Molecular changes in egg tempera paint dosimeters as tools to monitor the museum environment
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2. Determination of the degree of chemical change in museum exposed test paintings by mass spectrometry and discriminant analysis

The main objective of a chemical investigation of the egg tempera dosimetric test systems is to find molecular markers for changes in the chemical composition that occur as a result of artificial ageing under controlled laboratory conditions and by exposure in the museum environment. A further objective is to produce an index that expresses the degree to which the chemical composition of a test system has changed as a result of its environmental exposure. This chapter describes the methodology that was developed to derive molecular information from the tempera dosimetric test systems and to quantify the results. The procedures are illustrated with relevant examples of direct temperature-resolved mass spectrometry (DTMS) data obtained on the test systems.

2.1 Introduction

The analytical methodology applied to obtain dosimetric results from the test systems is schematically shown in Figure 1. After exposure of a test system to the museum environment or to laboratory ageing conditions small samples are taken from a test system that can be processed directly when DTMS is applied. The DTMS methodology allows the analysis of particulate material on an analytical probe. Information is obtained on volatile matter in vacuo such as lipids, sterols and organic dyes at low analysis temperatures. Information of polymerised substances or materials with strong chemical bonds, for example metal-bonded organic networks, is obtained at higher temperatures. At the highest temperatures information on metals and inorganic salts is obtained. Thus, a DTMS analysis provides mass spectrometric information on a wide variety of compounds in one analytical run. A detailed description of the technique is given by Boon [1].
Figure 1 **Analytical methodology flow chart for evaluation of chemical changes using DTMS and DA.**

Analysis of the large number of test systems that were aged under the different conditions results in an enormous data set. For example, each DTMS run consists of 120 scans over a mass range from 20-1000 amu. Analyses were performed in triplicate. Samples of field-exposed dosimeters are compared with the control test system and the set of laboratory light-aged test systems. Hence, rigorous data reduction methodology must be applied to visualise and quantify the extent to which the dosimetric test system had changed chemically as a function of environmental exposure. As a first step in data reduction, the spectra obtained in the DTMS run were summed. Then, Discriminant Analysis (DA) was performed on the summation spectra to estimate analytical reproducibility and perform the data reduction. Using DA, field site data are mathematically compared with the light ageing data to derive a quantitative number on the environmental stress experienced.

### 2.2 Experimental

The tempera paints and mock paintings were prepared according to the method described in **Chapter 1**. The previous chapter also describes the laboratory exposure to light, temperature and air pollutants and gives a summary of the environmental conditions at the field sites where the mock paintings have been exposed.
2.2.1 Direct temperature-resolved mass spectrometry (DTMS)

Although the sensitivity of the DTMS method allows analysis of samples that are much smaller, for the analysis of the dosimetric test systems samples of approximately 1 mg were scraped off the Melinex support and homogenised into ethanol (~ 100 μl). The exact sample size and volume of the ethanol added varied with the composition of the tempera test system, i.e. the pigment-volume concentration. Aliquots of 1 μl of the sample suspension was deposited on the 0.1 mm diameter, platinum/rhodium (90:10) filament (Drijfhout, The Netherlands) of the DTMS probe. DTMS analysis was performed on a JEOL SX 102A double focusing mass spectrometer with B/E geometry. In the ion source of this instrument, the wire was resistively heated by ramping the current as a rate of 0.5 A/min. Using this ramp the temperature was linearly increased from ambient to approximately 800°C in two minutes. Desorbed and pyrolysed material was ionised by 16 eV electron impact ionisation. The mass spectrometer was scanned over a m/z range of 20-1000 using a 1 s cycle time. Samples were analysed in triplicate for discriminant analysis, and the spectra were summed over the TIC.

2.2.2 Discriminant Analysis (DA)

Mass spectra were numerically analysed by discriminant analysis (DA) with the FOMpyroMAP multivariate analysis programme, a modified version of the ARTHUR package from Infometrix Inc. (Seattle, USA; 1978 release) and with the FOM developed Matlab® (The Mathworks Inc., Natick, MA, USA) toolbox ChemomeTricks. DA, as applied here, is a double stage principle component analysis (PCA) technique [2].

There are a few requirements that have to be met in order to perform discriminant analysis successfully. Data on a test system must be available in at least duplicate before DA can be performed. In the present research, results of triplicate measurements were subjected to DA. Furthermore, the application of the DA to evaluate the chemical change in the dosimetric test systems requires that all samples be measured within a single day to minimise variance due to variance in the operation of the mass spectrometer.
2.3 Qualitative description of chemical changes in tempera test systems

Water, proteins and lipids are the main constituents of an egg. The chemical composition of an average chicken egg as relevant to tempera painting is discussed in detail elsewhere [3, 4]. Oxidised lipids, mastic and polymerised proteinaceous material are the components of aged tempera paint in our tempera test systems. Of these components, the lipid and mastic fraction is detected with greatest sensitivity by DTMS. Due to their polymeric nature, proteins are not detected as intact molecules, but are pyrolysed to fragments of lower molecular weight. The proteinaceous fraction is observed at lower sensitivity compared to the lipid components, because the yield from pyrolysis is relatively low compared to the more quantitative desorption of apolar substances.

2.3.1 Unpigmented tempera

Figure 2A shows the DTMS summation spectrum of the unpigmented tempera control sample. There are three important mass peak windows in which components of the binding medium are observed. Triglyceride mass peaks are present between m/z 830 and 900. The cluster between m/z 852 and 862 represents the triglycerides consisting of 55 C-atoms (C55-TGs), and the cluster at m/z 876-890 triglycerides consisting of 57 C-atoms (C57-TGs). Diglycerides and fragments of triglycerides (TGs) and phospholipids show mass peaks between m/z 540 and 640. In the range from m/z 350 to 500 mass peaks from mastic are detected together with peaks originating from cholesterol (m/z 368 and 386). In the lower mass range fragment ions from di- and triglycerides, ions from fatty acids and dicarboxylic acids, and ions from pyrolysis products of (pre)polymeric compounds are observed. The peaks at m/z 262 and m/z 264 e.g. originate from acylium ions that are formed as fragments of glycerolipids which contain linoleic and oleic acid residues, respectively. Peaks at m/z 262 and 264 are also observed in the spectra of free linoleic and oleic acid [5].

Figure 2B shows the DTMS spectrum of 64-day light-aged unpigmented tempera. Comparison with Figure 2A shows that linoleic (m/z 262) and oleic (m/z 264) acid residues are drastically depleted upon exposure to light, as indicated by the decrease of the relative intensities of the peaks. Linoleic acid residues decrease more than oleic acid residues. Increased intensities of peaks at m/z 84, m/z 98 and m/z 152 are ions indicative of dicarboxylic acids formed by
Figure 2 DTMS summation spectra of unpigmented tempera control (A) and 64-day light-aged (B).
oxidative cleavage of unsaturated fatty acid moieties. The unresolved peak pattern between m/z 100 and m/z 300, especially in the high temperature window of the data, is indicative of polymeric networks that break down upon pyrolysis. Free palmitic and stearic acid are formed upon ageing due to hydrolysis of glycerolipids, as indicated by an increase of the peaks at m/z 256 and m/z 284 respectively. Increased intensities of m/z 384, 400, and 402 relative to m/z 386 indicate oxidation of cholesterol to cholestenone (m/z 384), 7-ketocholesterol (m/z 400), and oxo-cholesterols (m/z 402). The formation of these compounds has been confirmed by GCMS analyses [4] and by DTMSMS studies (see Chapter 5).

Focusing on the relative intensities of the peaks at m/z 854, 856 and 858, a decrease can also be observed in m/z 854, the molecular ion of a fourfold unsaturated C55 triglyceride, and m/z 856, a triply unsaturated C55 triglyceride. This indicates that the degree of unsaturation determines the degree to which some of the triglycerides are depleted. Furthermore, in the triglyceride mass window of the light-aged sample, a cluster appears between m/z 860 and 875. The mass difference between the most abundant triglyceride peak in unaged tempera (m/z 856) and the most abundant peak in the light-aged sample (m/z 872), viz. 16 a.m.u., suggests that insertion of oxygen has taken place. The novel technique of matrix-assisted laser desorption/ionisation Fourier transform mass spectrometry (MALDI-FTMS) was applied to study the changes in the TGs in more detail [6, 7]. The high resolution of the MS data obtained by this technique allowed unequivocal determination of the elemental composition of triglyceride ageing products and unambiguously demonstrates that light ageing induces oxygenation of the unsaturated TGs (see also Chapters 6 and 7).

Table 1 summarises the attribution of the most important peaks in the DTMS spectra of fresh and aged unpigmented tempera. The first column shows the m/z value, the second the mass spectrometric interpretation and the third column shows the molecular origin of the compound or the compound class.

Most of the peaks in the table originate from the lipidic components of the binding medium, such as the glycerolipids, cholesterol and the mastic triterpenoids. It must be noted that apart from oxidation and hydrolysis, cross-linking plays a role in the ageing of the lipid fraction as well. This is evidenced by the size exclusion chromatography results shown in Chapter 6. The fragments of cross-linked glycerolipids appear at the same m/z values as the oxidation and hydrolysis products, e.g. m/z 98, 256 and 284. Peaks originating from the proteinaceous fraction of the egg also contribute to the DTMS summation spectrum but are present at relatively low intensities. This is due to the fact that the proteins are pyrolysed so that a great variety of pyrolysis products

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**Table 1 Characteristic peaks in DTMS spectra.**

<table>
<thead>
<tr>
<th>m/z</th>
<th>Interpretation</th>
<th>Compound class of origin</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>Fragment of dicarboxylic acids</td>
<td>Glycerol lipids</td>
<td>aged</td>
</tr>
<tr>
<td>98</td>
<td>Fragment of dicarboxylic acids</td>
<td>Glycerol lipids</td>
<td>aged</td>
</tr>
<tr>
<td>99</td>
<td>Side chain fragment ion of 3-oxo-25,26,27-trinordammarano-24,20-lactone</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>109</td>
<td>Side chain fragment ion of hydroxydammaranonone</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>143</td>
<td>Side chain fragment ion of ocotiline</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>152</td>
<td>C₃ dicarboxylic acid diacylum ion</td>
<td>Glycerol lipids</td>
<td>aged</td>
</tr>
<tr>
<td>203</td>
<td>Pentacyclic triterpenoid fragment ion</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>205</td>
<td>Pentacyclic triterpenoid fragment ion</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>248</td>
<td>Fragment ion of oleic acid</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>256</td>
<td>Palmitic acid</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>262</td>
<td>Linoleic acid acylium ion</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>264</td>
<td>Oleic acid acylium ion</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>284</td>
<td>Stearic acid</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>Palmitic acid monoglyceride fragment</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>338,339</td>
<td>Oleic acid monoglyceride fragment</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>341</td>
<td>Stearic acid monoglyceride fragment</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>368</td>
<td>Cholesterol – H₂O (Cholestadiene)</td>
<td>Sterols</td>
<td></td>
</tr>
<tr>
<td>382</td>
<td>Cholestadienone</td>
<td>Sterols</td>
<td>aged</td>
</tr>
<tr>
<td>384</td>
<td>Cholestenone</td>
<td>Sterols</td>
<td>aged</td>
</tr>
<tr>
<td>386</td>
<td>Cholesterol</td>
<td>Sterols</td>
<td>aged</td>
</tr>
<tr>
<td>400</td>
<td>Hydroxycholestenone</td>
<td>Sterols</td>
<td></td>
</tr>
<tr>
<td>402</td>
<td>Hydroxycholesterol</td>
<td>Sterols</td>
<td>aged</td>
</tr>
<tr>
<td>408</td>
<td>28-nor-olean-17-en-3-one</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>414</td>
<td>3-oxo-25,26,27-trinordammarano-24,20-lactone</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>426</td>
<td>Dammaradienol (3β-hydroxy-dammar-20,24-diene)</td>
<td>Mastic TTP</td>
<td>aged</td>
</tr>
<tr>
<td>439</td>
<td>Ursonic and oleanic acid</td>
<td>Mastic TTP</td>
<td></td>
</tr>
<tr>
<td>454</td>
<td>Ursonic and oleanic acid</td>
<td>Mastic TTP</td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>Oxo-ursonic and oleanic acid</td>
<td>Mastic TTP</td>
<td></td>
</tr>
<tr>
<td>546-550</td>
<td>C₃₅ diglyceryl ions (2 – 0 unsaturations)</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>572-578</td>
<td>C₃₇ diglyceryl ions (3 – 0 unsaturations)</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>600-606</td>
<td>C₃₉ diglyceryl ions</td>
<td>Glycerol lipids</td>
<td></td>
</tr>
<tr>
<td>852-862</td>
<td>C₅₅ triglycerides (5 – 0 unsaturations)</td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td>866-876</td>
<td>Oxygenated C₅₅ triglycerides</td>
<td>Triglycerides</td>
<td>aged</td>
</tr>
<tr>
<td>878-890</td>
<td>C₅₇ triglycerides (6 – 0 unsaturations)</td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td>892-906</td>
<td>Oxygenated C₅₇ triglycerides</td>
<td>Triglycerides</td>
<td>aged</td>
</tr>
</tbody>
</table>

*TTP = triterpenoids

is formed. Such products include side chain fragments and dissubstituted diketopiperazines, pyrrolidindiones and diketopyrrolines [8]. An additional cause of the low intensity of the protein fragment peaks is that the pyrolysis products of proteinaceous material are less effectively ionised. Assignment of peaks based on unit mass is often very difficult because the peaks may originate from more than...
one pyrolysis products and hence have different exact masses. O’Connor [9] has shown by high resolution DT-FTMS that at least 7 pyrolysis products of different elemental composition but identical nominal mass of 97 are formed upon pyrolysis of bovine serum albumin (MW 66kDa). Nonetheless, differences can be observed between the DTMS spectra of the proteinaceous fraction of the light-aged egg samples, when the lipid fraction has been removed with dicholomethane/ethanol. Pilot studies with DTMS-DA show trends with ageing. The main differences relate to the peaks from CO₂ and SO₂ released from oxidised functional groups. The complete interpretation of the changes indicated in the discriminant mass spectra of the proteinaceous fractions of the light-aged temperas is very complex and has not been studied further.

2.3.2 Lead white pigmented tempera

Figure 3A shows the DTMS spectrum of an unaged lead white pigmented test system. Due to the high pigment concentration, the peaks in this spectrum at m/z 206-208 (Pb) and m/z 44 (CO₂), which originate from the lead white pigment itself, are plotted off-scale. Comparison of the DTMS spectrum of the unaged lead white pigmented tempera with that of unaged unpigmented tempera shows that some alteration of unsaturated triglycerides has already taken place in the curing stage of the lead white tempera. Early metal catalysed oxidation reactions in the dark are evidenced by lower relative intensities of the peaks at m/z 854, 856, 262 and 264 and the presence of a small cluster at m/z 866-876. Oxidation of cholesterol is also taking place in the curing stage. Furthermore, in the unaged lead white tempera, free fatty acids such as palmitic acid (m/z 256) and stearic acid (m/z 284) are observed with higher relative abundance than in the unpigmented equivalent. This is interpreted as hydrolysis of glyceryl ester moieties in phospholipids and di- and triglycerides. The formation of free fatty acids is also observed in DSC results [10, 11] where a low temperature peak/shoulder develops upon light exposure. The spectrum of 64-day light-aged lead white test system (Figure 3B) indicates that light ageing leads to the formation of similar reaction products as observed in the corresponding light-aged unpigmented test systems, albeit at a higher reaction rate. This is deduced from the relative intensities of m/z 854, 856 and 858, which point to a lower degree of unsaturation in the di- and triglycerides for the light-aged lead white tempera (64 days) as compared to the unpigmented equivalent. Furthermore, the intensity of the cluster of peaks from oxygenated triglycerides (m/z 866-876) has increased.
Determination of the degree of chemical change

Lead White Pigmented Tempera Control

64-Day light-aged

Figure 3 DTMS summation spectra of lead white tempera control (A) and 64-day light-aged (B).
2.3.3 Azurite pigmented tempera

The spectra of unaged and 64-day light-aged azurite pigmented test systems are shown in Figure 4 A and B. Azurite, a basic copper carbonate, decomposes and forms carbon dioxide at high temperature. The resulting m/z 44 dominates the spectra. Copper is not observed in the DTMS spectra of azurite tempera. Comparison of the spectrum of unaged azurite tempera with that of unaged unpigmented tempera strips shows that the effect of addition of azurite to the binding medium leads to severe changes in the curing stage. These changes are due to metal catalysed oxidation of the binding medium. Unlike lead white pigmented test systems, free fatty acids are not observed to a great extent in azurite test systems. Copper catalyses oxidation but does not affect the stability of the ester bonds in the triglycerides and phospholipids, to such a great extent as lead white. Comparison of the unaged with the 64-day light-aged azurite test system suggests that the lipid fraction of the paint undergoes only minor additional changes upon light ageing, such as further oxidation of cholesterol.

2.4 Description of chemical changes in light-aged test systems by Discriminant Analysis

The analytical results discussed above show that many processes in the test systems can be retrieved by DTMS. Since the DTMS spectra of the tempera dosimetric systems contain many mass peaks, quantification of the changes using peak ratios alone is insufficient. The multivariate technique of Discriminant Analysis (DA) was used to compare the spectra, determine the analytical reproducibility, and derive relevant sets of correlated mass peaks, which describe the changes quantitatively in the mathematical form of discriminant function scores.

In the case of the DTMS and DA of a light ageing series of a tempera test system, an increase in the degree of chemical change with ageing time can be expected. Figure 5 shows the result of DA of DTMS data from a light ageing series of unpigmented tempera strips. The abscissa represents the ageing time (days), while the ordinate represents the score on the first discriminant function. The spreading in the data is indicated with the grey band. This figure demonstrates that the light ageing of an unpigmented test system takes place very quickly in the first days of exposure. At longer exposure times the process proceeds at a much lower rate and the degree of chemical change appears to
Figure 4 DTMS summation spectra of azurite tempera control (A) and 64-day light-aged (B).
plateau. These observations agree with classical kinetic considerations that predict that reaction rates decrease as time progresses due to diminishing concentrations of reactants. By performing an inverted standardisation procedure on the loadings of the m/z values of the discriminant functions so-called discriminant mass spectra can be derived, which indicate which m/z peaks increase or decrease as a function of the ageing time. In this way, discriminant function data can be interpreted chemically.

Figure 5 Light ageing curve of unpigmented tempera obtained by DTMS and DA. The outer lines of the graph are the minimum and maximum, and the inner line is the average of three results.

Figure 6A shows the first discriminant function (DF1) mass spectrum from the light ageing series of unpigmented tempera. Peaks in this figure that decrease in relative intensity upon exposure are plotted as negative peaks, whereas those that increase as a function of light exposure are positive peaks. The DF1 spectrum, therefore, indicates the following: a decrease in triglycerides with unsaturated bonds (m/z multiplets at 856 and 882); a decrease in mastic pentacyclic triterpenoids (m/z 454, 439, 426, 248, 203) [12]; a decrease in C18:2 and C18:1 fatty acyl moieties (m/z 262 and m/z 264) and a decrease in cholesterol (m/z 386 and 368). Light induced oxidation is exemplified by: an increase in oxidised triglycerides (872 and 898); high intensity peaks for oxidised cholesterol at m/z 400 and 384; and the appearance of mass peaks for palmitic acid (m/z 256), stearic acid (m/z 284), and azelaic acid (m/z 152 and 98).
Determination of the degree of chemical change

Figure 6  First (A) and second (B) discriminant mass spectrum for light-aged unpigmented tempera.

The DF2 spectrum is shown in Figure 6B. In this figure m/z 262 and m/z 264 appear on opposite sides of the spectrum. The scores of the different samples in the light ageing series on the second discriminant function (0, 32 and 64 days score positive while 4, 8, and 16 days score negative) indicate that the m/z 262 : m/z 264 ratio decreases in the first stage of light ageing, and increases again upon progressed light ageing (data not shown). This is explained as follows. Although the relative intensities in the DTMS summation spectrum of both m/z 262 and m/z 264 decrease, the m/z 262 initially decreases at a faster rate than the 264 so
that the m/z 262 : m/z 264 ratio decreases upon light ageing. At longer exposure times (32 and 64 days) the linoleic acid moieties are almost completely consumed, so that the oleic acid moieties are depleted at a higher rate and the m/z 262 : m/z 264 ratio increases again. The same line of reasoning applies for the peaks at m/z 600 and m/z 602. The phenomenon is not observed in the diacylglycerol cluster at m/z 572-580 because these C$_{37}$ fragment ions contain the more saturated fatty acyl groups (see also Chapter 6) so that less linoleic acid moieties are present. Thus, these observations on Figure 6B are attributed to differences in reactivity of the oleic (m/z 264) and linoleic (m/z 262) acyl groups. Oleic acid and linoleic acid are reported to show significantly different autoxidation rates [13] and based the observations by Cho et al. [14] the same may be expected for photo-induced oxidation.

As many environmental factors interact in a museum environment, the changes in the chemical composition of a test system exposed in a museum cannot simply be seen as due to one process alone, but is an integration of a number of processes. The extent to which the processes have proceeded is a function of the different environmental factors. The presence of air pollutants such as nitrogen oxides and sulphur oxides in the museum atmosphere, for example, leads to oxidation of organic materials. Moreover, acidifying air pollutants in combination with high relative humidity accelerate hydrolytic processes, especially in the presence of higher temperatures.

The following example shows that different processes of chemical change can be identified in the tempera dosimetric test systems. In this case, the processes are determined by the pigments rather than by different environmental factors. DTMS data of the light ageing series of unpigmented, lead white pigmented and azurite pigmented test systems were subjected to discriminant analysis. Two different types of chemical reactions were detected. Figure 7 shows plots of the data points obtained on the test systems as coordinates in the score map of the first discriminant function (DF-1) and the second discriminant function (DF-2). The geometric distance between the data points is a measure of the difference in chemical composition. Two ageing phenomena are observed and interpreted as due to (1) oxidation and to (2) hydrolysis of ester bonds in the glycerolipids and in the protein lipid network polymer [4]. DF1 (oxidation) separates unpigmented test systems from lead white and azurite pigmented ones. DF2 (ester bond hydrolysis) separates lead white pigmented from azurite pigmented tempera. The series of aged azurite pigmented tempera data points is less resolved because the most of the chemical changes had already taken place during the curing stage in the dark.
Determination of the degree of chemical change

Figure 7  Map of scores on the first two discriminant functions for light ageing series of unpigmented, lead white pigmented and azurite pigmented tempera.

Figures 8 A and B show the corresponding discriminant mass spectra of the first (A) and the second (B) discriminant function. The first discriminant mass spectrum suggests that the process represented by the horizontal axis mainly involves oxidative processes, as indicated by m/z 386 (cholesterol) being plotted as reacting compound and m/z 384 and m/z 400 (cholesterol oxidation products) as ageing products. Oxidation is also indicated by the positions of intact TGs and oxidised TGs. The discriminant mass spectrum of the vertical axis shows di- and triglyceride peaks (m/z 570-620 and m/z 850-900) on the side of the reacting compounds and peaks at m/z 239, 256, 267, and 284 on the product side. This is interpreted as de-esterification of glycerolipids to form free fatty acids or fatty acid salts with the metal cations. Hence, the data shown in Figure 7 indicate that hydrolysis of ester bonds is an important process during light ageing of lead white pigmented tempera. The map also indicates that the degree of oxidation of the binding medium of azurite pigmented tempera is very high, but that the degree of hydrolysis is relatively low compared to lead white pigmented systems.

These data clearly show that DTMS & DA can be used successfully to bring the different processes into focus that take place in the tempera dosimetric test systems. Hydrolytic and oxidative processes in the test systems induced by environmental factors are important tracers of the degradation processes. Tracing
Figure 8 First (A) and second (B) discriminant mass spectrum for light ageing series of unpigmented, lead white pigmented and azurite pigmented tempera.

these phenomena in the field-exposed dosimetric test systems will give important indications on the quality of the exposure environment. A low severity environment should lead to chemical changes that correspond to only a few days of light ageing induced change under lab conditions. High severity environments on the other hand may lead to a degree of chemical change in the test systems considerably stronger than that seen in the 64-day light ageing experiments.
2.5 Effects on the chemistry of the test systems by exposure to NO\textsubscript{x} and SO\textsubscript{2}.

In order to test the sensitivity of the dosimetric test systems to air pollutants, all tempera test systems prepared were subjected to a period of exposure lasting four days to evaluate the effects of exposure to NO\textsubscript{x} and SO\textsubscript{2}. DTMS was used to investigate the chemical changes in the lipid marker compounds.

![Unpigmented tempera diagram](image)

**Figure 9** Map of scores on the first two discriminant functions for light-aged (16L), thermally aged (21T) and NO\textsubscript{x}/SO\textsubscript{2} exposed unpigmented tempera test systems.

**Figure 9** presents a discriminant function score map of a comparative study of controls, 21-day thermally aged, 16- and 64-day light-aged and NO\textsubscript{x}/SO\textsubscript{2} exposed unpigmented tempera dosimetric test systems by DTMS. The study was designed to probe the data set for differences in effects of thermal ageing, light ageing and ageing due to noxious gases. The 16-day light-aged, 21-day thermally aged, the NO\textsubscript{x}/SO\textsubscript{2} exposed and the control sample defined the training set. The 64-day light ageing results were used as test set. The geometric distance between controls (only curing) and 21 days thermal ageing is relatively small compared to the effect of light ageing. The conclusion can be drawn that thermal ageing effects (in the dark) on the lipids and mastic in the binder are small. The chemical effect of the noxious gases on the lipid fraction of the binding medium is similar to the effects of light ageing because triglycerides and sterols are oxidised. However, differences between the two conditions are detected in the second discriminant function (DF2). The mass spectral data in DF2 indicate that
the oxidation of sterols is less severe under NO$_x$/SO$_2$ conditions but that hydrolytic processes affecting the ester bond are more prominent. Incorporation of SO$_2$ in the form of sulphates or sulphinic acids is also evident from ions at m/z 64 which are due to pyrolytic elimination of SO$_2$ from the samples [15]. The presence of sulphates in the NO$_x$/SO$_2$ exposed unpigmented tempera test system was confirmed by FTIR spectroscopy [10]. Further comparative studies in the different tempera test systems have shown that NO$_x$/SO$_2$ effects are detected with good sensitivity in lead white, smalt, sienna and alizarin test systems.

2.6 DTMS studies of selected dosimetric test systems from the field sites

As mentioned in Chapter 1, mock test paintings were exposed at a selection of seven different sites, consisting of controlled (Tate, Rijksmuseum, Uffizi) and uncontrolled sites (Sandham Chapel and Alcázar). Control in this context means that the relative humidity and temperature are set by air conditioning systems. Furthermore, at controlled sites illuminance levels are such that the annual dose of illumination is below the generally accepted value of 650 klx·h for museum exposure of paintings [16]. At the uncontrolled sites the light levels are expected to exceed those values, and hence more drastic chemical changes in the test systems can be anticipated. On top of that, other environmental factors, such as variations in relative humidity, temperature and air pollution also contribute to the chemical changes in a test system. A detailed description of the field sites is given in Chapter 1.

The question was tested whether dosimetric test systems from field sites were similar to those subjected to the laboratory conditions of thermal ageing, light ageing or noxious gas exposure, respectively. The discriminant analysis with a training set consisting of the DTMS data of controls, thermally aged (21 days), NO$_x$/SO$_2$ and 16-day light ageing of the unpigmented tempera dosimeters shows that field site exposed dosimeters plot in the area of light-aged test systems of 8 to 16 days exposure. This implies that the chemistry at the field sites resembles mostly that of light ageing (vide infra).

This observation was used to define a protocol for the comparison of field-exposed dosimeters. The DTMS data from the light ageing under laboratory conditions during 4, 8, 16, 32 and 64 days were used for calibration. Discriminant analysis was performed with the artificially light-aged dosimeters as
the training set in order to calculate a multivariate solution space in which the geometric position of the DTMS data of field-exposed dosimeters was interpolated (i.e., field site data were included as a test set). The following example illustrates the approach and discusses results on the field-exposed unpigmented tempera test systems. **Figure 10** shows the scores on the first discriminant function for the field-exposed unpigmented dosimeters. The light ageing data, the data from NOx/SO2 and from the control sample for field exposure (FOM, stored under dark and oxygen free conditions in a copper lined anticorrosive bag) are given for comparison. As might be expected, field-exposed test strips from the uncontrolled environments (the Alcázar and Sandham Chapel) indicate a stronger chemical change than dosimeters exposed in the controlled museum environments. The result of the Rijksmuseum “Depot Oost” sample is remarkable because this dosimeter shows considerable chemical change, although it was exposed in the painting storage facility where light intensity is very low. It indicates that other factors than light intensity alone play an important role in environment-induced chemical processes.

**Figure 10** Comparison of the scores on the first discriminant function (DF1) of laboratory-aged and field-exposed unpigmented tempera dosimeters. The light ageing series defined the training set for discriminant analysis. Discriminant scores are normalised so that the score of the control for light ageing (00L) is zero. Legend to field sites: FOM, control dosimeter stored under exclusion of oxygen; ALC, Alcázar; RDO, Rijksmuseum Depot “Oost”; RNW, Rijksmuseum Nightwatch; SAC, Sandham Chapel; TAT, Tate Gallery; and UFF, Uffizi.
In the ideal situation the dosimetric test systems would be calibrated against all (combinations of) environmental factors. This, however, would require an extensive data set that would have been far too large for the explorative character of the ERA project. Therefore, the light ageing set was used for calibration of the degree of chemical change in the field-exposed dosimeters. This approach can only yield valid results if the chemical processes that take place during natural ageing at the field sites closely resemble those that take place during light ageing. Hence, it is important to compare the nature of the chemical change in the natural ageing process with that of the light ageing process. Discriminant analysis with both the light ageing and the field site DTMS data in the training set was used to determine whether the processes that occur during the light ageing differ substantially from those taking place during field exposure. This was done for the unpigmented tempera dosimetric test systems. **Figure 11 A** shows the map of the scores on the first discriminant function plotted against those on the second discriminant direction. The relative significance of the discriminant functions indicated along the axes is 88 % (DF1) and 11 % (DF2). A deviation of the score on DF2 (the ordinate) is far less important than the score on DF1 (the abscissa). This demonstrates that field site exposure in the museums is expressed by the same molecular characteristics as the light ageing.

In the case of the lead white pigmented test systems, however, a difference is observed between the artificially light-aged and the majority of the field site exposed dosimeters. The map of the scores on the two first DFs for the lead white temperas is shown in **Figure 11B.** The DF1 separates the light-aged systems from the field-exposed systems, with the exception of the Tate Gallery exposed dosimeter. The discriminant mass spectrum shown in **Figure 12** indicates that chemically the differences are caused by hydrolysis of glycerolipids, producing free fatty acids (m/z 256 and 284) and lead soaps (m/z 239 and 267), and by the oxidation of cholesterol (m/z 386) to hydroxycholestenone (m/z 400) and oxo-cholesterol (m/z 402) in the field-exposed systems. Furthermore, the peak of the mastic derived ageing product 3-oxo-25,26,27-trinordamarano-24,20-lactone (m/z 414) and its main EI fragment peak (m/z 99) [17] are more abundantly present in the spectra of the field-exposed dosimeters.

The ranking order of the field sites according to environmental light ageing stress, shown in **Figure 10** for the lead white tempera test system, is not the same as in unpigmented dosimeters because the chemical changes induced by field exposure are not entirely comparable. The FOM control is classified as similar to the light ageing control. The dark storage room in the Rijksmuseum Depot (RDO) has again a relatively high stress score and is in this respect
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Figure 11  Map of scores on the first two discriminant functions for light-aged and field-exposed unpigmented (A) and lead white tempera test systems (B). Both the light ageing results and the field exposure results were included in the training set.

comparable to the unpigmented test system. This suggests that there is an unknown oxidising agent active in the dark, which contributes to a chemical change comparable to very strong light ageing. The lowest museum gallery stress value is found in the Tate Gallery dosimeter. The highest value is found for the Sandham Chapel, which is the site with the highest relative humidity. Comparison of the unpigmented and lead white tempera test systems of Uffizi Gallery suggests that the lead white test system detected an additional contribution to the environmental ageing stress in the Uffizi Gallery. Possible extra factors are the air pollution exposure, and the higher local temperatures. It
must be noted that, although the processes that take place during field ageing are not identical to those during light ageing, the ranking of the field sites presented in Figure 10 is confirmed when the field site data alone are used as training set for DA.

**Figure 12** First discriminant mass spectrum for the lead white tempera test systems. Both the light ageing results and the field exposure results were included in the training set.

### 2.7 Extrapolation to museum exposure years

Referring to the dosimetric results obtained on the field-exposed unpigmented tempera dosimeters, it must be noted that even the “best” field sites, i.e. the ones with the smallest chemical change, show effects which are comparable with those observed upon eight days of artificial light ageing. This effect is even stronger in the case of the tempera systems with inorganic pigments (see Chapter 3). Using the reciprocity principle [18], this value of light exposure can be related to the number of years of exposure in a museum. The reciprocity principle states that the product of light intensity and exposure time determines the amount of damage to an object. Thus, eight days of artificial light ageing at 18 klx is 3.5 Mlx·h by reciprocity. This value exceeds the generally accepted annual dose of illumination of 650 klx·h [16] by more than a factor of 5.
The discrepancy between the actual effects of exposure to the museum environment observed and the theoretically expected value based on the reciprocity principle for light ageing may be explained in two different ways. Firstly, the assumption of the reciprocity is not valid because there are many factors that may affect the reciprocity principle, see [19]. The following factors may explain the relatively strong effects at lower light intensities. In several cases of radical initiated oxidative processes, the oxygen uptake is proportional to the square root of the light intensity. The depth of penetration of the light may vary when a layer of radiation absorbing material is formed on the surface. This factor becomes particularly relevant to the validity of the reciprocity principle when light intensities are so high that they allow two-photon processes to occur (very unlikely at 18,000 lx light intensity). Furthermore, the moisture content may affect the rates of light induced processes. In the present experiment moisture content may have played an important role; the artificial light ageing experiments were carried out at relatively low relative humidity (27-28%) and in many cases the relative humidity at the field sites was found to be much higher.

On the other hand, if the reciprocity principle is valid, the extreme effects cannot be explained when exposure to light is seen as the most important factor in ageing. Hence, the effects must originate from other factors that strongly contribute to the environmental stress (expressed as chemical change in the multivariate space) detected by the dosimeters. The composition (or reactivity) of the museum air may be such a factor. Dust is a potential factor as it may contain reactive pollutants or act as a nucleus for future deterioration. Gaseous air pollution, either diffusing into the museum from outside or originating from sources inside the museum, may strongly contribute to the deterioration processes. The results of the dosimeter that was exposed in the storage “Depot Oost” of the Rijksmuseum, where light intensity is very low, support the conjecture that light exposure is not the most important factor and that other factors must play an equally important role. One of these factors is NOx/SO2, which also contributes to the dosimeter’s DTMS signature in almost the same way as the light ageing effects. NOx is considered a component of air pollution that is very detrimental. Its action can be twofold. Firstly, it may increase the acidity of the environment by formation of nitric and nitrous acid. Secondly, it can oxidise organic materials that contain double bonds [20]. The action of nitrogen dioxide on polyunsaturated diacylphosphatidylcholines for instance has been studied by Balazy and co-workers [21]. Their results confirm the observation in this chapter that NOx can cause oxidation of unsaturated lipids even in the absence of light.
2.8 Conclusions

The validity of the principle of paint-based dosimetry has been demonstrated. The degree of chemical change in the light-exposed tempera dosimetric test systems can be correlated with the duration of light exposure. The results of field exposure show that the chemical composition of the paints is changed by exposure in museums: significant differences are observed among dosimeters exposed in different field sites.

The effect observed upon exposure of the dosimeters to museum environments is more drastic than anticipated. This suggests that either the reciprocity principle of light ageing is not valid, or that the chemical composition of the museum atmosphere plays an even more important role than the exposure to light alone. Testing of the reciprocity principle for light ageing under *ceteris paribus* conditions is strongly recommended. The results of such a survey can contribute to the understanding of light-induced deterioration processes of paint systems.

Direct temperature-resolved mass spectrometry (DTMS) is a rapid and adequate method to determine the chemical composition (of primarily the lipid fraction) of tempera paint systems and changes therein. Discriminant analysis (DA) was successfully applied to quantify the difference between DTMS data obtained on dosimeters exposed under different environmental conditions. Moreover, DA can be used to distinguish and identify different processes that take place in the temperas as a result of exposure.

The chemical changes observed upon exposure of the unpigmented dosimeters to NO\textsubscript{x}/SO\textsubscript{2} in the dark follow to a great extent the changes induced upon exposure to light. However, small chemical differences are observed. On the one hand cholesterol is less severely oxidised upon NO\textsubscript{x}/SO\textsubscript{2} exposure. On the other hand hydrolysis of glycerolipids plays a more important role. Thermal ageing has a very small effect on the composition of the lipid fraction of the unpigmented tempera dosimeters.

Comparison of the chemical changes in the field-exposed dosimeters with those in the laboratory-exposed (light, temperature and NO\textsubscript{x}/SO\textsubscript{2}) dosimeters indicates that the processes taking place during field exposure resemble most those taking place during light ageing.

The light ageing series of the dosimeters was used successfully for calibration the degree of chemical change in the field-exposed unpigmented tempera dosimeters. The degree of chemical change is expressed as numbers (i.e. DF scores) that indicate the “environmental stress” detected by the dosimeters.
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**References**


Chapter 2


