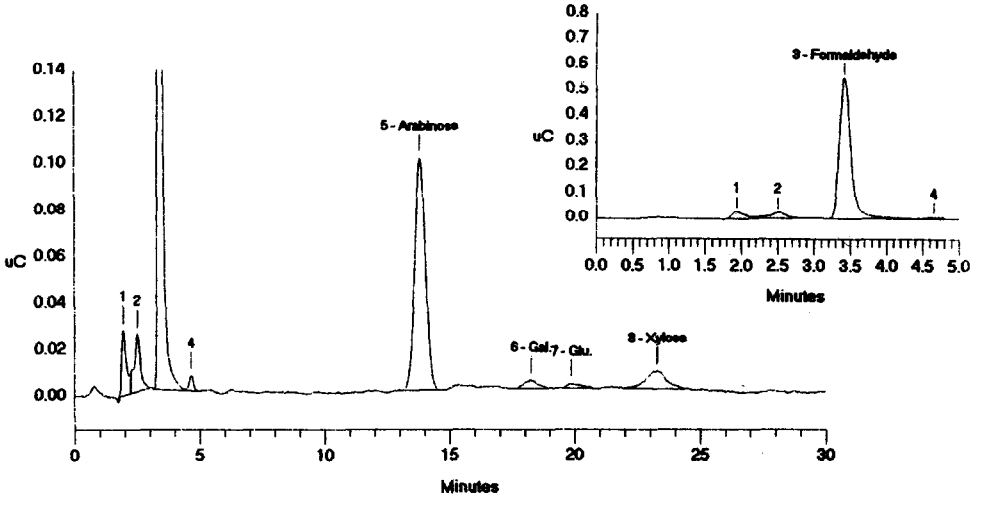

Fig. 5. Separation of monosaccharides on an anion-exchange resin using a step gradient of ionic strength and pulsed amperometric detection after ozonation of softwood kraft pulp (ozone charge 4%, temperature 65C).

posed [21]. However, an electrophilic attack of ozone on carbohydrates to liberate the anomeric carbon via an ozone-catalysed hydrolysis of glycosidic bonds would be also possible [21].

CONCLUSIONS

HPAEC–pulsed amperometric detection is a very versatile approach for the analysis of complex ozonation samples, in particular the separation of carbohydrate ozonation by-products in aqueous solution. The chromatograms reported in this paper show the applicability of this technique to follow the degradation of cellulose by ozone. The results demonstrate clearly the relatively higher concentrations of D-xylose and L-arabinose compared with D-glucose, which are not explicable by the random attack of ozone with scission on the macromolecules mainly formed by D-glucose.

Cellulose degradation, which gives oxidized and/

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code
1	2.78	1,6-Anhydro-glu	0.595	111445	1192136	2
2	3.45	Formaldehyde	0.621	233352	2707116	2
3	5.08	HMF	0.483	89815	1201634	1
4	6.30	Fucose	0.738	120641	1924180	1
5	12.65	Rhamnose	0.797	56794	1871400	1
6	13.93	Arabinose	1.025	79798	2113285	1
7	18.38	Galactose	1.036	63946	2641459	1
8	20.42	Glucose	1.567	89072	3851115	1
9	23.53	Xylose	1.752	92195	4849087	2
10	24.62	Mannose	1.876	34273	2138969	2
		Totals	10.490	971332	24490378	

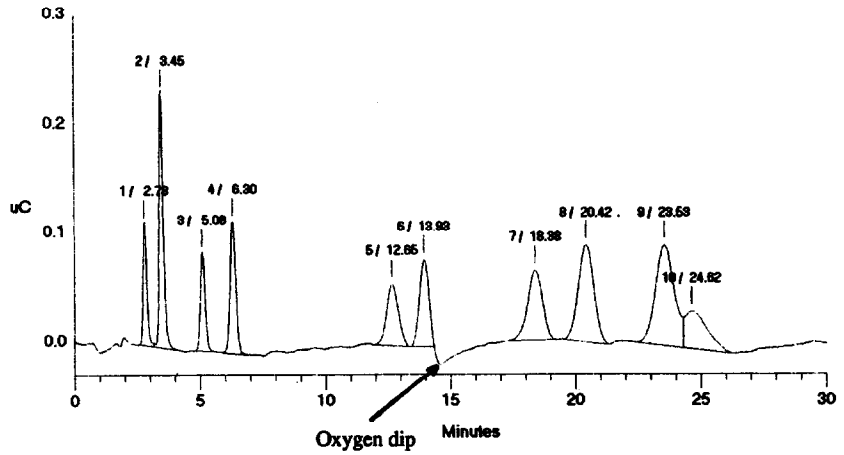

Fig. 6. Typical chromatogram of a standard mixture of formaldehyde, HMF and monosaccharides on an anion-exchange resin using a step gradient of ionic strength and pulsed amperometric detection.

TABLE I

IDENTIFIED SOLUBLE BY-PRODUCTS AFTER OZONATION OF CELLULOSE AND SOFTWOOD KRAFT PULP USING HPAEC

Original data obtained using different conditions of ozonation (ozone charge, temperature, pH). Absence of L-fucose, L-rhamnose and D-mannose.

Cellulose	Softwood kraft pulp
Formaldehyde	Formaldehyde
HMF	HMF
L-Arabinose	L-Arabinose
D-Glucose	D-Glucose
D-Xylose	D-Xylose
	D-Galactose
Cellobiose	Cellobiose
Cellotriose	
Cellotetraose	
Cellopentaose	
Cellohexaose	

or non-oxidized monosaccharides and oligosaccharides, might be caused by a free-radical attack and ozone electrophilic attack on carbohydrates. Anyway, cellulose seems less degraded at lower temperatures.

In addition, formaldehyde in aqueous solution can also be directly determined down to sub-parts per million levels by HPAEC separation with pulsed amperometric detection.

In further work, the purification and identification of unknown carbohydrate ozonolysis products by preparative chromatography and methods such as nuclear magnetic resonance and mass spectrometry will be carried out.

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