Emission of reactive oxygen species during degradation of iron gall ink

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Abstract

Iron gall inks are characterised by high contents of acids and transition metals, promoting degradation of cellulose due to hydrolysis and oxidation, respectively. Their chemical interaction with the environment is not well understood, especially in view of emissions of degradation products which could lead to spread of degradation processes.

In order to study the emissions, we employed gas chromatography / mass spectrometry following headspace micro-extraction, and liquid chromatography following hydroxyl radical scavenging with appropriate probes. We also studied chemiluminescence of cellulose affected by ink degradation.

We showed that while the emissions of organic volatile degradation compounds by inks are less intensive than those of surrounding paper, ink does promote the degradation of cellulose across big distances (from object to object). We were able to link this to emission of reactive oxygen species, probably hydrogen peroxide.

Keywords

Oxidation, cellulose, chemiluminescence, volatile organic products, hydrogen peroxide, Fenton reaction

Introduction

Paper is historically the most important information carrier. Cellulose, its most prominent structural component is a stable polymer in appropriate molecular environments. While acidity promotes hydrolysis of cellulose and certain transition metals promote its oxidation [1,2], they are both components of iron gall inks [3], arguably the most important Western inks.

Iron gall ink was in use until the first half of the 20th century and was generally a mixture of vitriol ($FeSO_4$ with impurities), gallotannins, Arabic gum, but could contain various additives. The extent of the problem is overwhelming: up to 60% of archival documents with this ink are extensively damaged [4] and it has been estimated that about 60-70% of Da Vinci’s works of art are affected by corrosion caused by the ink [5]. Various stabilisation treatments have been proposed tackling both the problem of acidity and that of transition metals. Treatments containing various antioxidants, from preventive antioxidants [6,7] to radical scavengers and peroxide decomposers [8,9] have been tested and some have been introduced into practice.

During the recent years, the chemistry of iron gall ink has become much better understood [10]. There are contradicting reports, however, on the interactions between the iron gall inks and their environment – Neevel [6] observed the Russell effect, i.e. reduction of Ag^+ on photographic negatives in close contact with the ink, which could be the consequence of light emission (chemiluminescence), or emission of volatile reductive compounds (e.g. hydrogen peroxide or aldehydes). On the other hand, Šelih et al. [2] reported on reduced light emission from transition metal containing paper in comparison with paper without transition metals, which was ascribed to a lower overall concentration of peroxides due to Fenton-type reactions, at high temperatures (180 °C).

Emissions of volatile organic compounds from degrading paper has become an interesting area of research and various studies on the identity [11], quantity and information content [12], and emission rates of volatile organic compounds [13] have recently been published. It is known that various aldehydes are formed as a consequence of cellulose degradation and reports have been published on more intensive emission of volatile organic compounds from freshly applied iron gall ink than from paper [14].

From these reports, it is difficult to hypothesise on the effect of the ink on its immediate microenvironment, which could be of potential concern in view of preservation of historic documents. Since transition metals decrease the intensity of chemiluminescence as a consequence of oxidative reactions [15], it therefore seems likely that the observed Russell effect is a consequence of emission of volatiles, or potentially, other species emitted by ink, but not detectable using gas chromatography.

This issue is not only of academic interest, since storage practices and display of objects containing iron gall inks might need to be re-examined if the emissions of volatiles from ink are shown to be substantial and could lead to pronounced degradation of objects in the immediate vicinity. In this work, we examine the emissions to discover the cause of the Russell effect and the potential negative effect iron gall inks exert on nearby objects due to emissions of reactive oxygen species.

Experimental

Samples

Several iron gall ink containing documents from the 19th century, donated by the Nationaal Archief, The Netherlands, were used in the study. Fibre analysis (SCAN-G3:90) showed that all were made of cellulose fibres (rags), with ink pH: 5.3 (S4), 5.1 (S5), 5.1 (S6), 5.2 (S12), 4.8 (S13). All the inks were tested for the presence of Fe(II) with bathophenantroline-containing indicator paper.

Filter paper made of pure cellulose (WH, Whatman filter paper No. 1, Maidstone, degree of polymerisation = 2900, R.S.D. = 1.6%) was used in the degradation experiments.

Determination of cellulose degree of polymerisation (DP)

Viscometric determinations of cellulose DP were performed according to the standard procedure SCAN-CM 15:88, using 1 mol L^{-1} cupriethylenediamine solvent prepared according to the standard SCAN C 16:62. DP was calculated from the intrinsic viscosity measurements using the equation $DP^{0.85} = 1.1[\eta]$ [16]. Typical R.S.D was below 3%.

Chemiluminometry

Chemiluminometric experiments were carried out using a Lumipol-2 chemiluminometer (Polymer Institute of the Slovak Academy of Sciences, Slovakia). A sample of 9 mm diameter was put onto an aluminium pan with the verso side facing upwards (ink side below), and placed onto the oven in the sample compartment. The following experiments were performed: oxidation in a flow of oxygen (3 L h^{-1}) for 60 min at 80 ^{o}C, after which the atmosphere was rapidly changed to nitrogen and the sample was cooled down to 40 ^{o}C in 15 min. The temperature was then steadily increased at the rate of 5 ^{o}C min^{-1} until 250 ^{o}C was reached.

Gas chromatography - mass spectrometry for determination of volatiles

We investigated the volatiles emission of iron gall ink containing paper relative to that of the same piece of paper without iron gall ink in 150-mL reaction vessels with a PTFE cap equipped with two valves with silicone/PTFE septa. The mass of paper was 0.91 g, and the vessels were conditioned at 80 ^{o}C for 5 days to accelerate the degradation and to allow for volatiles to build up. This procedure was not dissimilar to the standard procedure for assessment of paper stability using closed vessel ageing (ASTM D6819-02e3).

Extraction of the VOCs from the atmosphere inside the reactor was performed via a valve using a SPME device with a DVB/CAR/PDMS fibre for 60 min at room temperature. After this, the SPME was transferred to the GC injector system, where desorption took place.

An Agilent 7890A gas chromatograph, coupled to an Agilent 5975C quadrupole mass spectrometer equipped with a Gerstel cooled injection system CIS 4 was used, heated to 250 ^oC. A 60-m Restek RTX-20 column, I.D. 0.25 mm and 1 μm stationary phase thickness was used. The mobile phase used was helium (99.999%) at a flow of approximately 0.9 mL min^{-1}. The following oven temperature program was used: 1 min at 40 ^oC, then heating to 280 ^oC at the rate of 10.0 ^oC min^{-1}, after which the temperature was kept constant for 40 min.

Ionisation was performed using standard EI mode applying 70 eV at 230 ^oC. The interface was heated to 270 ^oC and the quadrupole mass analyser to 150 ^oC. The detection was initially performed using total ion current, and after the identification of the most abundant volatiles was performed using the NIST mass spectra library.

Liquid chromatographic determination of reactive oxygen species

The determination of reactive oxygen species was based on detection of hydroxylated *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide (NPG) derivatives: *N,N'*-(5-nitro-2-hydroxy-1,3-phenylene)bisglutaramide (*o*-HNPG) and *N,N'*-(5-nitro-4-hydroxy-1,3-phenylene)bisglutaramide (*p*-HNPG) [16,17]. Into 6-mL glass vials, 1 mL of 5.2 mmol L^{-1} NPG solution in phosphate buffer (pH 7.0) was transferred and dried in a stream of nitrogen. Following this, pieces of paper with and without iron gall ink were added, same mass into each vial. The vials were gas-tightly closed and conditioned for 7 days at 80 ^oC. Subsequently, the reacted NPG was dissolved in 1 mL of phosphate buffer (pH 7.0) and a 100 μL aliquot was injected using the Agilent 1100 HPLC system (Palo Alto) consisting of an autosampler, a binary pump and a variable wavelength detector set at 431 nm. A Phenomenex Gemini (150 x 4.6 mm i.d.) stainless-steel analytical column packed with C18, 3 μm particle size was used. The mobile phase flow rate was 1.5 mL min^{-1}. The separation was carried out using a gradient of acetonitrile and 0.1 mol L^{-1} phosphate buffer adjusted to pH 7.0. The initial mobile phase contained

3% of acetonitrile, which changed linearly to achieve 9% at 6.0 min. The gradient was then rapidly changed to 30% of acetonitrile at 6.1 min and remained the same until 10.0 min, after which the column was equilibrated with the initial mobile phase composition. Peak areas of *o*-HNPG and *p*-HNPG derivatives were determined.

Determination of the pro-degrading effect of ink

The effect of the presence of iron gall ink on the degradation of cellulose in the vicinity of the ink (but not in contact with the ink) was examined in the following way. In 8.84 mL glass vials the same mass ratio between rag paper with ink and WH was used (0.1215 g of each). However, the rag paper in each glass vessel contained a different amount of ink, the area of which was evaluated using a scanner and expressed in area of ink per mass of paper. The vials were conditioned at 80 ^oC for 5 days, following which the DP of WH was determined and plotted against the area of ink on the rag paper degraded in the same vial.

Results and Discussion

In order to understand the emissions of volatile degradation products from paper with iron gall ink compared to paper without ink, we selected two sacrificial historic 19th-century letters written with the ink. In each case, half of the page contained writing, the other half was empty. The two halves of identical mass (0.91 g) were inserted into 150-mL and subjected to accelerated degradation at 80 ^oC for 5 days in order to allow for volatile degradation compounds to build up in the vial.

After the experiment, the vials were cooled down and a SPME fibre was inserted through the septum in order to sample the volatiles. After GC/MS analyses, we found out that the emissions of organic volatiles from paper with ink were significantly less intensive than from the identical paper without ink (Figure 1). The differences observed in the two experiments may come from the different amount of applied ink, different ink or paper composition, or different area covered with ink, but nevertheless, the result that ink-containing paper emits less volatiles is in both cases significant and in disagreement with the previously reported research [14]. This difference could be a consequence of removal of VOCs through their oxidation by Fe(III) present in the ink itself, or by oxygen containing oxidative species (e.g. hydroxyl radicals), which are known to be present in elevated concentrations in papers containing transition metals [2].

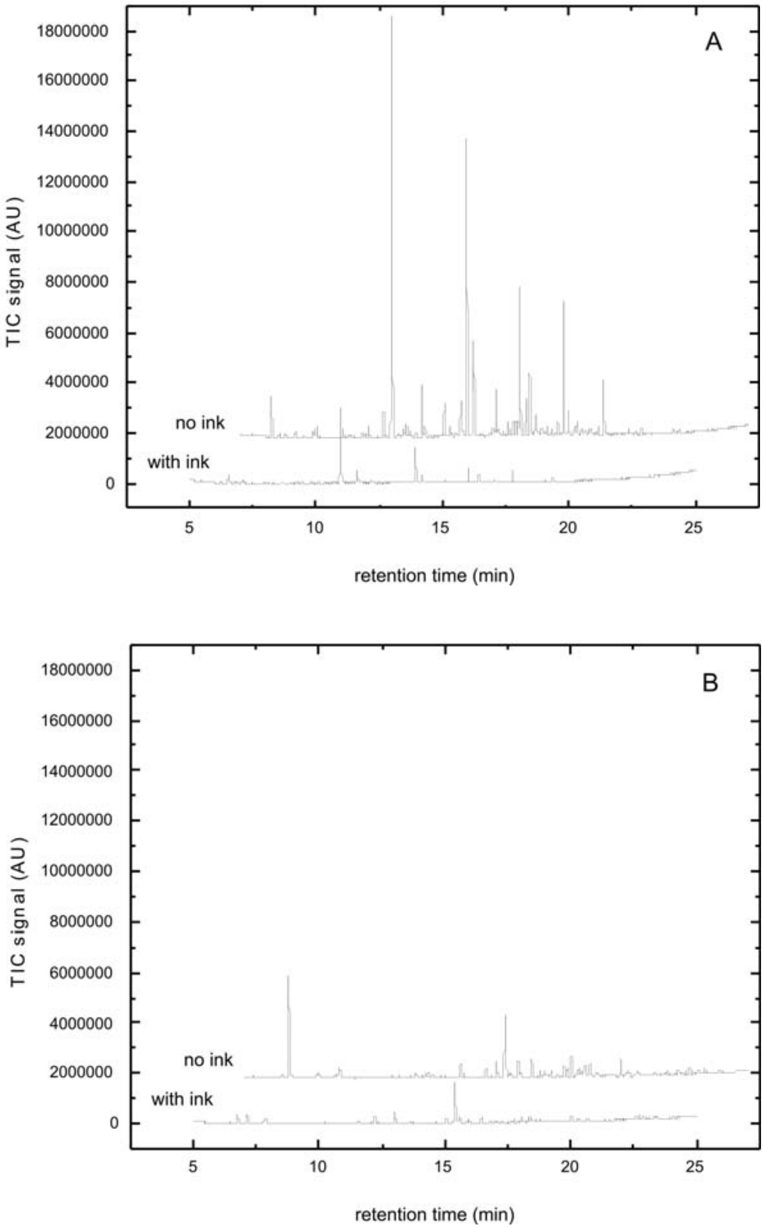

Fig. 1: Total ion chromatograms after SPME GC/MS analyses of volatiles emitted by two different papers, with and without iron gall ink, after 5-day degradation at 80 ^{o}C in closed vials. Paper pH: 5.5 (A) and 5.1 (B).

Considering that the inks emit less VOCs, it is difficult to explain the Russell effect [6] through reduction of Ag^+ by volatile reducing compounds, e.g. aldehydes. In addition, the low intensity of chemiluminescence observed in papers with high transition metal content [2] can also not explain the effect. The same authors showed, however, that the presence of transition metals leads to a higher concentration of reactive oxygen species in paper, which are detectable using hydroxyl radical scavengers, hence oxidative degradation of cellulose in the areas with ink is promoted. However, it has not been studied yet, whether transfer of reactive oxygen species (e.g. H_2O_2) could occur at larger distances or even between sheets of paper. This could lead to cross-infection and thus to faster degradation of the areas surrounding the ink, and could also explain the Russell effect.

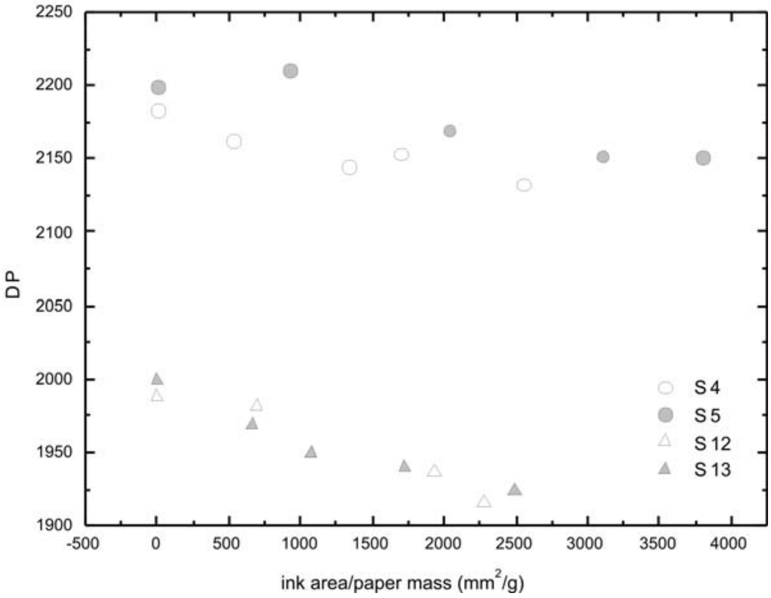

Fig. 2: DP of cellulose paper (WH) after a 5-day period of degradation in closed vials, in dependence of the presence of rag papers S4, S5, S12 and S13 with iron gall ink applications of different cumulative areas (ink area/paper mass). The total mass of paper in the vial was always the same, the mass ratio of WH vs. rag paper was the same, only the ink area on rag paper was varied.

In order to examine this possibility, we performed an experiment in closed glass vials (V = 8.84 mL) in which always the same mass ratio between rag paper with ink and pure cellulose paper (WH) was used. The rag paper sample in each glass vessel contained a different amount of ink, the area of which was evaluated using an office scanner. To quantify and normalise the surface area of ink, we calculated the ratio of inked area and paper mass, A_{ink}/m_{paper}.

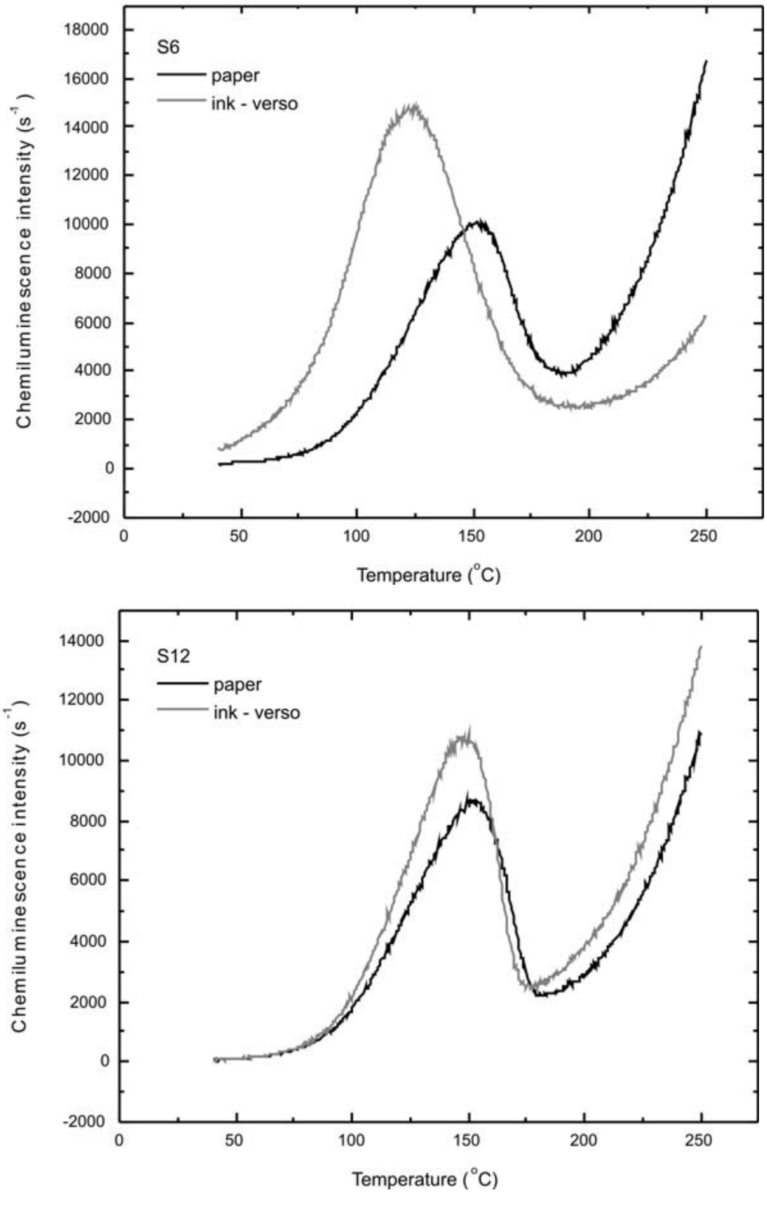

Fig. 3: Dynamic chemiluminometric experiments in nitrogen atmosphere (3 L/min flow) after 60-min preoxidation in oxygen at 80 ^oC, and annealing in nitrogen for 15 min at 40 ^oC, with two different samples with iron gall ink.

The DP of cellulose paper samples (WH) after an accelerated degradation experiment (5 days, 80 ^oC) was determined in dependence of the area of the ink. If there is any promotion of degradation of the cellulose paper degraded in the vicinity of rag paper with ink, it could only occur due to volatile compounds emitted by the ink. To our surprise, the effect of the presence of ink was measurable (Figure 2). While the ink does not emit VOCs in higher amounts than the surrounding paper, it does however, promote the degradation of paper in the immediate proximity. It is possible that reactive oxygen species (e.g. H_2O_2), which are not detectable using gas chromatography, lead to more pronounced degradation of cellulose in the vicinity.

An attractive possibility to study paper in the immediate vicinity of ink is to examine the one beneath an ink line. Its oxidative degradation can be studied using chemiluminometry of reverse side of the link line. Studies of the emission of light during oxidation of organic materials are established in research on degradation of synthetic polymers [18], but also of cellulose [1]. Chemiluminescence during degradation of cellulose has been attributed to many processes, including decomposition of peroxides, transglycosidation, charge transfer reactions [1] and singlet oxygen emission [19]. Peroxide-related emission is best observed in dynamic experiments with pre-oxidised samples, where the emission appears as a distinct peak at 120-150 ^{o}C [20]. In Figure 3, we show two such experiments. After a pre-oxidation step, the paper beneath ink shows a higher peroxide peak than paper without ink application. This result confirms that there is more oxidative degradation of cellulose as a result of the presence of ink – however, while this could be the result of migration of peroxides and other oxygen reactive species from the ink to the immediately adjacent areas of paper, it could also be the result of other migration processes, i.e. of non-volatile ink components or of degradation compounds. Most notably, migration of acids from the ink and enhanced acid-catalysed degradation of cellulose in paper beneath the ink application may lead to more extensive degradation of cellulose and thus a higher content of short-chain cellulose degradation products prone to oxidation [21].

In order to establish the emission of reactive oxygen species from paper into its environment and possible long-distance transfer, we therefore had to devise a different experiment. We used the hydroxyl radical scavenger, *N,N'*-(5-nitro-1,3-phenylene)bisglutaramide (NPG), which on reaction with hydroxyl radicals leads to production of hydroxylated derivates, easily determined using HPLC. Experiments were performed in closed vials, with a dry layer of NPG beneath a degrading paper sample. After the degradation, the vials were opened, the NPG dissolved and analysed using HPLC. The peak areas of the hydroxylated derivates were summed up and are compared in Figure 4.

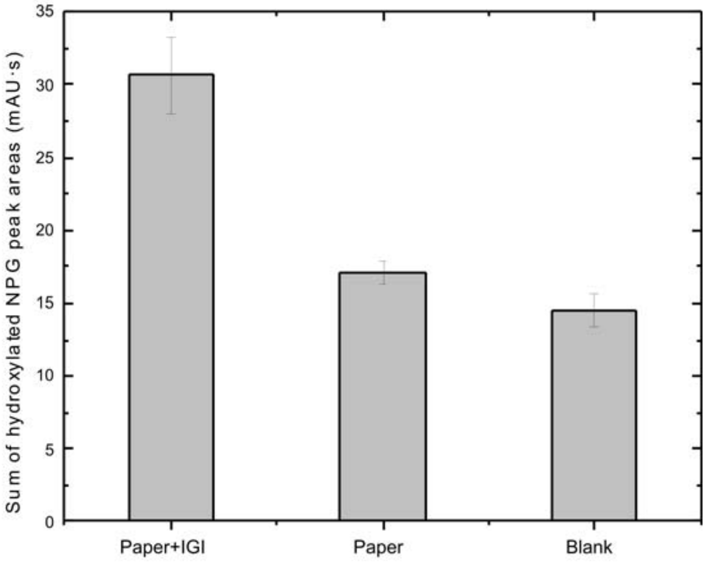

Fig. 4: Sum of peak areas of hydroxylated NPG derivates formed in the vicinity of degrading paper with iron gall ink (IGI), paper only, and in a vial without any added paper (blank). The error bars represent the standard deviation for three parallel experiments.

The data in Figure 4 undoubtedly demonstrate that emission of reactive oxygen species from paper takes place, especially in the presence of iron gall ink. Even if there is no ink present, the measurement is still statistically significantly higher from the blank experiment. The amount of hydroxylated derivates of NPG in the case of paper with iron gall ink is surprisingly high, however.

The question remains, what reactive oxygen species could lead to hydroxylation of NPG under the conditions used. Hydroxyl radicals are probably too reactive to escape the fibrous network, and from among the other non-ionic species, which could potentially escape from paper into the surrounding atmosphere, hydrogen peroxide could be put forward as a viable candidate. The hydroxylation of NPG probably takes place as a result of formation of hydroxyl radicals once hydrogen peroxide is absorbed in the NPG layer and reacted with traces of transition metals. While it has been assumed [22] that superoxide is formed during reaction of atmospheric oxygen with carbonyl (aldehyde) groups of a monosaccharide with simultaneous formation of H_2O_2, there are strong indications that similar mechanisms take place during oxidation of polysaccharides [21].

The hypothesis that hydrogen peroxide is the emitted reactive oxygen species is corroborated by the Russell effect experiment, for the success of which a reducing compound is necessary, such as hydrogen peroxide.

Summary

Degradation of iron gall inks on paper was examined using a variety of techniques in order to examine the emission of volatile species formed during the process. We have shown the following:

- the emission of volatile organic compounds from iron gall ink is lower than from the neighbouring paper,
- chemiluminescence observed on the verso of ink is higher than that of the neighbouring paper, which was ascribed to possible formation and transfer of reactive oxygen species, but could also be a consequence of the presence of a higher content of degradation products,
- despite the less intensive emissions of volatile organic degradation products, iron gall ink has a measurable negative effect on paper degrading in its vicinity,
- emission of reactive oxygen species, possibly hydrogen peroxide, from paper containing iron gall ink was shown to be significant. Even paper without ink was shown to emit such peroxide.

The above findings explain the observed Russell effect and shed more light on the emission of volatiles during degradation of paper. The effect of the emitted hydrogen peroxide on oxidative degradation of paper in the vicinity of iron gall inks could be mitigated through the use of appropriate absorbing media during long-term storage or display.

Acknowledgements

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