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# Comprehensive characterisation of cellulose- and lignocellulose-degradation products in aged papers: Capillary zone electrophoresis of low-molar mass organic acids, carbohydrates, and aromatic lignin derivatives

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**Abstract**

Capillary zone electrophoresis was used for a comprehensive characterisation of several classes of degradation products from old books. Using different methods with direct and indirect UV detection, three categories of organic compounds were investigated, namely low-molar mass organic acids, aromatic lignin derivatives, and simple carbohydrates. The occurrence of these compounds in paper depended on the state of degradation and on the type of pulp. Formic, acetic, glycolic, succinic, and lactic acids originated from holocellulose degradation. In lignocellulosic papers, acetosyringone, 4-hydroxyacetophenone, 4-hydroxybenzaldehyde, vanillin, vanillic acid, furoic acid, and 4-hydroxybenzoic acid were attributable to the lignin fraction. These two classes of compounds could be analysed directly from the aqueous extracts of papers. Glucose and cellooligomers formed through cleavage of the cellulose chain, while the hydrolysis of hemicelluloses yielded mainly pentoses among which arabinose and xylose were the most abundant. The carbohydrates were derivatised with 4-aminobenzoic acid ethyl ester prior to analysis. All the organic compounds identified were found in a panel of papers from various origin (rag, kraft, and groundwood pulps) and diverse states of deterioration, whether naturally or artificially aged. The production of these compounds was followed during the accelerated ageing of the papers and an attempt to pointing to possible molecular indicators of paper degradation was made.


*Keywords:* Paper; Cellulose; Lignocellulose; Degradation; Capillary zone electrophoresis

## 1. Introduction

The evaluation of the state of conservation of aged paper artefacts in relation to paper lifespan prediction is currently a prime issue in the cultural heritage field. The concern is essential to collection managers, but remains nonetheless a real challenge for the conservation physical chemist. One important research objective that would help in designing preservation strategies is the characterisation of the long-term chemical and physical stability of paper-based collections. The first problem to be addressed is a better knowledge of the degradation of paper taking place during the ageing, both natural and artificial ageing. Paper is made primarily from cellulose but can contain also other constituent polymers (hemicelluloses and lignin) and a number of substances, such as rosin, starch, gelatine, alum, and mineral fillers that are added to the pulp or after the sheet formation. Ageing sub-products of these polymers and substances can be found in the paper after a period of time and can also be released in the surrounding atmosphere. Their occurrences mostly depend on the environmental conditions the paper is being or has been subjected to. A number of studies have been

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dedicated to understanding the degradation of paper with time. It is now widely accepted that the state of (de)polymerisation of cellulose is the key parameter that will govern the persistence of the physical strength of a paper. Some works have been devoted to finding correlations between the mechanical strength decay of paper and the macromolecular characteristics of cellulose (Berggren, Molin, Berthold, Lennholm, & Lindström, 2003; Chamberlain & Priest, 1998; Gurnagul, Page, & Paice, 1992; Jerosch, 2002; Zou, Gurnagul, Uesaka, & Bouchard, 1994). In this respect, size-exclusion chromatography, which allows for a direct characterisation of the macromolecules, has proved an utmost performing tool for the precise assessment of cellulose degradation in pulps (Schult, Moe, Hjerde, & Christensen, 2000; Silva & Laver, 1997) and paper (Dupont, 2003a, 2003b; Jerosch, Lavédrine, & Cherton, 2001; Strlič & Kolar, 2003). A few studies have been committed to the analysis of the sub-products from the age degradation of paper, using liquid chromatography (LC), gas chromatography with mass spectrometry (GC-MS) (Buchbauer, Jirovetz, Wasicky, & Nikiforov, 1995; Havermans, de Feber, Genuit, & van Velzen, 1999; Parks, Guttman, Jewett, & Brinckman, 1990), and solid-phase microextraction (SPME) coupled with GC-MS (Lattuati-Derieux, Bonnassies-Termes, & Lavédrine, 2004). A very versatile, sensitive, and selective technique, capillary electrophoresis (CE) appears very well adapted to the characterisation of paper degradation products, yet it had only been marginally explored for these applications. Moreover, at comparable level of sensitivity, CE has the advantage over GC that it can separate very low-molar mass (LMM) analytes such as the sub-products from paper deterioration without derivatisation. Based on capillary zone electrophoresis (CZE) using UV detection with a photodiode array (PDA), the present study was dedicated to the characterisation of compounds formed during holocellulose (cellulose and hemicelluloses) and lignocellulose degradation with the aim of better comprehending the phenomena of paper decay. Through the identification of a few target compounds and the estimation of their abundance, the second objective was to examine possible molecular indicators or markers of paper alteration. Molecular indicators will help explore potential new diagnostic strategies of the changes occurring in paper with time. In order to be selected as molecular indicators of paper decay, the target compounds should be key sub-products from paper degradation. These products vary in nature depending on the type of pulp, and occur in different classes of organic compounds. Carbohydrates (mono-, di-, and oligosaccharides) and LMM aliphatic organic acids arise from the holocellulose fraction of paper. Aromatic, phenolic and furanic, compounds arise from the degradation of lignin. Target compounds should be sought among these three most prominent classes. Accordingly, the model compounds chosen to serve as standards for identification purposes in the unknown paper samples were selected in these categories based on notorious degradation products from cellulose, hemicelluloses, and lignin mentioned in the literature (ASTM Report, 2002; Buchbauer et al., 1995; Dahlman, Jacobs, Liljenberg, & Olsson, 2000; Havermans et al., 1999; Nevell, 1985; Parks et al., 1990; Shahani & Harrison, 2002). Different CZE methods were applied for the determination and quantitation of these compounds. For a representative sampling, and in order to encompass a wide range of states of deterioration of paper, naturally aged books of varied origins were selected. In addition, a model paper was submitted to artificial ageing and analysed. The similarity between the compounds evidenced in the naturally aged books and in the model paper was evaluated, and the evolution in their production as degradation proceeded during ageing was followed.

## 2. Experiments

### 2.1. Paper samples and extraction

Five books covering a period of 300 years and composed of different pulps were chosen. A modern acidic paper made of groundwood pulp (cold extract pH 5.9), henceforth denoted 'Mod', was used as model and underwent accelerated ageing tests. It was washed in deionised water and allowed to dry in a climatic room at 23 °C and 50% rH before analysis in order to eliminate possible pre-occurring degradation products. The books and paper description as determined by microscopic examination and histological staining with specific dyes (Herzberg and Lofton-Merrit) is given in Table 1. The paper samples were cut in 5 $mm^2$ pieces and were immersed in 2 mL deionised water at room temperature for 1 h. For organic acids and lignin derivatives extraction, 0.3 g of paper were used. For carbohydrates analysis, 0.9 g of paper was used. After 1 h, the water extract was filtered on a Millex®-GV filter (0.22 μm; PVDF, Millipore®).

Table 1
Books description and their average $M_w$ and PD

| Book abbreviation | Date | Pulp | Average $M_w$ (g $mol^{-1}$) | | Average PD ($M_w/M_n$) | |
|---|---|---|---|---|---|---|
| | | | Edges | Central zone | Edges | Central zone |
| SE | 18th century | Rag paper (linen) | $1.451 \times 10^5$ (STD% 4.2) | $2.484 \times 10^5$ (STD% 0.52) | 2.21 | 2.61 |
| OC | 1834 | Rag paper (linen or hemp) | $2.380 \times 10^5$ (STD% 0.72) | $3.054 \times 10^5$ (STD% 1.45) | 2.79 | 3.08 |
| FA | 1931 | Groundwood pulp (hardwood) | | | | |
| PDP | 1941 | Groundwood pulp and bleached kraft pulp | | | | |
| PB | 1968 | Groundwood pulp (minor) and bleached kraft pulp | | | | |
| Mod | 1990 | 75% groundwood pulp and 25% softwood cellulose | | | | |

The model compounds stock solutions were a mix of the different LMM organic acids or lignin derivatives in deionised water (DirectQ, Millipore®). For carbohydrate analysis, derivatisation was necessary prior to the injection. The derivatisation was carried out using the method based on the reductive amination of saccharides (Vorndran, Grill, Huber, Oefner, & Bonn, 1992). A stock solution of 4-aminobenzoic acid ethyl ester (ABEE) (100 mg mL$^{-1}$) and acetic acid (100 mg mL$^{-1}$) was prepared in methanol, to which cyanoborohydride (10 mg mL$^{-1}$) was added. The new solution was immediately mixed 1:1 (v/v) to the aqueous solution of saccharides (3 mM each in the model compounds stock solution) in a conical bottom vial. After heating to 80 °C for 1 h the excess unreacted ABEE was precipitated by adding 1–1.5 parts of a 450-mM borate buffer (pH 8.6) prepared by dissolving boric acid in deionised water (DirectQ, Millipore®). The reaction mixture was then vortexed vigorously for a few seconds. The solution was cooled to ambient temperature and the precipitate removed by filtering on a Chromafil® filter (0.20 μm; PTFE, Macherey Nagel).

### 2.2. Molar mass determination

The average molar mass of cellulose from SE and OC, the two rag paper books, was determined using size-exclusion chromatography (SEC) with dual online detection, multiangle laser light scattering (MALS), and differential refractive index (DRI). The analysis could not be carried out on the other books, as their lignin content was too high to allow their direct dissolution in the solvent used. A LC solvent vacuum degasser 1100 series (Agilent), isocratic HPLC pump 515 (Waters), and manual injector (Vici AG, Valco International) were part of the chromatographic set-up. The MALS detector was a Dawn EOS (Wyatt Technologies) and the DRI detector was a 2414 (Waters). The separation was carried out on a set of four columns packed with polystyrene divinyl benzene Phenogel Linear (2) (5-μm particle diameter mixed bed pores columns, $L \times D$ 300 mm × 4.6 mm, Phenomenex) preceded by a guard column Phenogel (5-μm, $L \times D$ 30 mm × 4.6 mm, Phenomenex). The columns compartment (Interchim, Model 102) and the MALS were thermostated at 60 °C, the DRI was set to 55 °C. The system was operated at a flow rate of 0.4 mL min$^{-1}$ with an injection volume of 100 μL, and the run time was 50 min. The mobile phase was *N*,*N*-dimethylacetamide with 0.5% lithium chloride (LiCl/DMAc). The dissolution of the paper samples prior to the SEC analysis was carried out in 8% LiCl/DMAc, according to a procedure developed and detailed in a previous publication (Dupont, 2003b). The data acquisition was carried out with ASTRA software version 4.90.07 (Wyatt Technologies) in 0.5-s intervals. Each sample solution was run three times non-consecutively. The average values are reported in Table 1. The repeatability of the SEC–MALS/DRI method as previously determined was RSD% = 2.5 on $M_w$ for three separate pure cellulose paper samples analysed two to three times non-consecutively. More details on the chromatographic procedure are given elsewhere (Dupont & Harrison, 2004).

### 2.3. Accelerated ageing

The accelerated ageing was carried out with the paper 'Mod' for periods of 2, 3.5, and 8 days inside hermetically sealed glass tubes (Alltech Associates) in a dry heat oven (Memmert) at 100 °C following the ASTM standard method D6819-02e2 (ASTM Report, 2002; D6819-02e2, 2002). This accelerated ageing was preferred over a method where sheets of paper are individually suspended in a climatic chamber since it has been recently proposed to better mimic natural ageing (ASTM Report, 2002; Shahani & Harrison, 2002). Before insertion in the glass tubes, the paper was preconditioned to 23 °C and 50% rH. In the present work, the sample size was miniaturised by using 1.36 g of paper instead of 4 g. The volume of the glass tubes was 49 mL instead of 145 mL in order to maintain the ratio of paper weight to the volume of air inside the tube identical (w/v = 0.028 g mL$^{-1}$) and thus achieve similar partial pressure of water vapour and rH inside the tube as in the ASTM standard method. The rH inside the tube has been estimated to approach 90–100% (ASTM Report, 2002. Ch. Temperature aging studies at CCI). Once out of the oven, the glass tubes were left to cool in the climatic room before opening and the paper samples were subsequently reconditioned for 24 h to 23 °C and 50% rH before analysis. In order to project future degradation of naturally aged paper and better appraise natural ageing versus accelerated ageing, samples of the old books (PDP, OC, PB, and FA) were aged in sealed tubes under approximately the same conditions as above (w/v of 0.02 g mL$^{-1}$) for 2 and 5 days, respectively.

### 2.4. Chemicals

#### 2.4.1. Model LMM organic acids

Glycolic acid (99%, Merck), lactic acid (85%, Aldrich), succinic acid (>99.5%, Fluka), potassium formate (99%, Aldrich), and sodium acetate (>99%, Fluka).

#### 2.4.2. Model lignin derivatives

3,5-Dimethoxy-4-hydroxyacetophenone (acetosyringone) (97%, Aldrich), 4-hydroxy-3-methoxyacetophenone (acetovanillone) (98%, Aldrich), 4-hydroxyacetophenone (99%, Aldrich), 4-hydroxy-3-methoxybenzaldehyde (vanillin) (⩾98% Fluka), 4-hydroxybenzaldehyde (98%, Aldrich), 4-hydroxy-3-methoxybenzoic acid (vanillic acid) (⩾98 %, Merck), 2-furancarboxylic acid (2-furoic acid) (98%, Aldrich), and 4-hydroxybenzoic acid (99+%, Aldrich).

#### 2.4.3. Model carbohydrates

D-glucose (Labosi), D-mannose (Touzart et Matignon), D-galactose, D-xylose and L-arabinose (Prolabo), D-(+)-cellobiose (99%, Fluka), D-(+)-cellotriose (98%, Fluka), and cellotetraose (90+%, Fluka).

*2.4.4. Reagents*

Cetyltrimethylammonium bromide (CTAB, Aldrich), 2,6-pyridinedicarboxylic acid (PDC, 99%, Aldrich), ABEE (Sigma), sodium cyanoborohydride (95%, Aldrich), boric acid (99.79%, Fisher Chemicals), acetic acid glacial (99.6%, Carlo Erba Reagents), and sodium hydroxide (Fisher Chemicals).

*2.5. CE conditions*

A P/ACE MDQ equipped with a PDA detector was employed (Beckman Coulter). The system operation, data acquisition, calibration, and quantitation were performed using 32 Karat 5.0 software (Beckman Coulter). The compounds were identified according to their migration time $t_m$ compared to the model compounds, and their UV absorption spectra when applicable (aromatic compounds). Spiking with corresponding model compounds was carried out when attributions needed to be confirmed.

*2.5.1. LMM organic acids analysis*

Indirect UV detection at 350 nm was used with the reference at 200 nm and a bandwidth of 20 nm. Data collection rate was set to 4 Hz. A bare fused silica capillary with 75 μm internal diameter (ID) (Beckman Coulter) was cut to a total length of 61 cm (50.4 cm effective length). Prior to injection the capillary was rinsed by flushing 1 min with NaOH 0.1 M followed by 1.5 min with deionised water. It was then conditioned for 1 min with the running buffer. The buffer was prepared with PDC 5 mM as background electrolyte (BGE), and CTAB 0.5 mM, pH 5.6 (adjusted with NaOH 1 M). The injection was made in hydrodynamic mode by applying a pressure of 0.7 psi for 4.5 s. A separation voltage of −25 kV was applied to the anodic end. The resulting current was 15–16 μA. The run temperature was fixed to 25 °C. After each analysis the capillary was rinsed for 2 min with deionised water.

*2.5.2. Lignin derivatives analysis*

Direct UV detection using three wavelength channels was used with a bandwidth of 20 nm. The channels were selected in order to optimise the sensitivity of the detection for each analyte by choosing their absorption maxima or near-maxima: 325 nm was used for acetosyringone, acetovanillone, 4-hydroxyacetophenone, vanillin, and 4-hydroxybenzaldehyde, 242 nm was chosen for 2-furoic acid, and 210 nm for vanillic acid and 4-hydroxybenzoic acid. Data collection rate was set to 4 Hz. The bare fused silica capillary with 75 μm ID (Beckman Coulter) was cut to a total length of 60.3 cm (50.3 cm effective length). The capillary was rinsed by flushing 1 min with NaOH 0.1 M followed by 1.5 min with deionised water. It was then conditioned for 1 min with the running buffer. The electrolyte was a borate buffer 50 mM, pH 9.1, prepared with boric acid (99.79%, Fisher Chemicals) and sodium tetraborate anhydrous (⩾99%, Fluka). The injection was made in hydrodynamic mode by applying a pressure of 0.7 psi for 4.5 s. The separation voltage was +25 kV applied to the anodic end, and the resulting current was in the range 66–70 μA. The temperature was fixed to 25 °C. After the analysis the capillary was rinsed for 2 min with deionised water.

*2.5.3. Carbohydrates analysis*

Direct UV detection at 305 nm of the pre-derivatised carbohydrates was used with a bandwidth of 30 nm. Data collection rate was set to 4 Hz. A bare fused silica capillary with 20 μm ID (Beckman Coulter) was cut to a total length of 48.5 cm (40 cm effective length). The capillary was rinsed by flushing 1 min with NaOH 0.1 M followed by 1.5 min with deionised water. It was then conditioned for 3 min with the running buffer, a borate buffer 450 mM, pH 9.94, prepared with boric acid and NaOH (300 mM). The injection was made in hydrodynamic mode by applying a pressure of 0.5 psi for 20 s. The separation voltage was +28 kV applied to the anodic end, and the resulting current was 17 μA. The run temperature was fixed to 20 °C. After the analysis the capillary was rinsed for 2 min with deionised water.

*2.6. Method validation*

The parameters used to evaluate robustness and specificity of the methods were precision, linearity, and sensitivity. Within-day precision, assimilated to repeatability ($r$) and day-to-day precision, assimilated to reproducibility ($R$) were determined on peak areas and $t_m$. $R$ and $r$ were calculated in terms of relative standard deviation, with RSD% = (Standard Deviation/average) × 100. The value of $R$ was established based on five injections of different solutions made on different days, and that of $r$ was based on five injections of a given solution made on the same day. The limit of detection (LOD) and the limit of quantitation (LOQ) used to assess sensitivity were calculated as $3S/N$ and 3.33 LOD respectively, as recommended for HPLC methods (Snyder, Kikland, & Glajch, 1997). The linearity of the response was established for each model analyte building calibration curves with five to six levels of concentration using individual stock solutions. Each calibration point corresponded to five injections.

## 3. Results and discussion

*3.1. Sample preparation*

Working on books and other original graphic documents entails certain limitations in terms of sampling possibilities. In this context, the analytical work has to compromise between a miniaturisation of the amount of paper sample and the requirements for reliability and significance of the data bound to the LOD and LOQ. Previous work reported the use of 1 g of paper in 5 mL of water and a time of extraction of 30 min (Shahani & Harrison, 2002). The possibility of increasing the injection volume of a less

concentrated sample was not envisaged as it would amplify background noise and cause peak widening. Instead, the weight of paper and volume of water were reduced, and various extraction times were tested. We compared extractions made on 0.5 g with w/v = 0.1 g mL$^{-1}$, 0.3 g with w/v = 0.15 and 0.06 g mL$^{-1}$, and 0.15 g with w/v = 0.075 g mL$^{-1}$. Extraction times were 30, 45, and 60 min. The best compromise between sample consumption and goodness of the signal was obtained with 0.3 g of paper (w/v = 0.15 g mL$^{-1}$) in 60 min. The extractions were carried out at room temperature. Efforts to concentrate the samples by centrifugation and high-temperature extraction both resulted in a depletion of the most volatile analytes from the aqueous solutions. Ethanol and methanol were tested as extraction solvents but resulted in current and baseline instability during the CZE separation.

### 3.2. LMM organic acids

The analysis of LMM organic acids is of interest in various research areas. Numerous publications have been dedicated to the separation of these acids in a variety of organic products such as food and beverage samples (Castiñeira, Peña, Herrero, & García-Martín, 2000; Esteves, Lima, Lima, & Duarte, 2004; Galli & Barbas, 2004; Kampfl, Buchberger, & Haddad, 2000; Mato, Huidobro, Simal-Lozano, & Sancho, 2006; Soga & Ross, 1997), plants and plant products (Galli, Olmo, & Barbas, 2000; Wang, Qu, Shan, & Lin, 2003), environmental samples (Hagberg, Dahlén, Karlsson, & Allard, 2000; Levart, Guček, Pihlar, & Veber, 2000), and industrial samples (Chen, Preston, & Zimmerman, 1997). To our knowledge, despite the potential of CE for applications related to cultural heritage, only one study has been devoted to the separation of LMM organic acids in paper (Shahani & Harrison, 2002). Due to their low absorptivities above 220 nm, most CZE separations of LMM organic acids are based on indirect UV detection, which relies on the addition of a UV absorbing co-ion in the buffer as BGE. For their separation, the use of phthalate, benzoate, and PDC has been most often reported (Weinberger, 2000). The mobility of these three BGEs is close to those of the short-chain organic acids under study, which is one of the requirements to produce fine peaks presenting no fronting or tailing (Soga & Ross, 1997). In the present study, the three chromophore ions were tested in the same electrophoretic conditions as described in *LMM organic acids analysis* unless otherwise stated (see *CE conditions*). Benzoate-based buffers gave unexploitable data, with very noisy baselines [benzoic acid 10 mM in phosphate buffer 50 mM ($NaH_2PO_4$/$Na_2HPO_4$) pH 7; benzoic acid 5 mM, CTAB 5 mM in phosphate buffer 500 mM pH 6.5; benzoic acid 5 mM, CTAB 0.5 mM in phosphate buffer 12 mM pH 6.5; benzoic acid 5 mM and CTAB 0.5 mM in acetate buffer 25 mM pH 5.5–2 s injection at 0.3 psi]. The phthalate buffer yielded a fine peak with an acetate standard but the repeatability on $t_m$ was very poor [methylphthalate 5 mM, CTAB 0.25 mM pH 6.8–UV detection at 340 nm, reference at 200 nm]. As detailed in *Method optimisation*, PDC yielded the only usable results.

#### 3.2.1. Method optimisation

In order to achieve an optimised separation of LMM organic acids, the following parameters were tested.

*3.2.1.1. Coating of the capillary.* Cationic surfactants such as CTAB are used to dynamically coat the capillary and reverse the electroosmotic flow (EOF), which is frequently done when separating analytes in their anionic form to shorten the migration times. Several concentrations of CTAB in the running buffer were tested, from 0.25 to 2 mM. Migration times obtained were not significantly different but with 1 and 2 mM, the baseline noise increased considerably thereby worsening the LOD. This was attributed to the fact that these concentrations were above the critical micelle concentration of CTAB (CMC = 0.92 mM), and the availability of the surfactant for dynamically coating the capillary was lesser. In the lower concentration range, based on four runs, $t_m$ of formate, glycolate, acetate, and lactate showed globally smaller RSD values when 0.5 mM CTAB was used in the buffer (RSD% 0.085, 0.09, 0.32, and 0.12, respectively) than when 0.25 mM CTAB was used (RSD% 0.39, 0.47, 0.1, and 1.7, respectively). A concentration of 0.5 mM CTAB was thus selected for the running buffer.

*3.2.1.2. Ionic strength of the buffer.* High PDC concentration (high ionic strength) generates slower EOF. Fig. 1 shows that at constant CTAB concentration of 0.5 mM the sensitivity decreased with increasing PDC concentration in the running buffer (1, 5, 10, and 25 mM). The fastest separation combined with the highest sensitivity was achieved with 1 mM PDC. However, selectivity was not optimal as glycolate and acetate co-migrated. A concentration of 5 mM PDC was thus chosen as the best compromise between good resolution of glycolate and acetate, separation speed, and sensitivity.

*3.2.1.3. pH of the buffer.* The pH has an effect on the separation by affecting both the mobility of the analytes and the EOF. The model LMM organic acids have p$K_a$ values between 3.75 and 5.61. The best reproducibility on the $t_m$ of most organic acids chosen using PDC as BGE has been previously shown to fall in the pH range 5–6, where the mobilities are less sensitive to small pH variations (Soga & Ross, 1997). A pH range from 5 to 7 was explored. We can see in Fig. 2 that in the range pH 5–5.9 no significant change in $t_m$ of formate, glycolate, acetate, and lactate was observed. Beyond pH 5.9, baseline instability, widening, and tailing of peaks were observed. The resolution also decreased significantly with a co-migration of glycolate and acetate. It was decided to use pH 5.6, in order to possibly compare the data with previous work (Shahani & Harrison, 2002; Soga & Ross, 1997).

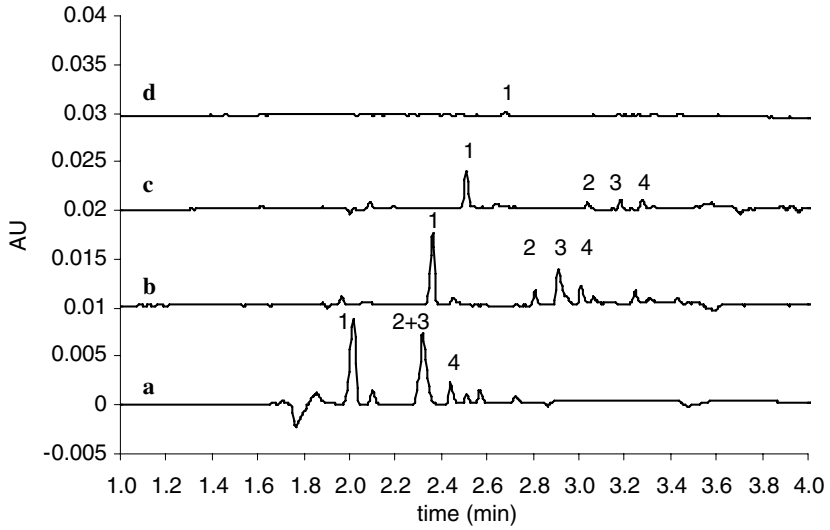


Fig. 1. Effect of PDC concentration in the buffer (with CTAB 0.5 mM). (a) 1 mM, (b) 5 mM, (c) 10 mM, (d) 25 mM, with (1) formate, (2) glycolate, (3) acetate, (4) lactate, respectively (0.1–2 mg $L^{-1}$ each).

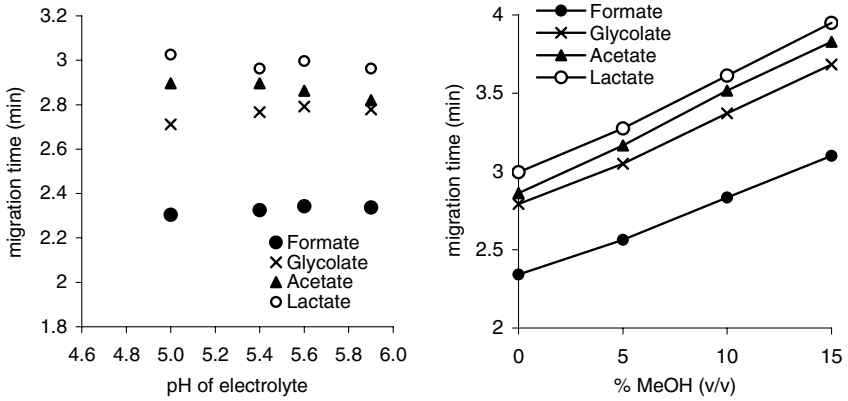


Fig. 2. Effect of electrolyte pH (left graph) and effect of methanol in the electrolyte (right graph) on the separation.

*3.2.1.4. EOF modifier.* Organic solvents can be used in small proportion in buffers to enhance the selectivity of the separation by modifying the zeta potential and thus the EOF. By modifying the mobility (variation of the size of the solvated particle) and the p$K_a$ of the analytes, solvents can also influence their migration (Sarmini & Kenndler, 1997). The influence of adding methanol (0–15%) in the running buffer is shown in Fig. 2. The $t_m$ increased linearly with increasing methanol content, and the resolution between glycolate and acetate was slightly improved. However, widening and tailing of the peaks increased as well. With reasonable resolution between the two acids without methanol it was thus decided not to add solvent in the buffer.

### 3.2.2. Method validation

The method was found fairly repeatable and reproducible with values of $r$ and $R$ which varied slightly for each acid, and were better on $t_m$ ($0.16\% < r < 0.99\%$ and $0.07\% < R < 0.44\%$) than on peak areas ($2.46\% < r < 7.2\%$ and $0.95\% < R < 3.66\%$) (Table 2). The linearity evaluated using six solutions (5, 10, 20, 50, 75, and 100 mg $L^{-1}$) of each model LMM acid yielded coefficients of linear correlation of 0.999 indicating a fair precision. LODs below 0.5 mg $L^{-1}$ and LOQs below 1.5 mg $L^{-1}$ indicated a good sensitivity (Table 3).

### 3.2.3. Naturally aged books

The electropherograms obtained with the books OC, PB, and FA are shown in Fig. 3. The five model LMM organic acids were effectively found in various amounts in the three books extracts. This confirmed earlier work, which also identified these LMM organic acids in papers using CZE

Table 2
Repeatability ($r$) and reproducibility ($R$), RSD% ($n = 5$) of migration times ($t_m$) and peak areas for LMM organic acids

| | Formate | | Succinate | | Glycolate | | Acetate | | Lactate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area |
| $r$ | 0.21 | 2.5 | 0.99 | 3.79 | 0.16 | 2.16 | 0.63 | 3.35 | 0.17 | 7.2 |
| R | 0.07 | 3.66 | 0.44 | 1.35 | 0.16 | 0.95 | 0.25 | 3.60 | 0.15 | 3.60 |
| Average $t_m$ (min) | 2.45 | | 2.70 | | 2.90 | | 3.00 | | 3.11 | |

Table 3
Calibration curves equations, LODs, and LOQs for LMM organic acids standards, lignin derivatives standards, and carbohydrate standards, with $x$ = peak area and $y$ = acid concentration (mg $L^{-1}$)

| | Calibration curve | Correlation coefficient | LOD | | LOQ | |
|---|---|---|---|---|---|---|
| | | | (mg $L^{-1}$) | (μmol $L^{-1}$) | (mg $L^{-1}$) | (μmol $L^{-1}$) |
| Formate | $y = 1.4540 \times 10^{-3}x$ | 0.9992 | 0.06 | 1.3 | 0.20 | 4.3 |
| Succinate | $y = 1.0740 \times 10^{-3}x$ | 0.9993 | 0.52 | 4.4 | 1.7 | 14.4 |
| Glycolate | $y = 9.0802 \times 10^{-4}x$ | 0.9999 | 0.35 | 4.6 | 1.17 | 15.4 |
| Acetate | $y = 1.2326 \times 10^{-3}x$ | 0.9996 | 0.43 | 7.2 | 1.43 | 23.8 |
| Lactate | $y = 1.8857 \times 10^{-3}x$ | 0.9992 | 0.37 | 4.1 | 1.23 | 13.7 |
| Acetosyringone | $y = 1.0071 \times 10^{-3}x$ | 0.99998 | 0.42 | 2.1 | 1.40 | 7.1 |
| Acetovanillone | $y = 5.1570 \times 10^{-4}x$ | 0.9997 | 0.19 | 1.1 | 0.63 | 3.8 |
| 4-Hydroxyacetophenone | $y = 3.2923 \times 10^{-3}x$ | 0.9989 | 0.09 | 0.7 | 0.30 | 2.2 |
| 4-Hydroxybenzaldehyde | $y = 1.9648 \times 10^{-4}x$ | 0.9989 | 0.15 | 1.2 | 0.50 | 4.1 |
| Vanillin | $y = 3.7970 \times 10^{-4}x$ | 0.99996 | 0.09 | 0.6 | 0.30 | 2.0 |
| Vanillic acid | $y = 1.6707 \times 10^{-3}x$ | 0.998 | 0.35 | 2.1 | 1.18 | 7.0 |
| Furoic acid | $y = 1.3439 \times 10^{-3}x$ | 0.998 | 0.11 | 0.9 | 0.37 | 3.3 |
| 4-Hydroxybenzoic acid | $y = 5.8579 \times 10^{-4}x$ | 0.998 | 0.05 | 0.4 | 0.17 | 1.2 |
| Cellobiose | $y = 8.46055 \times 10^{-2}x$ | 0.9991 | 72 | 210 | 240 | 700 |
| Xylose | $y = 3.87580 \times 10^{-2}x$ | 0.9984 | 36 | 240 | 120 | 800 |
| Glucose | $y = 3.51453 \times 10^{-2}x$ | 0.9993 | 35 | 190 | 117 | 650 |
| Mannose | $y = 3.85534 \times 10^{-2}x$ | 0.9990 | 40 | 220 | 133 | 740 |
| Arabinose | $y = 3.03211 \times 10^{-2}x$ | 0.9997 | 35 | 230 | 117 | 780 |
| Galactose | $y = 3.06014 \times 10^{-2}x$ | 0.9984 | 37 | 210 | 123 | 680 |

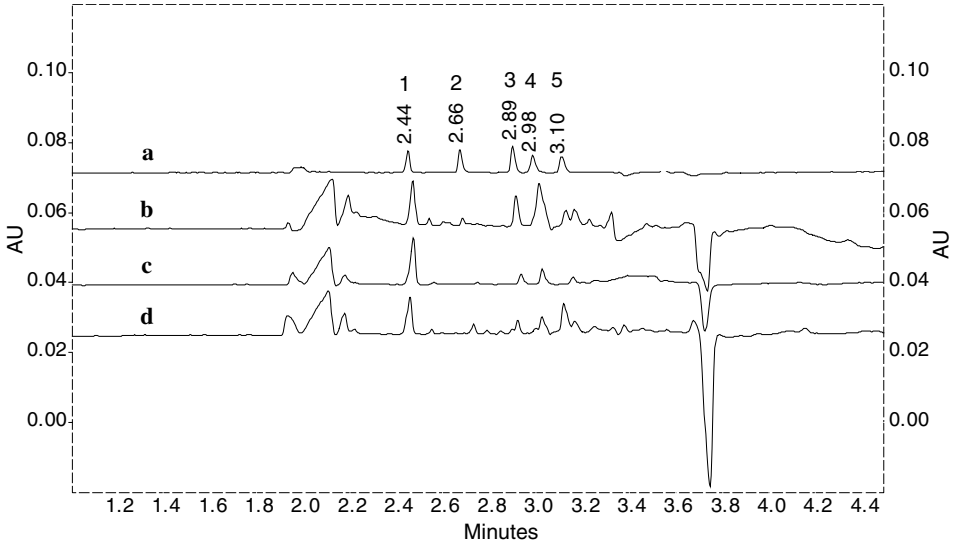


Fig. 3. Electropherograms of (a) model LMM organic acids stock solution with (1) formate, (2) succinate, (3) glycolate, (4) acetate, and (5) lactate, 10 mg $L^{-1}$ each in deionised water (MilliQ, Millipore®); (b) aqueous extract FA; (c) aqueous extract PB; (d) aqueous extract OC. Capillary 75 μm × 61 cm ($l$ = 50.4 cm). Electrolyte PDC 5 mM, CTAB 0.5 mM pH 5.6. Hydrodynamic injection 0.7 psi, 4.5 s; applied potential −25 kV; temperature 25 °C. Detection, signal = 350 nm, reference = 200 nm.

(Shahani & Harrison, 2002). The method was very fast; the five acids were separated within little more than 3 min. The complexity of the sample matrix did not affect the selectivity and sensitivity of the method as a system peak appeared at $t_m$ 3.75 min, distanced enough from the $t_m$ of the acids. Very few and minor peaks were unidentified. This result infers that the number of different short-chain aliphatic acids produced during the decay of paper is limited, and that they occur in all the papers regardless of the pulp, origin, and additives. They are produced from the holocellulose fraction of the paper. The large fronting peak at $t_m$ 2.1 min corresponds to unresolved small inorganic ions (aluminate from the internal sizing, residual chloride from bleaching, sulphate, and nitrate from atmospheric pollution).

The age and/or the state of deterioration of the books as visually evaluated and characterised using SEC-MALS/DRI was overall consistent with the relative abundance of the LMM organic acids found in the naturally aged books (Fig. 4). Very yellowed and brittle, a typical condition of groundwood pulp books of the first half of 20th century, FA which was in the most advanced state of degradation,

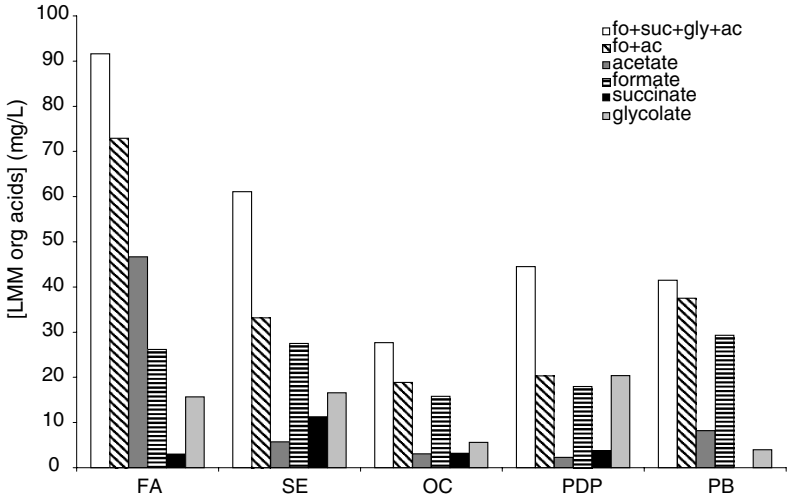


Fig. 4. Quantitation of LMM organic acids in the naturally aged books.

showed the highest proportion of total organic acids present.

SE ranked second in the production of total organic acids. SE is made of rag paper (almost pure cellulose) and is in fair general conservation condition. SE is the oldest of the books (18th century) which is consistent with the high organic acids content quantified. Certainly, the values of $M_w$ of the cellulose of SE sampled from several pages in the edge (typically the most degraded area) and in a more central area between the edge and the inked zone (typically a less degraded area) were $1.45 \times 10^5$ and $2.48 \times 10^5\,g\,mol^{-1}$, respectively. These values indicated a book relatively degraded, in particular on the edges of the pages, as they approached $10^5\,g\,mol^{-1}$ which has been proposed as a critical molar mass of cellulose below which the paper can be considered as mechanically fragile (Jerosch, 2002).

OC, also made of rag paper but more recent than SE and visibly in good conservation condition, showed the lowest yield in total LMM acid-degradation products of all the books analysed. The values of $M_w$ of cellulose from OC (sampled in the same areas of the pages as described for SE) were found significantly higher than those of SE, with $2.38 \times 10^5$ and $3.05 \times 10^5\,g\,mol^{-1}$ for the edge and the central page zone, respectively. In SE and OC, lower $M_w$ and higher polydispersity (PD) values compared to a new rag paper (cotton or linen) are a sign of degradation (both parameters would typically be in the ranges $M_w = 4.5 - 5 \times 10^5\,g\,mol^{-1}$/PD = 1.6 – 1.8). However, in relative terms, the determination of the molar mass confirmed the results obtained with CZE that OC was clearly less degraded than SE.

The coherence in these results corroborates the discussion but should be taken as an indication rather than a proof as the initial values of the molar masses of the cellulose at the moment of the production of the particular books are of course unknown. In order to try to circumvent this constraint, it was decided to superimpose the effect of accelerated ageing on the papers from the books, already aged naturally, and to observe the progression in the production of the LMM organic acids. Acetate and formate were found to increase during artificial ageing with a quasi-linear progression in PDP, PB, and OC (and a slightly different evolution in FA) (Fig. 5). We chose to follow the production of these two acids because they were major components in all the books extracts, and their evolution seemed the most uniform. They were thus the more susceptible to be used as degradation indicators. Conversely, glycolate and succinate were absent or very minor (OC, PB),

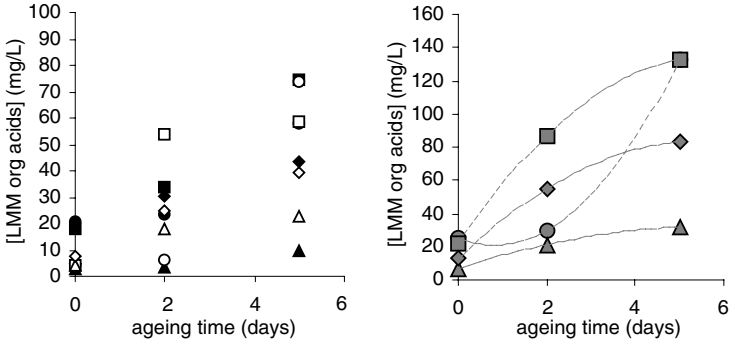


Fig. 5. Production of LMM acids during accelerated ageing of FA (circles), PDP (squares), OC (triangles) and PB (diamonds). Left graph: acetate (full marks) and formate (void marks). Right graph: sum of formate and acetate.

or showed a more random progression during ageing (FA), which is consistent with findings from earlier work (ASTM Report, 2002). In FA, succinate was quantified as 3.0, 14.3, and 3.0 mg $L^{-1}$ and glycolate as 15.7, 0, and 0 mg $L^{-1}$ at 0, 2, and 5 days of ageing, respectively. Lactate was excluded as potential degradation marker as lactic acid is also produced by metabolism reactions of microorganisms.

### 3.2.4. Artificially aged model paper

Similarly as in the naturally aged books, the five model LMM aliphatic acids were identified in the paper Mod as major analytes, and only a few minor other peaks were present. This corroborates the fact that the accelerated ageing method in sealed tube relates quite closely to natural ageing, thus allowing for better life expectancy predictions than other ageing methods where sheets of paper are hung in a heat/humidity climate chamber, and allowed to off-gas in the ventilated oven space. In the sealed tube, the organic compounds produced during the degradation remain in contact with the paper during the entire ageing period (as in a closed book) and are believed to accelerate the degradation rate (Shahani & Harrison, 2002). The evolution in the production of the acids in the paper 'Mod' during the accelerated ageing can be seen in Fig. 6. The total production of acids and the sum of formate and acetate showed a linear progression, with correlation coefficients above 0.9. This result further supported the plausible use of these two acids as indicators of paper degradation.

Following the production of acetic acid in paper as an indicator of the evolution of the degradation has been proposed earlier (Ligterink & Pedersoli, 2001). The authors evaluated the occurrence of acetic acid and furfural in paper using SPME/GC-MS. The main difficulty in finding a reliable marker of degradation is of course its chemical significance. The key issues are twofold: choosing a compound (or several compounds) that is representative of the major degradation pathway taking place and using sampling/measurement techniques that are appropriate to evaluate the occurrence and the evolution in the production of this compound. As the organic acids analysed in the papers are more or less volatile ones, a gas-phase sampling method such as SPME is justified (Lattuati-Derieux et al., 2004; Ligterink & Pedersoli, 2001). However, LMM organic acids are polar and show a great affinity to cellulose. A sampling method in aqueous phase seemed therefore more suitable than a gas-phase sampling method to evaluate global abundances. In aqueous phase, the acids adsorbed in the cellulose fibres can more easily dissociate and dissolve in the water, while in gas phase, only the equilibrium gas fraction between the paper and the surrounding air can possibly be analysed.

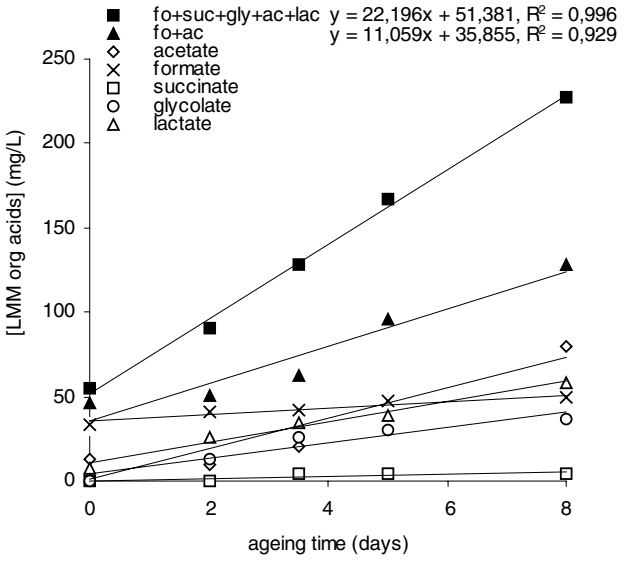


Fig. 6. Production of LMM organic acids in paper 'Mod' during accelerated ageing.

### 3.3. Lignin derivatives

As in the case of LMM organic acids, the main advantages of CE over GC methods in the analysis of aromatic lignin derivatives are reduced sample preparation (derivatisation, usually esterification, is unnecessary) and extremely short separation times. However, few studies have been dedicated to the analysis of lignin and its degradation products using CZE, these compounds being of interest almost exclusively to the pulp and paper industry, and biomass control (Fournand & Lapierre, 2001; Javor, Buchberger, & Faix, 2003; Sjöholm, Nilvebrant, & Colmsjö, 1993; Sjöholm, Norman, Störker, & Colmsjö, 2000; Sjöholm, Störker, & Norman, 2000). To our knowledge, only one study has looked at lignin derivatives in degraded historic papers (ASTM Report, 2002. Ch. Temperature aging studies at LOC) and served as a base to the present study. The method required no further optimisation besides modifying the UV detection channels for improved sensitivity, as described in the *Experiments* section.

### 3.3.1. Method validation

The values of $r$ and $R$ varied slightly for each model compound, and were generally better on peak areas ($0.01\% < r < 0.04\%$ and $0.07\% < R < 1.69\%$) than on $t_m$ ($0.5\% < r < 0.7\%$ and $0.38\% < R < 2.85\%$), but values not exceeding 3% proved the method is precise (Table 4). Linearity evaluated using six solutions (0.5, 2, 10, 20, 50, and 100 mg $L^{-1}$) of each of the eight model lignin derivative compounds gave coefficients of linear correlation of at least of 0.999 for most analytes, 0.998 for vanillate and furoate, which showed acceptable precision. The method was sensitive with LODs below 0.4 mg $L^{-1}$ and LOQs below 1.4 mg $L^{-1}$ (Table 3).

### 3.3.2. Naturally aged books

The separation of the model lignin derivatives was achieved within 9 min (Fig. 7). In the papers containing groundwood pulp (FA, PDP, and PB) acetosyringone, 4-hydroxyacetophenone, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 2-furoic acid, vanillin, and vanillic acid were found in measurable amounts (Fig. 8 and Table 5). Vanillic

Table 4
Repeatability ($r$) and reproducibility ($R$), RSD% ($n = 5$) of migration times ($t_m$) and peak areas for lignin derivatives with (1) acetosyringone, (2) acetovanillone, (3) 4-hydroxyacetophenone, (4) vanillin, (5) 4-hydroxybenzaldehyde, (6) vanillic acid, (7) furoic acid, and (8) 4-hydroxybenzoic acid

| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area |
| $r$ | 0.54 | 0.01 | 0.54 | 0.02 | 0.57 | 0.02 | 0.58 | 0.02 | 0.62 | 0.02 | 0.50 | 0.04 | 0.64 | 0.02 | 0.70 | 0.02 |
| $R$ | 0.88 | 1.69 | 1.02 | 0.07 | 1.68 | 0.08 | 0.38 | 0.09 | 0.97 | 0.1 | 2.85 | 0.2 | 2.29 | 0.13 | 0.92 | 0.13 |
| Average $t_m$ (min) | 5.80 | | 6.08 | | 6.35 | | 6.77 | | 7.31 | | 7.91 | | 8.53 | | 8.70 | |

acid was the most abundant analyte with 103 mg $L^{-1}$ in PB, 168 mg $L^{-1}$ in FA, and 339 mg $L^{-1}$ in PDP (Table 5). Vanillin was found to occur proportionally an order of magnitude less than vanillic acid. The less abundant were 4-hydroxybenzaldehyde and 2-furoic acid; acetovanillone was not detected in any of the books. Very few unidentified peaks were present on the electropherograms, which indicated a selective method and a non-perturbing sample matrix effect on the separation.

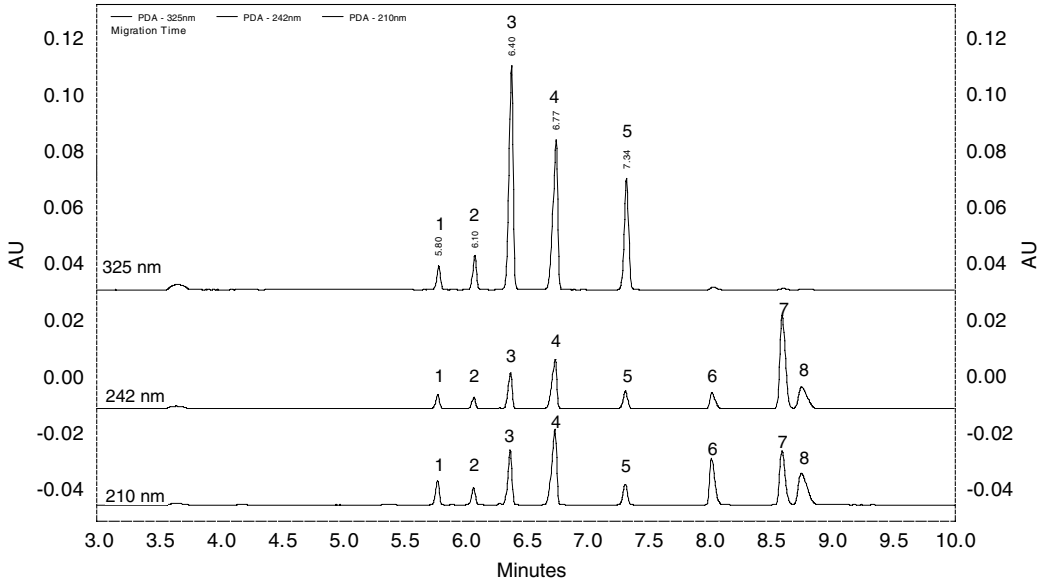


Fig. 7. Electropherograms of model lignin derivatives stock solution with (1) acetosyringone, (2) acetovanillone, (3) 4-hydroxyacetophenone, (4) vanillin, (5) 4-hydroxybenzaldehyde, (6) vanillic acid, (7) 2-furoic acid, and (8) 4-hydroxybenzoic acid, 20 mg $L^{-1}$ each in deionised water (MilliQ, Millipore®). Capillary 75 μm × 60.3 cm ($l = 50.3$ cm). Borate buffer 50 mM pH 9.1. Hydrodynamic injection 0.7 psi, 4.5 s; applied potential +25 kV; temperature 25 °C. Detection signals = 325, 242, and 210 nm.

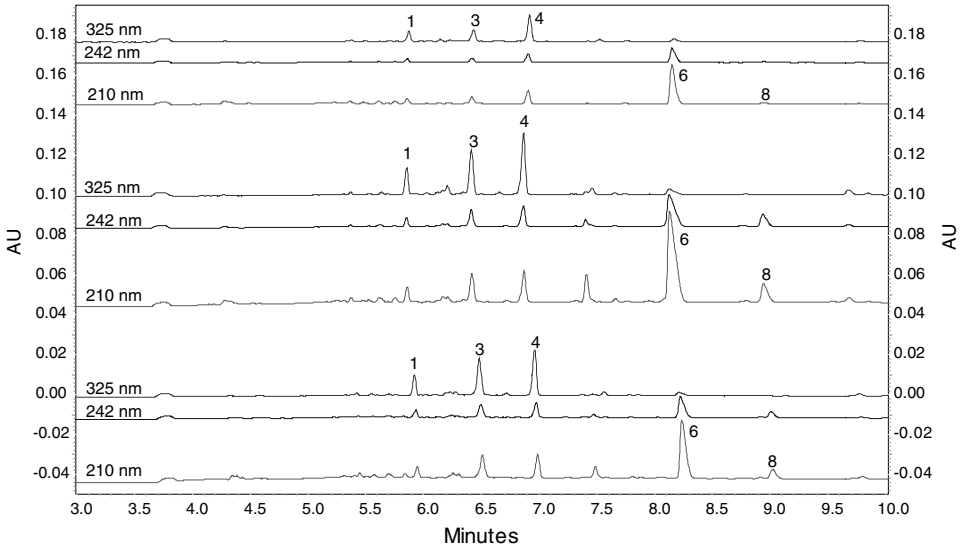


Fig. 8. Electropherograms of the aqueous extracts of PB (top 3), PDP (middle 3) and FA (bottom 3) (same experimental conditions as in Fig. 7).

Table 5
Quantitation of lignin derivatives in books during accelerated ageing

| | Ageing (days) | FA (mg $L^{-1}$) | PDP (mg $L^{-1}$) | PB (mg $L^{-1}$) | SE (mg $L^{-1}$) |
|---|---|---|---|---|---|
| Acetosyringone | 0 | 12.4 | 25.5 | 9.7 | 0 |
| | 2 | 4.4 | 9.4 | 9.0 | 3.7 |
| | 5 | 3.1 | 5.9 | 9.1 | 5.2 |
| 4-Hydroxyacetophenone | 0 | 15.8 | 16.3 | 10.3 | 0 |
| | 2 | 7.1 | 10.5 | 7.4 | 0 |
| | 5 | 7.5 | 13.6 | 6.3 | 0 |
| 4-Hydroxybenzaldehyde | 0 | 0.8 | 2.3 | 0.5 | 0 |
| | 2 | 1.0 | 1.6 | 0.8 | 0 |
| | 5 | 0.9 | 2.0 | 1.2 | 0 |
| 4-Hydroxybenzoic acid | 0 | 10.1 | 20.4 | 1.8 | 0 |
| | 2 | 38.4 | 24.2 | 2.5 | 0 |
| | 5 | 27.7 | 20.3 | 2.9 | 0 |
| 2-Furoic acid | 0 | 2.4 | 3.4 | 0 | 0.5 |
| | 2 | 14.3 | 7.4 | 1.6 | 3.3 |
| | 5 | 18.9 | 20.3 | 4.3 | 10.2 |
| Vanillic acid | 0 | 167.8 | 339.2 | 102.9 | 6.5 |
| | 2 | 734.0 | 561.9 | 142.7 | 10.8 |
| | 5 | 461.1 | 624.9 | 205.9 | 15.0 |
| Vanillin | 0 | 19.6 | 28.3 | 11.3 | 1.1 |
| | 2 | 52.1 | 35.8 | 16.5 | 1.2 |
| | 5 | 39.0 | 48.8 | 20.3 | 0 |
| Vanillic acid/vanillin (mole ratio) | 0 | 8 | 11 | 8 | 5 |
| | 2 | 13 | 14 | 8 | 8 |
| | 5 | 11 | 12 | 9 | – |

It is noteworthy that PB, which among the ligneous papers contains the smallest quantity of groundwood pulp, also showed the lowest abundance of lignin-degradation products, compared to PDP and FA in which lignin is present in higher proportions. In the two rag papers, SE and OC, minute amounts of vanillin and vanillic acid were found. This was consistent with the linen rag composition of the two books, as linen contains only very small amount of lignin, and most of it is removed during the textile process. The detection of these two analytes in SE confirmed that the method is very sensitive.

During the whole period of the accelerated ageing, the production of vanillic acid and vanillin increased in PDP and PB, where as in FA both compounds increased only until the second day before they started to decrease (Fig. 9). The other lignin derivatives showed more irregular evolutions in all the books extracts, which precluded their use as possible degradation indicators. The production of vanillin and vanillic acid evolved in the same proportion, and the mole ratio of vanillic acid to vanillin remained in the range 5–14 during the whole ageing period (Table 5). From these results it seems that vanillin was produced at a certain kinetic rate during the ageing with a quasi-simultaneous oxidation to vanillic acid at approximately the same rate. We can also conclude that the ageing method used appeared to reproduce quite well natural ageing, which confirmed previous results (ASTM Report, 2002; Shahani & Harrison, 2002).

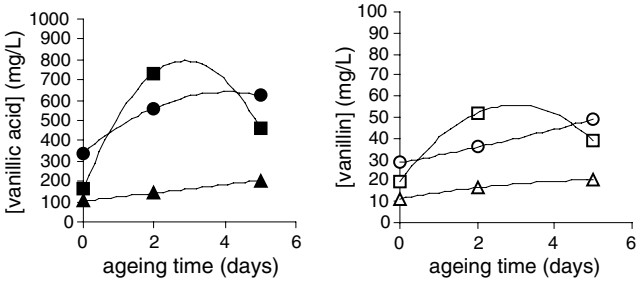


Fig. 9. Production of vanillic acid (left graph, full marks) and vanillin (right graph, void marks) in FA (squares), PDP (circles), and PB (triangles) during accelerated ageing.

### 3.4. Carbohydrates

Complex carbohydrate mixtures are the most difficult compounds to separate using CE. The chief reasons are the minute structural differences between the compounds (often only in the configuration of their hydroxyl groups), their lack of easily ionisable functions and their lack of UV absorption (El Rassi, 2002; Honda, 1996). Ionisation can be generated by chelation with strong alkaline ions such as borate or metal cations. The detection problem can be addressed by labelling with UV-absorbing or fluorescent tags. The configuration similarities between monosaccharides however, remain an inherent limitation to the separation. Despite these difficulties, successful CZE methods for the analysis of monosaccharides, and their di-, tri-, and oligomers have been elaborated (Honda, 1996), the most recent, with and without pre-capillary derivatisation, having applications for food and beverages products (Cortacero-Ramírez, Segura-Carretero, Cruces-Blanco, Hernáinz-Bermúdez de Castro, & Fernández-Guttiérrez, 2004; Soga, 2002, pp. 483–502; Soga & Serwe, 2000) and for ligno- and holocellulose products (Dahlman et al., 2000; Mosier et al., 2005; Rydlund & Dahlman, 1996; Sartori et al., 2003).

The characterisation of glucose and cellooligomers in papers using high pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been studied previously (ASTM Report, 2002. Ch. Temperature aging studies at LOC). Using GC as separation method, the use of glucose and its oligomers as indicators of degradation in aged papers has been proposed (Erhardt, Tumosa, & Mecklenburg, 2001). In the field of cultural heritage, CZE has been used to characterise monosaccharides in plant gums used as binding media in historic and artistic works (Gröbl, Harrison, Kaml, & Kenndler, 2005) but has never been explored for identifying these compounds in books. In the present method, the separation was carried out in a borate buffer and pre-capillary derivatisation with ABEE, by means of reductive amination was used to label the carbohydrates, following earlier work (Dahlman et al., 2000; Sjöberg, Adorjan, Rosenau, & Kosma, 2004). In order to suit our purpose of analysis of carbohydrates present in papers a few modifications were brought to these methods as detailed in *Method optimisation*.

### 3.4.1. Method optimisation

In order to achieve an optimised separation of carbohydrates, the following parameters were tested.

*3.4.1.1. pH of the buffer.* The pH range explored was 9.7–10. The best compromise between resolution and time of analysis was found at pH 9.94. Below that pH, arabinose and mannose co-eluted, and above, despite an optimal resolution, the separation lasted over 25 min.

*3.4.1.2. Ionic Strength of the buffer.* In an attempt to improve the resolution of the peaks by increasing the ionic strength of the borate buffer, concentrations between 225 and 900 mM were tested. The optimal separation was obtained with 450 mM, which confirmed previous work (Sjöberg et al., 2004). Below that concentration the sensitivity decreased, and above it, resolution between arabinose and mannose did not improve further, while the current reached too high levels.

*3.4.1.3. Temperature.* It was decided to use 20 °C as no significant influence on the separation of the run temperature in the range 15–25 °C was found.

*3.4.1.4. Injection volume.* The time of the hydrodynamic injection was increased from 5 to 20 s as the latter resulted in increased peak heights and areas without significant loss in resolution.

### 3.4.2. Method validation

The repeatability was good but the reproducibility was poor, and both were better on $t_m$ ($0.22\% < r < 0.49\%$ and $3.02\% < R < 4.04\%$) than on peak areas ($0.81\% < r < 1.89\%$ and $5.28\% < R < 10.84\%$) (Table 6). Linearity evaluated using five solutions (75, 250, 500, 750, and 1000 mg $L^{-1}$) of each model carbohydrate gave coefficients of linear correlation of 0.999 (0.998 for xylose) signifying acceptable precision of the method. LODs ranging from 35 to 72 mg $L^{-1}$ and LOQs ranging from 117 to 240 mg $L^{-1}$ were found (Table 3). This result indicated the method was not very sensitive and substantiated previous works underlining the difficulty of separating carbohydrates with CZE.

### 3.4.3. Naturally aged books

Glucose and its oligomers cellobiose, cellotriose and cellotetraose, are expected to arise from cellulose hydrolysis. Xylose and arabinose are the most frequent pentoses found in hemicelluloses (xyloglycans and xyloglucans) (Ebringerová, Hromádková, & Heinze, 2005), but other monomers such as glucose, mannose, and galactose are also present in various amounts. Electropherograms of the model carbohydrates and aqueous extracts from some of the naturally aged books can be seen in Fig. 10. Residual unreacted ABEE appeared at $t_m$ of 6.7–6.8 min, which did not mask any of the analytes.

The electropherograms of PB and OC showed a total absence of peaks. It can be noted that these two books were those containing the lesser amounts of organic acids and, for PB, also the lesser amounts of lignin-degradation products. Coincidentally they also were visually the less degraded books. The abundance of carbohydrates in PDP, FA, and SE is shown in Fig. 11. As expected arabinose was the major peak in groundwood pulp books, where hemicelluloses are present in large quantities, with 480 mg $L^{-1}$ in FA and 236 mg $L^{-1}$ in PDP. Xylose was also present in significant amount in FA with 126 mg $L^{-1}$ while in PDP only arabinose was quantifiable. It can be noted that arabinose has been observed to appear very fast during the ageing of lignocellulosic papers compared to other carbohydrates (ASTM Report, 2002. Ch. Temperature aging studies at LOC). The nature and the proportion of each monosaccharide in hemicelluloses depend greatly upon the variety of plant and wood. It was thus not possible to draw conclusions on the origin of the plant based only on the type and

Table 6
Repeatability ($r$) and reproducibility ($R$), RSD% ($n = 5$) of migration times $t_m$ and peak areas for carbohydrates

| | Cellobiose | | Xylose | | Glucose | | Mannose | | Arabinose | | Galactose | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area | $t_m$ | Area |
| $r$ | 0.22 | 2.50 | 0.31 | 1.89 | 0.37 | 1.70 | 0.38 | 0.81 | 0.38 | 1.80 | 0.49 | 1.80 |
| $R$ | 3.02 | 5.28 | 2.95 | 7.80 | 3.60 | 8.40 | 3.60 | 6.90 | 3.70 | 5.99 | 4.04 | 10.84 |
| Average $t_m$ (min) | 11.85 | | 12.14 | | 13.85 | | 14.12 | | 14.33 | | 16.70 | |

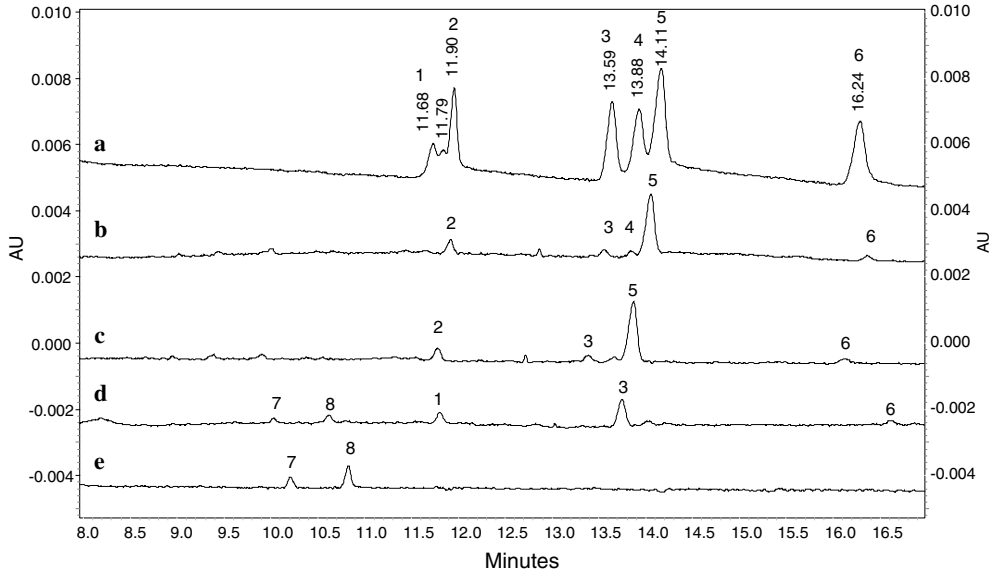


Fig. 10. Electropherograms of (a) model carbohydrates stock solution with (1) cellobiose, (2) xylose, (3) glucose, (4) mannose, (5) arabinose, and (6) galactose, 500 mg $L^{-1}$ each in deionised water (DirectQ, Millipore®), (b) aqueous extract FA, (c) aqueous extract PDP, (d) aqueous extract SE, and (e) model carbohydrates stock solution of (7) cellotriose and (8) cellotetraose, 500 mg $L^{-1}$ each in deionised water (DirectQ, Millipore®). Capillary 20 μm × 48.5 cm ($l = 40$ cm). Borate buffer 450 mM, pH 9.94. Hydrodynamic injection 0.5 psi, 20 s. Applied potential +28 kV; temperature 20 °C; Detection signal = 305 nm.

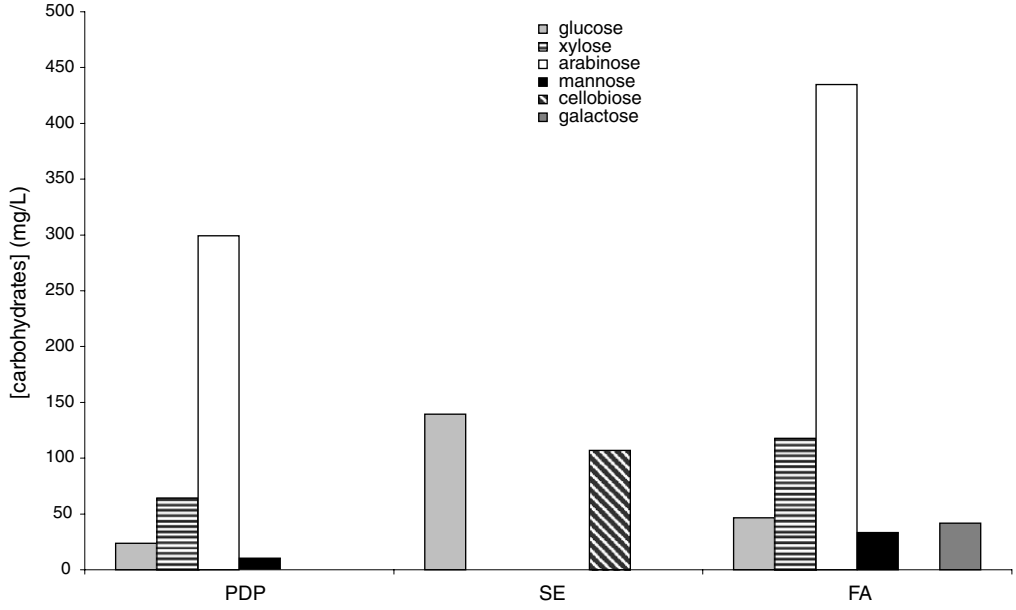


Fig. 11. Quantitation of carbohydrates in the naturally aged books.

amount of monosaccharides found, all the more that the latter were sub-products of degradation and were thus not representative of the initial carbohydrate composition. With a total of 606 mg $L^{-1}$, FA showed the highest total amount of monosaccharides. This result was consistent with those obtained previously indicating that with the highest abundance of LMM organic acids and lignin-degradation products FA was the most degraded book.

The electropherogram of SE showed glucose as major peak with 147 mg $L^{-1}$, as well as the presence of cellobiose, cellotriose, and cellotetraose. This result jointly with the absence of pentoses was consistent with the almost pure cellulose composition of SE. Even though it is also a component of hemicelluloses, glucose appeared as the main carbohydrate sub-product of cellulose degradation. Hemicelluloses are more readily hydrolysable than cellulose mainly because of their more amorphous structure. The results from the panel of papers studied confirmed that when present, hemicelluloses degraded faster than cellulose, yielding a majority of pentoses from which arabinose formed the major part. Although the method would benefit from better optimisation, the sensitivity was sufficient for

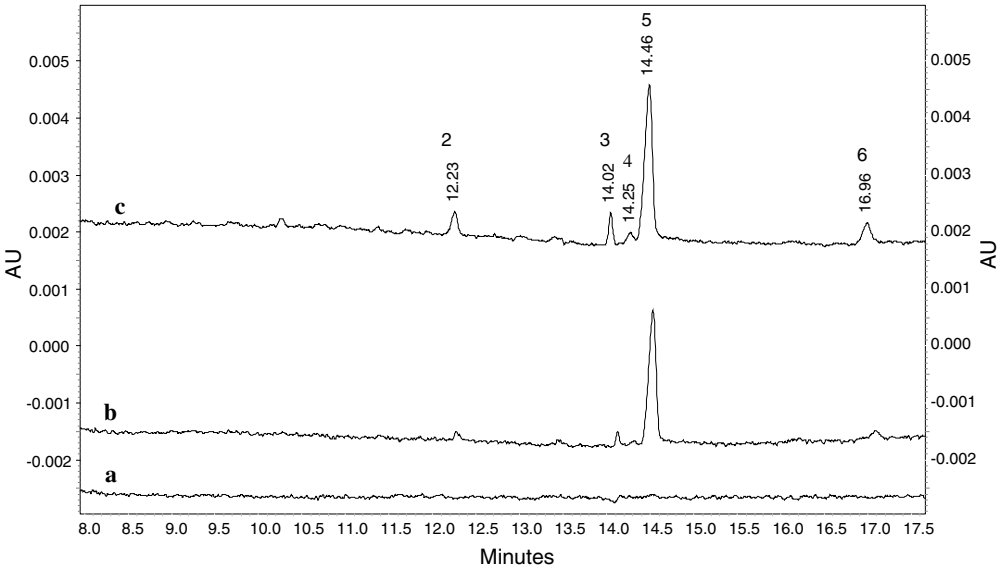


Fig. 12. Electropherograms of paper 'Mod' unaged (a), aged 3.5 days (b), aged 8 days (c) with (2) xylose, (3) glucose, (4) mannose, (5) arabinose, and (6) galactose (same experimental conditions as in Fig. 10).

the detection and quantitation of most monosaccharides and their occurrence in naturally aged papers.

### 3.4.4. Artificially aged model paper

Before ageing, no peak was detectable on the electropherogram of paper 'Mod'. The production of carbohydrates was quantifiable only after accelerated ageing, and showed a regular progression with time (Fig. 12). A steady increase in the amount of carbohydrates in paper during accelerated ageing has been observed earlier (ASTM Report, 2002. Ch. Temperature aging studies at LOC). Arabinose was the major analyte with 426 mg $L^{-1}$ after 3.5 days, and 520 mg $L^{-1}$ after 8 days, as expected for a lignocellulosic pulp paper. The other carbohydrates were found near-LODs. After 3.5 and 8 days, respectively, 25 and 76 mg $L^{-1}$ of xylose, 25 and 57 mg $L^{-1}$ of glucose, 0 and 28 mg $L^{-1}$ of mannose, and 22 and 67 mg $L^{-1}$ of galactose were quantified (Fig. 13).

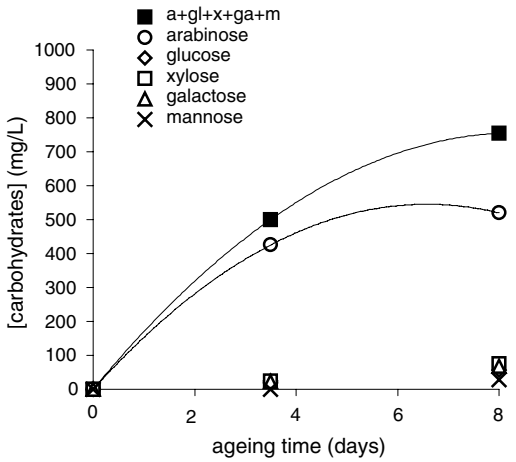


Fig. 13. Production of carbohydrates in paper 'Mod' during accelerated ageing.

## 4. Conclusions

This study has proved the usefulness of CZE in the comprehensive analysis of organic compounds found in aqueous extracts of ligno- and holocellulosic papers and originating during their degradation. The three methods for LMM organic acids, lignin derivatives, and carbohydrates were validated, and the separation of the three types of compounds was achieved within 4, 9, and 17 min respectively, which was considerably faster than any LC or GC method. The methods used were accurate and moderately to very sensitive and precise to carbohydrates and LMM organic acids/lignin derivatives, respectively. The compounds found in the books and in the model paper after accelerated ageing, were identical to those produced during their lifespan through natural ageing. No major degradation compound was unidentified. A clear link could be made between a high quantity of the compounds from the three classes investigated and a more advanced state of degradation of the paper. The quantity of these products increased during the ageing with a quasi-linear trend in most cases. The nature of the organic compounds could be related to the type of paper. In particular, considerable amounts of vanillic acid and a major quantity of arabinose were found in lignocellulosic papers. A significant presence of glucose was more specific to the degradation of pure cellulose papers. LMM organic acids were found in all papers regardless of their origin as they arose from both cellulose and hemicellulose degradation.

The choice of molecular indicators of paper degradation cannot rely merely on their presence in aged papers as analysed by some analytical technique. It can be argued that the diversity of organic compounds present in paper calls for great caution when trying to point out to specific indicators or markers of degradation. Among the three classes explored, only a few paper sub-products would likely fulfil

the conditions to be designated as indicators. Their production was correlated with the state of degradation of the paper, and their nature depended on the origin and the fabrication process of the latter. Acetic acid being found in all types of paper could represent a general marker. Vanillic acid, vanillin, and arabinose can represent plausible indicators of lignocellulosic papers, where as glucose would be an indicator of cellulose degradation. The quantitation of these compounds in papers can be foreseen as useful in evaluating the degradation state, but clearly more research needs to be done. Particularly, the panel of papers analysed needs to be extended as well as the accelerated ageing tests in order to attain a better level of statistical significance. In our opinion, the likelihood that these molecular indicators alone could be used for an early warning of degradation is nevertheless disputable. Demonstrating the existence of threshold levels that could indicate a decrease in durability of paper was not the purpose of the present investigation, but it can be argued that it is very difficult to evidence threshold levels choosing markers only at the molecular level. The presence of specific sub-products of the transformation of paper with time and their abundance may not be sufficient to unequivocally rank papers as more or less degraded. Indicators should be investigated among diverse parameters relative to different properties and characteristics of paper. The examination of the molar masses of cellulose in the case of AC and SE provided a complementary type of indication. It is now important to understand better the relationships between the molecular degradation and the macromolecular condition of the cellulose. In this respect, it must be underlined that this research is part of a vaster work undertaken to evaluate the rate of decay of paper under various conditions through an integrated approach encompassing molecular and macromolecular characterisation. The development of methods for molar mass and molar mass distribution determination, based on microdestructive sampling applicable to historic papers, are essential for the appraisal of paper degradation and would complement the methods employed to assess molecular degradation which were investigated in the present article.

## References


ASTM Report RR#D06-1004 (2002). *ASTM Paper aging research program*. ASTM International, West Conshohocken, PA.

Berggren, R., Molin, U., Berthold, F., Lennholm, H., & Lindström, M. (2003). Alkaline degradation of birch and spruce: influence of degradation conditions on molecular mass distributions and fibre strength. *Carbohydrate Polymers, 51*, 255–264.

Buchbauer, G., Jirovetz, L., Wasicky, M., & Nikiforov, A. (1995). On the odour of old books. *Research note. Journal of Pulp and Paper Science, 21*, 398–400.

Castiñeira, A., Peña, R. M., Herrero, C., & García-Martín, S. (2000). Simultaneous determination of organic acids in wine samples by capillary electrophoresis and UV detection: optimization with five different background electrolytes. *Journal of High Resolution Chromatography, 23*, 647–652.

Chamberlain, D. C., & Priest, D. J. (1998). Comparison of physical and chemical methods for analysing cellulose degradation following artificial ageing treatment. *Cellulose Chemistry and Technology, 32*, 35–41.

Chen, J., Preston, B. P., & Zimmerman, M. J. (1997). Analysis of organic acids in industrial samples comparison of capillary electrophoresis and ion chromatography. *Journal of Chromatography A, 781*, 205–213.

Cortacero-Ramírez, S., Segura-Carretero, A., Cruces-Blanco, C., Hernáinz-Bermúdez de Castro, M., & Fernández-Guttiérrez, A. (2004). Analysis of carbohydrates in beverages by capillary electrophoresis with precolumn derivatization and UV detection. *Food Chemistry, 87*, 471–476.

D6819-02e2 (2002). *Standard test method for accelerated temperature aging of printing and writing paper by dry oven exposure apparatus*. ASTM International, West Conshohocken, PA.

Dahlman, O., Jacobs, A., Liljenberg, A., & Olsson, A. I. (2000). Analysis of carbohydrates in pulps employing enzymatic hydrolysis and subsequent capillary zone electrophoresis. *Journal of Chromatography A, 891*, 157–174.

Dupont, A.-L. (2003a). *Gelatine sizing of paper and its impact on the degradation of cellulose during aging. A study using size-exclusion chromatography*. Ph.D. Thesis, University of Amsterdam, pp. 256.

Dupont, A.-L. (2003b). Cellulose in lithium chloride/*N,N*-dimethylacetamide, optimisation of a dissolution method using paper substrates and stability of the solutions. *Polymer, 44*, 4117–4126.

Dupont, A.-L., & Harrison, G. (2004). Conformation and *dn/dc* determination of cellulose in *N,N*-dimethylacetamide containing lithium chloride. *Carbohydrate Polymers, 58*, 233–243.

Ebringerová, A., Hromádková, Z., & Heinze, T. (2005). Hemicellulose. In *Polysaccharides I, structure, characterization and use* (pp. 1–67). Springer.

El Rassi, Z. (2002). Electrophoresis and electrochromatography: fundamentals, methodologies and applications. In *Carbohydrate analysis by modern chromatography and electrophoresis* (pp. 597–676). Elsevier.

Erhardt, D., Tumosa, C. S., & Mecklenburg, M. F. (2001). Chemical and physical changes in naturally and accelerated aged cellulose. In *Historic textiles, papers and polymers in museums* (pp. 23–37). Washington, DC: ACS.

Esteves, V. I., Lima, S. S. F., Lima, D. L. D., & Duarte, A. C. (2004). Using capillary electrophoresis for the determination of organic acids in Port Wine. *Analytica Chimica Acta, 513*, 163–167.

Fournand, D., & Lapierre, C. (2001). Capillary zone electrophoresis of coniferyl alcohol oxidation products. *Journal of Agricultural and Food Chemistry, 49*, 5727–5731.

Galli, V., & Barbas, C. (2004). Capillary electrophoresis for the analysis of short-chain organic acids in coffee. *Journal of Chromatography A, 1032*, 299–304.

Galli, V., Olmo, N., & Barbas, C. (2000). Development and validation of a capillary electrophoresis method for the measurement of short-chain organic acids in natural rubber latex. *Journal of Chromatography A, 894*, 135–144.

Gröbl, M., Harrison, S., Kaml, I., & Kenndler, E. (2005). Characterisation of natural polysaccharides (plant gums) used as binding media for artistic and historic works by capillary zone electrophoresis. *Journal of Chromatography A, 1077*, 80–89.

Gurnagul, N., Page, D. H., & Paice, M. G. (1992). The effect of cellulose degradation on the strength of wood pulp fibres. *Nordic Pulp and Paper Research Journal, 3*, 152–154.

Hagberg, J., Dahlén, J., Karlsson, S., & Allard, B. (2000). Application of capillary zone electrophoresis for the analysis of low molecular weight organic acids in environmental samples. *International Journal of Environmental Analytical, 78*, 385–396.

Havermans, J.B., de Feber, M.A., Genuit, W.J., & van Velzen, G.J. (1999). Emission of volatile organic compounds from paper objects affected with iron-gall ink corrosion. In J. Bridgland (Ed.), *Preprints of ICOM 12th triennial meeting* (pp. 513–516). ICOM Committee for Conservation, Lyon.

Honda, S. (1996). Separation of neutral carbohydrates by capillary electrophoresis. *Journal of Chromatography A, 720*, 337–351.

Javor, T., Buchberger, W., & Faix, O. (2003). Capillary electrophoresis determination of lignin degradation products obtained by permanganate oxidation. *Analytica Chimica Acta, 484*, 181–187.

Jerosch H. (2002). *Évaluation de l'état de dégradation de la cellulose/holocellulose dans différents types de papiers par chromatographie d'exclusion stérique*. Thèse de doctorat, Université Versailles-St-Quentin-en-Yvelines, pp. 204.

Jerosch, H., Lavédrine, B., & Cherton, J.-C. (2001). Study of the stability of cellulose–holocellulose solutions in *N,N*-dimethylacetamide–lithium chloride by size exclusion chromatography. *Journal of Chromatography A, 927*, 31–38.

Kampfl, C. W., Buchberger, W., & Haddad, P. R. (2000). Determination of organic acids in food samples by capillary zone electrophoresis. *Journal of Chromatography A, 881*, 357–364.

Lattuati-Derieux, A., Bonnassies-Termes, S., & Lavédrine, B. (2004). Identification of volatile organic compounds emitted by a naturally aged book using solid-phase microextraction/gas chromatography/mass spectrometry. *Journal of Chromatography A, 1026*, 9–18.

Levart, A., Guček, M., Pihlar, B., & Veber, M. (2000). Determination of organic acids in air by capillary electrophoresis and ion-exclusion chromatography. *Chromatographia Supplement, 51*, 321–324.

Ligterink, F.J., & Pedersoli Jr., J.L. (2001). Development of the SPME book-mark method for the determination of furfural and acetic acid concentrations in books. In *Abstracts of ICOM-CC interim meeting, Working Group on Graphic Documents*, Vantaa, Finland.

Mato, I., Huidobro, J. F., Simal-Lozano, J., & Sancho, M. T. (2006). Simultaneous determination of organic acids in beverages by capillary zone electrophoresis. *Analytica Chimica Acta, 565*, 190–197.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Lasdich, M. (2005). Features of promising technologies for pre-treatment of lignocellulosic biomass. *Bioresource Technology, 96*, 673–686.

Nevell, T. P. (1985). Degradation of cellulose by acids, alkalis, and mechanical means. In T. P Nevell & S. H. Zeronian (Eds.), *Ellis Horwood Series Chemical ScienceCellulose chemistry and its applications* (pp. 223–265). John Wiley & Sons.

Parks, E.J., Guttman, C.M., Jewett, K.L., & Brinckman, F.E. (1990). *Studies on the degradation products of paper with and without pollutants in a closed environment I. Preliminary results*. Report NISTIR 4456. National Institute of Standards and Technology, Gaithersburg, MD.

Rydlund, A., & Dahlman, O. (1996). Efficient capillary zone electrophoresis separation of wood-derived neutral and acidic mono- and oligosaccharides. *Journal of Chromatography A, 738*, 129–140.

Sarmini, K., & Kenndler, E. (1997). Influence of organic solvents on the separation selectivity in capillary electrophoresis. *Journal of Chromatography A, 792*, 3–11.

Sartori, J., Potthast, A., Ecker, A., Sixta, H., Rosenau, T., & Kosma, P. (2003). Alkaline degradation kinetics and CE-separation of cello- and xylooligomers. *Carbohydrate Research, 338*, 1209–1216.

Schult, T., Moe, S. T., Hjerde, T., & Christensen, B. E. (2000). Size-exclusion chromatography of cellulose dissolved in LiCl/DMAc using macroporous monodisperse poly(styrene-co-divinylbenzene) particles. *Journal of Liquid Chromatography and Related Technologies, 23*, 2277–2288.

Shahani, C. J., & Harrison, G. (2002). Spontaneous formation of acids in the natural aging of paper. In V. Daniels, A. Donithorne, & P. Smith (Eds.), *IIC congress: works of art on paper, books, documents and photographs, techniques and conservation* (pp. 189–192). London: International Institute for Conservation.

Silva, A. A., & Laver, M. L. (1997). Molecular weight characterization of wood pulp cellulose: dissolution and size exclusion chromatographic analysis. *Tappi Journal, 80*, 173–180.

Sjöberg, J., Adorjan, I., Rosenau, T., & Kosma, P. (2004). An optimised CZE method for analysis of mono- and oligomeric aldose mixtures. *Carbohydrate Research, 339*, 2037–2043.

Sjöholm, E., Nilvebrant, N.-O., & Colmsjö, A. (1993). Characterization of dissolved kraft lignin by capillary zone electrophoresis. *Journal of Wood Chemistry and Technology, 13*, 529–544.

Sjöholm, E., Norman, E., Störker, M., & Colmsjö, A. (2000). Charge density of lignin samples from kraft cooking of birch. *Journal of Wood Chemistry and Technology, 20*, 337–356.

Sjöholm, E., Störker, M., & Norman, E. (2000). A method for evaluating lignin mobility distributions obtained by capillary zone electrophoresis. *Journal of Wood Chemistry and Technology, 20*, 113–132.

Snyder, L. R., Kikland, J. J., & Glajch, J. L. (1997). *Practical HPLC method development* (second ed.). John Wiley & Sons.

Soga, T. (2002). Analysis of carbohydrates in food and beverages by HPLC and CE. In *Carbohydrate analysis by modern chromatography and electrophoresis* (pp. 483–502). Elsevier.

Soga, T., & Ross, G. A. (1997). Capillary electrophoretic determination of inorganic and organic anions using 2,6-pyridinedicarboxylic acid: effect of electrolyte's complexing ability. *Journal of Chromatography A, 767*, 223–230.

Soga, T., & Serwe, M. (2000). Analysis of carbohydrates in food samples by capillary electrophoresis with indirect UV detection. *Food Chemistry, 69*, 339–344.

Strlič, M., & Kolar, J. (2003). Size exclusion chromatography of cellulose in LiCl/*N,N*-dimethylacetamide. *Journal of Biochemical and Biophysical Methods, 1681*, 1–15.

Vorndran, A. E., Grill, E., Huber, C., Oefner, P. J., & Bonn, G. K. (1992). Capillary zone electrophoresis of aldoses, ketoses and uronic acids derivatized with ethyl *p*-aminobenzoate. *Chromatographia, 34*, 109–114.

Wang, M., Qu, F., Shan, X.-Q., & Lin, J.-M. (2003). Development and optimisation of a method for the analysis of low-molecular-mass organic acids in plants by capillary electrophoresis with indirect UV detection. *Journal of Chromatography A, 989*, 285–292.

Weinberger, R. (2000). *Practical capillary electrophoresis* (second ed.). Academic Press p. 106.

Zou, X., Gurnagul, N., Uesaka, T., & Bouchard, J. (1994). Accelerated aging of papers of pure cellulose: mechanism of cellulose degradation and paper embrittlement. *Polymer Degradation and Stability, 43*, 393–402.