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Pushing the limits: Quantification of chromophores in real-world paper samples by GC-ECD and EI-GC-MS

A. Schedl[a,b], T. Zweckmair[a,b], F. Kikul[a,b], M. Bacher[a,b], T. Rosenau[a,b], A. Potthast[a,b,*]

[a] University of Natural Resources and Life Sciences-Vienna, Konrad-Lorenz-Straße 24, A-3430 Tulln, Austria
[b] Department of Chemistry, Division of Chemistry of Renewable Resources, Austria

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ABSTRACT

Widening the methodology of chromophore analysis in pulp and paper science, a sensitive gas-chromatographic approach with electron-capture detection is presented and applied to model samples and real-world historic paper material. Trifluoroacetic anhydride was used for derivatization of the chromophore target compounds. The derivative formation was confirmed by NMR and accurate mass analysis. The method successfully detects and quantifies hydroxyquinones which are key chromophores in cellulosic matrices. The analytical figures of merit appeared to be in an acceptable range with an LOD down to approx. 60 ng/g for each key chromophore, which allows for their successful detection in historic sample material.

1. Introduction

Since the introduction of Gutenberg's movable letters, multitudes of copies of printed matter can be readily produced within a short time frame. Since then, books became available not only for the rich, but also for the common people. Printing of text – previously always handwritten – allowed for much faster spreading of information. In those early days of mass printing, the printing substrate was still rag paper, made out of old cotton and linen rags. Preserving such goods, and storing the information printed on them is the concern of preservation scientists. They are especially interested in the prevention of degradation of documents of historical value, with yellowing being a common and obvious effect of such degradation.

To understand the complex process of paper aging, several factors which are influencing the degradation, have to be investigated. Over the last decade three important degradation compounds that are indicative of the yellowing have been identified in several cellulosic materials [1–6]. They are usually denoted as key chromophores since they occur almost ubiquitously upon aging of cellulosic matrices, arising from degraded carbohydrates rather than lignin [1,7,8] or nitrogen-containing species as known for Maillard processes [9–11]. Deterioration of paper substrates mainly occurs due to mechanical, irradiation, hydrolytic and oxidative stress. However, several other factors such as ink [12], pollution [13–16] and microbial attack [17–21] have additional substantial impact on the degradation of cellulosic material. The first method to isolate and identify chromophoric compounds in cellulosic matrices has been introduced by Rosenau et al. [6]. This method enables extraction of quinoid and aromatic compounds. It has become known as the CRI (Chromophore Release and Identification) approach and has been applied successfully to different native (cellulose I allomorph) and regenerated (cellulose II allomorph) cellulose samples. [6]. Elucidating the structure of the main chromophores contributing to cellulose yellowing was an important step to gain information about the complex degradation processes in cellulosic substrates [1]. Nevertheless, the CRI method is time-consuming as it applies several washing, extraction and manipulation steps which do not allow for automatization, and requires a significant amount of sample material which limits the applicability of this method even further. It is unsuitable for large sample numbers, as in mechanistic studies, or small sample amounts, as in cases of historic paper materials.

Recently, paper spray mass spectrometry has been demonstrated to be a fast and useful analytical tool to tackle chromophores even in small samples of valuable historic sample material [22,23], albeit with false positive results as a possible drawback. For a reliable qualitative detection sophisticated instrumentation, such as accurate mass spectrometry combined with ion mobility approaches, are used in the paper spray setup. These demanding requirements limit the routine use of the paper spray mass spectrometry approach.

Gas chromatographic separation with its enormous availability and capacity appears to offer a way out. The chromophores to be detected are present in a low ppm to ppb range which requires a sensitive detector to successfully perform trace analysis. Hence, two detection techniques were selected, namely negative chemical ionization (NCI) prior to mass spectrometric detection [24] as well as the less elaborate

* Corresponding author at: University of Natural Resources and Life Sciences-Vienna, Konrad-Lorenz-Straße 24, A-3430 Tulln, Austria.
E-mail address: antje.potthast@boku.ac.at (A. Potthast).

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electron-capture detection (ECD) [25,26]. Both approaches provide useful sensitivity, but typically require a halide being attached to the analyte to facilitate trace-level detection. However, naphthoquinones show high electron affinity which allows for their detection in ECD even without having any halide attached [27]. To cover all key chromophore compounds while keeping the benefits of enhanced sensitivity and improved peak shape we decided to apply compound derivatization [28]. We have selected fluoride – as present in trifluoroacetate moieties – to be incorporated into the analytes by derivatization to facilitate their gas chromatographic separation and trace-level detection by both NCI-MS and ECD. Previous investigations on reacting substituted *p*-benzoquinones and 1,4-naphthoquinones with trifluoroacetic anhydride [29,30] were used as a starting point. We present a method comprising an extraction technique, a solid phase extraction (SPE) step to enrich chromophores, a derivatization reaction converting hydroxyl into trifluoroacetate groups, and the application of GC-ECD for the separation and detection of derivatized chromophores in historic cellulosic substrates on a routine basis.

2. Materials and methods

2.1. Chemicals

Trifluoroacetic anhydride (TFAA, 99.5%) was obtained from ABCR (Karlsruhe, Germany). Acetonitrile and chloroform were purchased from VWR-International (Vienna, Austria). Methanol (99.99%) was from Fischer Scientific (Loughborough, UK). HPLC-grade water was supplied by Fisher Scientific GmbH (Vienna, Austria).2,5-dihydroxyacetophenone (DHAP),2,5-dihydroxy-1,4-benzoquinone (DHBQ), 5,8-dihydroxy-1,4-naphthoquinone (DHNQ) D-glucose, D-xylose and trifluorotoluene (≥99.9%) were purchased from Sigma-Aldrich and Fluka (Schnelldorf, Germany).

All chemicals were of the highest purity available and were used without further purification. SPE-cartridges (Strata™-X 33 µm Polymeric Reversed Phase, 30 mg / 1 mL) were obtained from Phenomenex Inc. (Torrance, USA).

2.2. Solid phase extraction (SPE) procedure

The SPE cartridge was conditioned with methanol according to the procedure recommended by the manufacturer. Subsequently the tube was rinsed with deionized water and loaded with the diluted sample dissolved in deionized water. For the washing step, acetonitrile/deionized water (1:9, v/v) was used. Finally, the target compounds were eluted with acetonitrile /deionized water (9:1, v/v).

2.3. Derivatization of standard compounds

10 µg of each chromophore were weighted into 10 mL headspace vials which were closed with a crimp cap. The compounds were dissolved in 5 mL of $CHCl_3$ followed by the addition of 2 mL of trifluoroacetic anhydride. The solution was overlaid with argon and sealed afterwards. The samples were left on a shaker for 48 h at room temperature. TFAA and $CHCl_3$ were removed under N_2-flow. The dry samples were then redissolved in 1 mL of $CHCl_3$ and transferred into 1.5 mL GC vials with crimp caps for measurement. The trifluoroacetylated compounds are stable in acetone, CH_2Cl_2 and $CHCl_3$ for at least 7 days.

2.4. Nuclear Magnetic Resonance (NMR) of trifluoroacetylated chromophores

All NMR spectra were recorded on a Bruker Avance II 400 (resonance frequencies 400.13 MHz for ^1H and 100.61 MHz for ^{13}C) equipped with a 5 mm observe broadband probe head (BBFO) with z-gradients at room temperature with standard Bruker pulse programs.

1 mg of each DHBQ and DHNQ were individually dissolved in 400 µL of $CDCl_3$ (99.8% D, euriso-top, France). After the addition of 5 µL of trifluorotoluene (standard) the derivatization reaction was started by the addition of 200 µL of TFAA. ^1H NMR spectra were recorded directly from the crude reaction product without quenching any reactant or applying any sample preparation step.

In case of DHAP, internal calibration was carried out using the acetyl CH_3 signal. No internal standard (trifluorotoluene) was required in this specific case.

Chemical shifts are given in ppm, referenced to residual solvent signals (7.26 ppm for ^1H, 77.0 ppm for ^{13}C). ^1H NMR data were collected with 32k complex data points and apodized with a Gaussian window function (lb = −0.3 Hz,gb = 0.3 Hz) prior to Fourier transformation. ^{13}C-jmod spectra with WALTZ16 ^1H decoupling were acquired using 64k data points. Signal-to-noise enhancement was achieved by multiplication of the FID with an exponential window function (lb = 1 Hz). Bruker TopSpin 2.1 was used for the acquisition and processing of the NMR data.

2.5. Sample material

Real world samples: rag paper from historical books and handwritten letters which had turned yellowish upon natural aging were obtained from museums or from second-hand stores.

The sample material was analyzed without further pretreatment. Due to the uncertain origin, no more detailed information about storage circumstances and material condition is available. Surface pH measurements were carried out according to Ahn et al., 2011 [31]. A plain description of the historic samples is shown in the Supplementary Material. Sample book “B5” was obtained from the Austrian National Library, where it was received as a foundling.

2.6. Artificially aged samples

Whatman filter paper (6 × 10 cm, density: 87 g/m^2) was immersed in an aqueous solution containing (a) 26.6 mM xylose or, (b) 22.2 mM glucose. In both cases, approximately 1.2 g of the aqueous monosaccharide solution was taken up by the paper sheet. Hence, a monosaccharide concentration of about 0.1 mg/cm^2 was obtained in each case.

All sample papers were conditioned at room temperature for six hours before being transferred into a Q-SUN Xe-3-HC test chamber (Q-Lab Deutschland GmbH, Saarbrücken, Germany). Aging was carried out at 80 °C and 65% relative humidity according to ISO standard 5630-3.

2.7. Sample preparation of real-world specimen for ECD measurements

2.7.1. Automated solvent extraction

Historic paper samples as well as artificially aged samples (5 g each) were extracted with acetone in an automated solvent extractor (ASE350, Dionex Corporation, Salt Lake City, UT, USA). The following procedure was used: 2 times with 4 cycles, each cycle 15 min. The ASE thermostat was set to ambient conditions.

2.7.2. Soxhlet extraction

Paper samples (5 g) were extracted with 300 mL of acetone in a Soxhlet apparatus for 4 h. The extractant was a round bottom flask (500 mL) with a magnetic stirrer while the heater temperature was set to boiling temperature (> 60 °C).

2.7.3. Derivatization of real-world specimen

The extracts of both extraction methods were separately concentrated to a volume of approximately 1 mL by rotary vacuum evaporation (BÜCHI Labortechnik AG, Flawil, Switzerland), diluted with 4 mL of deionized water and then subject to solid-phase extraction (Strata™-X 33 µm Polymeric Reversed Phase, Phenomenex Ltd.,