Analytical chemical studies on traditional linseed oil paints

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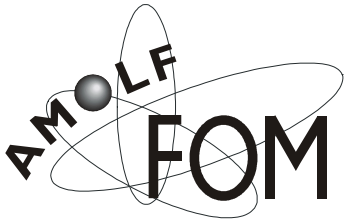

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The chemistry of the drying of the linseed oil is so complex as to be unintelligible to anyone who is not a student of chemistry.
(A.P. Laurie, The Painter's methods and materials)

In the ideal drying oil the first or "drying stage" of polymerisation is short; the second or "deteriorative stage" is prolonged.
(M. W. Formo, Bailey's industrial oil and fat products)

Art has to be forgotten:
Beauty must be realized.
(Piet Mondriaan)

MOLART Reports

MOLART - Molecular Aspects of Ageing of Painted Art - is a 5-year co-operative project between art historians, restorers, analytical chemists and technical physicists funded by the Dutch Organisation for Scientific Research (NWO). Technical support and advice is given by Shell-SRTCA (Amsterdam), AKZO NOBEL (Arnhem), Instituut Collectie Nederland (ICN, Amsterdam) and the Dutch art museums. The project was launched on 1 February 1995 and will end early 2003. The object of MOLART is to contribute to the development of a scientific framework for the conservation of painted art on the molecular level. The focus of MOLART is the determination of the present chemical and physical condition of works of art produced in the period from the 15th to the 20th century. Studies of historical paint manufacturing and workshop practice must give insight into the nature of the painters' media and the painting technique used originally. Fundamental studies on varnishes, paint and colorants are undertaken to understand the molecular aspects of ageing since this is thought to be a main cause for the continued need to treat paintings.

This thesis is the sixth in a series of MOLART reports that will summarise all research results obtained in the course of the project. Information about MOLART can be obtained from the project co-ordinator Prof. Dr. J.J. Boon, FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands.

Previously issues of the MOLART series:

1. Molecular studies of fresh and aged triterpenoid varnishes, Gisela A. van der Doelen, 1999. ISBN 90-801704-3-7
2. A mathematical study on craquelure and other mechanical damage in paintings, P. de Willigen, 1999. ISBN 90-407-1946-2
3. Solvent extractable components of oil paint films, Kenneth R. Sutherland, 2001. ISBN 90-801704-4-5
4. Molecular changes in egg tempera paint dosimeters as tools to monitor the museum environment, Oscar F. van den Brink, 2001. ISBN 90-801704-6-1
5. Discoloration in renaissance and baroque oil paintings, Margriet van Eikema Hommes, 2002.

This thesis is based on the following publications:

Chapter 3

van den Berg, J. D. J., Vermist, N. D., Carlyle, L., Hol apek, M., and Boon, J. J., "The effects of traditional processing methods of linseed oil on the composition of its triacylglycerols", submitted to Z. Kunsttechn. Kons.

Chapter 4

van den Berg, J. D. J., and Boon, J. J., "Direct temperature resolved mass spectrometry of oil paint constituents and aged oil paints", submitted to Journal of Analytical and Applied Pyrolysis

Chapter 5

van den Berg, J. D. J., van den Berg, K. J., and Boon, J. J., "Identification of non-cross-linked compounds in methanolic extracts of cured and aged linseed oil-based paint films using gas chromatography – mass spectrometry", Journal of Chromatography A, 950 (2002) 195-211

van den Berg, J. D. J., and Boon, J. J., "Unwanted alkylation during direct methylation of fatty acids using tetramethyl ammonium hydroxide reagent in a Curie-point pyrolysis unit", Journal of Analytical and Applied Pyrolysis, 61 (2001) 45-63

Chapter 6

van den Berg, J. D. J., van den Berg, K. J., and Boon, J. J., "Determination of the degree of hydrolysis of oil paint samples using a two-step derivatisation method and on-column GC/MS", Progress in Organic Coatings, 41 (2001) 143-155

Other publications

van den Berg, J. D. J., van den Berg, K. J., and Boon, J. J., "GC/MS analysis of fractions of cured and aged drying oil paints", in 14th International Conference on Mass Spectrometry, Ed. Karjalainen, E. J., Hesso, A. E., Jalonen, J. E., and Karjalainen, U. P., Advances in Mass Spectrometry, Vol. 14, Elsevier, Tampere, Finland (1997) D05 THPO121

van den Berg, J. D. J., Boon, J. J., van den Berg, K. J., Fiedler, I, and Miller, M. A., "Identification of an original non-terpenoid varnish from the early 20th century oil painting "The White Horse" (1929), by H. Menzel", Analytical Chemistry, 70 (1998) 1823-1830

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Rimer, B., Fiedler, I., M. A. Miller, Cunningham, M. and van den Berg, J. D. J., “Investigation of fatty acid migration in alizarin crimson oil paint in two works by Frank Stella”, in 27th annual meeting of the American Institute for Conservation of Historic and Artistic Works, St. Louis, Missouri, Ed. Wallace, F. A., AIC Paintings Specialty Group Postprints, AIC, Washington, (1999) 1-14

van den Berg, J. D. J., Vermist, N. D., and Boon, J. J., “MALDI-TOF-MS and ESI-FTMS of oxidised triacylglycerols and oligomers in traditionally prepared linseed oils used for oil paintings”, in 15th International Conference on Mass Spectrometry, Ed. Gelpi, E., Advances in Mass Spectrometry, Vol. 15, Elsevier, Barcelona, Spain (2000) in press

Contents

Chapter 1

General introduction

1.1 Paints and painting

Paints[1] have been used for decorative purpose for many centuries. As early as 40,000 years ago prehistoric man decorated his caves with paintings and sketches. Although the binding media that were used are not known, the pigments were shown to be natural earths, essentially oxides of iron. In ancient Egypt (5000 BC) paintings were produced which have shown to incorporate gum arabic, gelatine, egg white and beeswax [1-3]. The variety of colours and quality of these paints had improved markedly. Egyptian art featured blues, greens, reds and gold. The Persians also used the gum arabic, whereas the Greeks and Romans mostly used size for decoration of their walls and statues [3]. At about 1200 BC lacquers were developed to a high degree of sophistication in China [1].

The early renaissance was the real beginning of paints as we know them today, so most of the media used by artists in our hemisphere have the tradition of centuries of use behind them. In Europe, prior to the industrial revolution, the evolution of paints was mainly in the hands of craftsmen. One of the vehicles used was egg medium, although the precise history of its usage is not very clear. The first full description of the use of the yolk of the egg has been given by Cenninio Cennini at the beginning of the fifteenth century, but earlier reports on the use of egg medium are known, especially by painters from Italy [3]. Vegetable oils gradually replaced the egg medium since the fifteenth century because oil made the paint more transparent and less opaque.

The first use of a drying oil[2] has been conveyed by Ætius in the fifth century, who reported the preparation of linseed oil and stated that it was used by gilders and encaustic painters to preserve their work owing to its property of drying. An oil varnish, made by dissolving resin in a drying oil, is found in the

[1] Paint: A mixture of a pigment and a vehicle, such as oil, that together form a liquid or a paste that can be applied to a surface to provide an adherent coating that imparts colour to and often protects the surface.

[2] Drying oil: Relatively highly unsaturated oil, such as cottonseed, soybean and linseed oil, that is easily oxidized and polymerized to form a hard, dry, film on exposure to air.

Lucca manuscript, supposed to be of the eight century [3]. It was not till the eleventh or twelfth century when a first written account is available on the use of drying oil as a binding medium in a document by Theophilus [4], and a manuscript by Eraclius from that same period [3]. However, it is thought that there has been a long northern tradition in the use of vegetable oils as binding medium before the fifteenth century [3, 5].

1.2 Drying oil paints

Not all vegetable oils are suitable for painting because some of the oils are not able to form a dry film upon exposure to air. Since medieval times it has been known that the oils of linseed, poppyseed, and walnut form a dry film. Of all the reported media these drying oils are the most important ones and are still frequently used by artists today. Interestingly, they also are raw binding materials for other modern paint systems such as alkyd resins, epoxy esters and uralkyds. Although oil paint has been used for centuries, there are still many questions, even in our times, about the properties, use and curing and ageing of the oil paint system, despite all our physical and chemical knowledge and the availability of a wide range of analytical instruments and methods. This lack of understanding can be ascribed mainly to the complexity of the curing and ageing processes of oil paint systems and the large variety of parameters that play a role in these processes.

1.3 Degradation of linseed oil paint

Numerous factors like temperature, moisture, light and reactive species in the atmosphere or materials that have been added to the painting by the "conservator" during treatment influence the stability of an oil painting. Apart from the oil vehicle other components of a painting such as the varnish, pigments, and the support are subjected to deterioration. Yellowing crazing, and embrittlement of varnishes, fading, (dis)coloration or breakdown of pigments and destruction of the support are all unwanted processes observed in paintings [6]. The curing and ageing of the oil binding medium itself can lead to a large variety of defects of which the exact cause is most often unknown. This is due to the complexity of the different processes involved and unknown synergetic effects. Furthermore, both pigments and additives, such as driers or waxes, influence the onset of the different reactions. Actually, the whole process of curing, drying and ageing may be determined by the quality of the production process of the oil, which makes it very difficult to directly relate observation and cause. In a number of cases explanations on the ageing process of oil paints have been proposed to account for the observed defects. These ideas seem to make sense but unfortunately hard evidence is rarely available. Paint failure that has been observed includes yellowing, crack formation, wrinkling, Bénard cell formation, blooming,

blistering, flaking, and peeling [6]. Because all these defects are physical and/or chemical changes that relate to the chemical composition upon curing and ageing, it is necessary to study the evolution of the paint system on a molecular level. This thesis is intended as a scientific contribution in this area.

1.4 Analytical chemical research on linseed oil paints

Analytical chemical research on (model) oil paints and paintings by conservation scientists and chemists has been performed for at least five decades now. Initially, the application of scientific research was directed towards the identification of the type of materials that had been used. This research facilitated the authentication of the work of art and gave a better picture of the painting techniques used by the artist. Two other categories of scientific research related to (oil) paint systems are deterioration and environmental studies, and research into improved methods and materials for conservation.

Within the scope of the MOLART project several PhD. studies have been performed that can be placed into these last two categories: "Molecular studies of fresh and aged triterpenoid varnishes"[7], "Solvent extractable components of oil paint films" [8], and "Molecular changes in egg tempera paint dosimeters as tools to monitor the museum environment" [9]. Many references in these MOLART Reports will provide the reader with a good overview of the scientific studies that have been done on these subjects so far, including theoretical approaches of the problems encountered.

Traditionally, one of the most common analytical techniques used for the analysis of the oil-derived fraction of paints is gas chromatography, often combined with chemical work-up and derivatisation. The application of this technique in conservation science started in the '60s with the work by Mills [10], who identified the three different types of oils, linseed, walnut and poppyseed, used in Western European paintings based on the ratio of palmitic- (C16) and stearic acid (C18). This ratio is obtained with GC analysis after methylation of the hydrolysed oil paint. The C16 and C18 fatty acids were believed to be stable marker compounds in drying oil media. The P/S ratios for the three oils are so widely spaced that the range of values for each does not overlap significantly [11, 12]. It should be noted however that other traditional paint materials e.g. egg yolk, beeswax, and animal glues contain fatty acids as well, which can lead to a wrong classification of the type of oil used. More recently, Schilling *et. al.* quantified the fatty acid and glycerol content of oil paints after artificial ageing of test paints [13, 14] showing that the amount of C16 and C18 saturated fatty acids decreases unequally upon exposure to light and to a lesser extent upon exposure to heat. This was particularly true for the slow-drying paint formulations, thus compromising the validity of their P/S ratio. In a later study on the formation of so-called ghost-images[1] by this group it has been shown that there is unequal evaporation of C16

1 Ghost image: A hazy film of free fatty acids that has appeared on the inside of a protective glass layer in front of a painting.

and C18 fatty acids upon heating in an alumina crucible [15]. Schillings' data imply that the common practice of identification of the type of oil based on the P/S ratio may not be reliable. However, the authors realise that in a well-defined paint system the actual evaporation of saturated fatty acids, formed upon hydrolysis of the triacylglycerols of the oil, may be much slower, primarily because of the strong interactions with the oil matrix and/or pigments in the oil paint. This possible relation can also be deduced from a number of painting studies related to the formation of ghost images and blooming[1], which were thoroughly reviewed by Skaliks [16]. In general, it has been shown that slow-drying paint films have a tendency to produce ghost images or bloom. Pigments that have been shown to be involved are vine-black, alizarin, titanium dioxide and ochres [17, 18]. In addition to these observations, it has also been noted that paints made with a reasonable amount of white lead or verdigris, a copper containing pigment, hardly show the formation of ghost images or bloom. The variation in the relative amounts of the fatty acids deposited seems to be related to the ease of formation of metal salts. These observations indicate that these two phenomena may be strictly due to the quantity of free fatty acids present in the paint, which are thought to be affected by specific combinations of pigment, medium, and environment.

More detailed semi-quantitative information on the overall organic composition of aged paint samples by gas-chromatography has been obtained by Koller [19-21], who analysed the apolar-, polar-, triacylglycerol- and metal salt fractions in oil paints using a multiple extraction and derivatisation scheme. The paint sample was extracted with hexane, methanol (or another alcohol), chloroform and a solution of oxalic acid in methanol, respectively. These extracts were subsequently saponified, methylated and analysed by GC(/MS). In this way, a more detailed picture was obtained on the relative amounts of compounds formed upon hydrolysis, cross-linking and metal salt formation. Although the idea of separating the different fractions of oil-based paints prior to analysis is an improvement of the scientific examination of oil paint, there is some concern about the overlapping of the different classes of compounds extracted as already indicated by the author. GC and IR studies by Stolow [22, 23] on solvent extracts of pigmented and unpigmented paint films identified a non-cross-linked fraction, composed of (esterified) fatty acids and low-molecular weight degradation products formed upon oxidation, e.g. diacids and aldehydes. These authors also indicate the presence of a low-molecular weight cross-linked material fraction that could be present in the extracts, but could not be analysed with their analytical strategy. Erhardt and Tsang [24], Schilling *et. al.* [14] and Sutherland [8] focussed on the leachable components in oil paint films. Some of these studies also address the effect of solvents with different polarities on the paint films. These studies show that soluble fractions of numerous oil paint films consist of free fatty acids, and smaller amounts of monounsaturated fatty acids and fatty diacids. It was shown that esterified fatty acids are present in the form of acylglycerols. Simultaneous analysis of fatty acids, mono-, di- and triacylglycerols present in extracts with high temperature on-column GC/MS has been reported by Tumosa *et.*

1 Blooming: The deposition of free fatty acids on the surface of the painting after hydrolysis and migration from within the paint to the surface.

al. [25] . However, no oxidised acylglycerols were detected despite the age of the paint sample (18th century). In another recent study an extended range of (oxidised) mono-, di-, and triacylglycerols are identified in solvent extracts of relatively young oil paints by LC/MS [26]. These studies show that improvements in analytical methodology in the last decades are still expanding our knowledge on the various constituents in oil paints.

Very few studies are known in the literature that are dealing with the effect of removal of oil paint constituents by solvent extraction due to cleaning procedures of paintings. An extensive analytical study using GC-MS and scanning electron microscopy (SEM) has been performed by White and Roy [27] on a number of paintings ranging from the early fifteenth century to the end of the nineteenth century. The authors conclude that there is no evidence for the leaching of potentially plasticizing low molecular weight components from the cross-linked matrix as is the case for young paint test films when immersed in solvents. A similar study by Sutherland [8] on a number of paintings from the seventeenth to nineteenth centuries, however, has shown that for a number of paintings a small, but measurable proportion of fatty acids was found to be extracted. In other cases the effect was too small to be reliably determined [8] because of the rather large variability of the composition of the oil paint even in apparently similar paints. I would like to emphasise that the extraction of constituents due to solvent action, either by immersion or cleaning, will depend on the balance of the different processes known to happen within oil paint films, like oxidation, hydrolysis and the interaction of components of the binding medium with (inorganic) pigments.

1.5 Scope of the thesis

This thesis contributes to the determination and understanding of the internal chemistry of oil paint systems at the different stages in their lifetime. This is of fundamental importance for most aspects of the conservation and restoration of the works of art since the oil medium makes up the major part of the painting. The fate of every oil paint will be different due to the large number of variables that determine its quality and drying properties and due to the methods of paint modification and application by the painter. Although this may hamper a thorough and precise understanding of the detailed course of the life of oil paints, a general model has been proposed that describes the curing and ageing of oil paint [28, 29] based on previous analytical work and conscientious observations on oil paintings, which are available in literature. The work described in this thesis focuses on analytical chemical investigations of oil paint systems while using this model as a guiding principle.

The chemistry of traditional linseed oil begins with the method of preparation of the oil itself. The traditional manufacturing processes used for the oil production and work-up may lead to an alteration of the initial triacylglycerol composition. The relevant theoretical considerations are described in Chapter 2. This chapter also reviews the processes of chemical drying of oil and oil paint by

auto-oxidation and photo-oxidation. It examines the effects of addition of inorganic or organic pigments to the oil with a special focus on the resulting chemical composition as a function of ageing.

Different analytical methods used for investigation of the formation of degradation products and cross-linked materials upon processing of a number of traditionally prepared linseed oils are compared and evaluated in Chapter 3. These techniques include Fourier transform infra red spectroscopy (FTIR), size exclusion chromatography (SEC), high performance liquid chromatography atmospheric pressure ionisation mass spectrometry (HPLC-APCI-MS), direct temperature resolved mass spectrometry (DTMS), matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). The various 19th century methods for preparation of the oil are found to have a great influence on the composition of the oil used for making the paint.

After the paint is applied on a (primed) support the chemical drying of the viscous mixture starts due to the reaction of the unsaturated triacylglycerols with oxygen. Now the oil is changing rapidly, both chemically and physically, depending on both internal and external factors as will be described in more detail in the second part of Chapter 2. The outcome of all these processes can not be predicted beforehand for each individual artwork. Various analytical methods presented in Chapter 4-7 were developed to map the chemical condition of the complex oil paint system.

The combination of desorption/pyrolysis and mass spectrometry for the fingerprinting of linseed oil and paints derived from it is explored in Chapter 4. Direct temperature resolved mass spectrometry (DTMS) of relevant reference materials as well as cured and aged paint is investigated. The applicability, advantages and disadvantages of this relatively quick fingerprinting technique are discussed in detail, based on presented total ion currents and 16 eV ionisation mass spectra. The DTMS technique not only gives information on the relatively volatile non-cross-linked fractions within a paint sample but also on the presence of non-volatile cross-linked material and/or metal salts.

Apart from cross-linked systems, low molecular weight breakdown and hydrolysis products are formed within the oil paint due to oxidative cleavage of the triacylglycerols. This fraction has been characterised reasonably well in the past. However, often only a single analytical method was used, sometimes in combination with infrared analysis. The strategy mostly involved the hydrolysis of the complete paint sample, followed after wet chemical work-up by methylation of all liberated acid groups, and subsequent analysis with gas-chromatography/(mass spectrometry). Identification of extractable material from the oil paint was mostly performed in the same way. Because this approach may give an unrealistic reflection of the type of compounds that are present in the paint, we have investigated other analytical approaches as well to characterise the (non-volatile) non-cross-linked and cross-linked materials in more detail. The methods and results obtained are described in Chapter 5 and 6.

Chapter 5 describes the comparison of the analytical results obtained by on-column injection GC(/MS) analysis after a traditional wet chemical workup and by on-line Curie point pyrolysis GC/MS using in-situ (trans)methylation with tetramethyl ammonium hydroxide (TMAH). A variety of paint samples of different age, history and composition is analysed in order to investigate the chemical changes in the non-cross-linked fraction upon ageing. New compounds are identified on the basis of 70 eV electron ionisation mass spectra, isobutane chemical ionisation data and the additional data obtained with the derivatisation using deuterated TMAH.

The second part of Chapter 5 is a report on the formation of -methylated fatty acids as by-products of the use of TMAH as a (trans)methylation reagent in combination with Curie-point pyrolysis GC/MS.

Hydrolysis of the glycerol ester bonds within paint samples is addressed in Chapter 6. A validated two-step analytical method is presented suitable for the determination of the degree of hydrolysis. An extended range of oil paint samples was investigated with this method. A general relationship is established between the age of the oil paint and the progression of the hydrolytic processes.

Chapter 7 reports typical case studies in which the variety of analytical methods previously described in this thesis were used to obtain a better understanding of typical defects of oil paint systems. The first project described involves the problem of bloom formation, in combination with the formation of protrusions and ground staining, noticed on primed pieces of canvas by the American artist F. Church. The composition of the bloom and paint have been investigated in more detail using several mass spectrometric techniques, including secondary ion mass spectrometry (SIMS), GC/MS with on-column injection, and DTMS. An in-depth study on the exudation of organic crystals observed on some of the painted works of art by the American painter Frank Stella is described in the second part of this chapter. A number of analytical techniques have been used to identify the paint materials, including DTMS, GC/MS, and SEM. The crystals were identified as saturated C16 and C18 fatty acids derived from the binding medium. A mechanism to account for the bloom formation is proposed.

Chapter 2

Oil paint: developmental stages from an oil to a hard dry film

This chapter reviews the literature on the properties of oil, the chemical drying of oil and oil paint, the curing processes that take place when oil paint dries to a firm film and the ageing and degradation processes. It starts with a description of the production of raw linseed oil from seeds, the initial chemical composition and the subsequent refining and processing methods of the oil and how they affect the chemical composition. A detailed description of the chemistry of drying, the autoxidation and photoxidation processes, the effects of transition metals, and other factors that affect the composition of the oil constituents in oil paint are presented. The knowledge on the effect of paint formulation on the composition, including the role of the pigments in the curing and ageing processes as derived from a number of analytical studies on oil paints, is summarised. Degradation of oil paint systems, although less understood, will be discussed briefly in the last part of this chapter. A theoretical descriptive model of the development of fresh, cured and mature stage of linseed oil-based paint will be presented.

2.1 Production of linseed oil

The most important drying oils, walnut, poppy seed and linseed, are derived from seeds. Linseed oil, the largest volume drying oil, is obtained from flax, *Linum usitatissimum*. Flax is grown on different locations throughout the world in temperate zones located in the latitudes of roughly 30º to 60º nowadays. Nowadays the plant can be found on all continents: Argentina, Canada, Europe, India and the USA being major producers. The varieties can be divided into oleaginous flax (linseed) and textile flax (fibre flax). The conditions of farming and the effects of the climate will accentuate the differences between the two types. These factors are also important for the final composition of the oil that is obtained from the seeds. In general, the oil content of the seeds varies from 35 to 45 % of the dry weight.

Two methods, either a pressure method or a solvent extraction process are used to obtain the oil from the seeds of the flax plant. In both cases the seeds first have to be cleaned and separated from materials by subjecting them to processes such as blowing with air (to remove chaff, etc.) or screening (to remove particles of appreciably different size). In a next step, the seeds are ground to a fine meal, which facilitates the oil extraction. The meal then can be heated in a kettle prior to pressing, a process called "cooking". The principle objective of this heat treatment is to coagulate the proteins in the walls of the oil containing cells and make the cells permeable to the flow of oil. At the same time, the lowered viscosity of the oil at elevated temperatures also assists the flow of the oil from the seeds.

These basic principles – reduction of the size of the seeds, the heat treatment, and pressing- have remained the same over the centuries, with the difference that in the modern oil production process after the first pressing often a extraction with an organic solvent is applied to increase the yield of oil [30-32].

2.2 The composition of linseed oil

The properties of the drying oils depend on their chemical composition. By far the largest proportion of the oil constituents are triacylglycerols, triesters of glycerol (1,2,3-propanetriol) with mixtures of fatty acids. The general structure of a triacylglycerol (TAG) and some fatty acids are depicted in Figure 1. The typical fatty acid composition of the three drying oils used in traditional oil paints shown in Table 1 points out that there are only a limited number of fatty acid moieties involved. Doubly and triply unsaturated C18 fatty acids make up the largest fraction. The reactivity of a drying oil with oxygen is a result of the reaction with these C18 non-conjugated unsaturated fatty acids, i.e. fatty acids with double bonds that are separated by single methylene groups (see also Figure 1). The range of fatty acid compositions of linseed oils is depicted in Table 2 [33, 34]. It is clear there are differences in the oil compositions dependent on its source. Besides the 5 major fatty acids smaller amounts of saturated (C12, C14, C20, C22 and C24) and unsaturated fatty acids (C16:1, C20:1) can be found in linseed oils.

Table 1. Typical fatty acid compositions of the three most common drying oils used for traditional oil painting [12, 34].

	Fatty Acid (% of total FA)				
Oil	Palmitic[a]	Stearic	Oleic	Linoleic	Linolenic
Linseed	4-10	2-8	10-24	12-19	48-60
Poppyseed	9-11	1-2	11-18	69-77	3-5
Walnut	3-8	0.5-3	9-30	57-76	2-16

[a] These are the common names. The systematic names are hexadecanoic-, octadecanoic-, (9Z)-octadec-9-enoic-, (9Z,12Z)-octadeca-9,12-dienoic-, and (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid, respectively. Short-hand notation C16, C18, C18:1, C18:2, and C18:3 FA, respectively.

Table 2. Fatty acid distribution in different linseed oils [33, 34]

Oil	Fatty Acid (% of total FA)				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Europe	4-6	2-3	10-22	12-18	56-71
Russia	6-7	3-6	15-23	14-19	49-60
Canada	5-6	3-4	19-20	14-16	54-61
India	9-10	7-8	10-21	13-15	50-61
Argentina	4-5	5-6	19-21	15-24	45-53

TAGs are composed of three (un)saturated fatty acids. A large number of possible combinations exist. Kartha and co-workers investigated the TAG composition of linseed oil and compared the values obtained with two theoretical FA distributions, based on the "even" and "restricted random" distribution theories [35]. The numbers obtained agreed very well with the latter. They found the oil to be composed of 5% GS_2U, 29 % GSU_2, and 66% GU_3. G stands for the glycerol backbone, S is a saturated fatty acid and U an unsaturated fatty acid. For example, GS_2U is a TAG composed of two saturated FAs and one unsaturated FA. The research showed that all TAGs of linseed oil contain of at least one unsaturated fatty acid moiety.

Other (semi-)quantitative studies on the linseed oil composition using capillary gas chromatography (CGC), reversed phase high performance liquid chromatography (RP-HPLC), direct chemical ionisation mass spectrometry (DCI-MS) [36] or direct inlet mass spectrometry (DI-MS) [37] have given a more exact picture of the ratio of the different TAGs present. The data are presented in Table 3. Each TAG identified is represented by a three-letter code, e.g. POL. These three letters represent the individual fatty acids (P=palmitic, Ln=linolenic, L=linoleic, O=oleic, and S =stearic acid).

Oils are classified as drying oil, when they chemically dry to form solid films on exposure to air; semi-drying oils when they form tacky, that is, sticky, films; and non-drying oils, which do not form highly viscous material upon exposure to air [38, 39]. Furthermore, oils can be differentiated as non-conjugated and conjugated, depending on whether the double bonds are separated by a methylene group or not. Tung oil is an example of a drying oil with a high amount of conjugated C18 fatty acids (-eleostearic acid). These acids are even more reactive with oxygen and account for the fast drying of tung oil. In the case of non-conjugated oils a useful empirical relationship is given by the drying index, which is greater than 70 for drying oils. This is calculated as follows:

Drying index = (% linoleic acid) + 2(% linolenic acid)

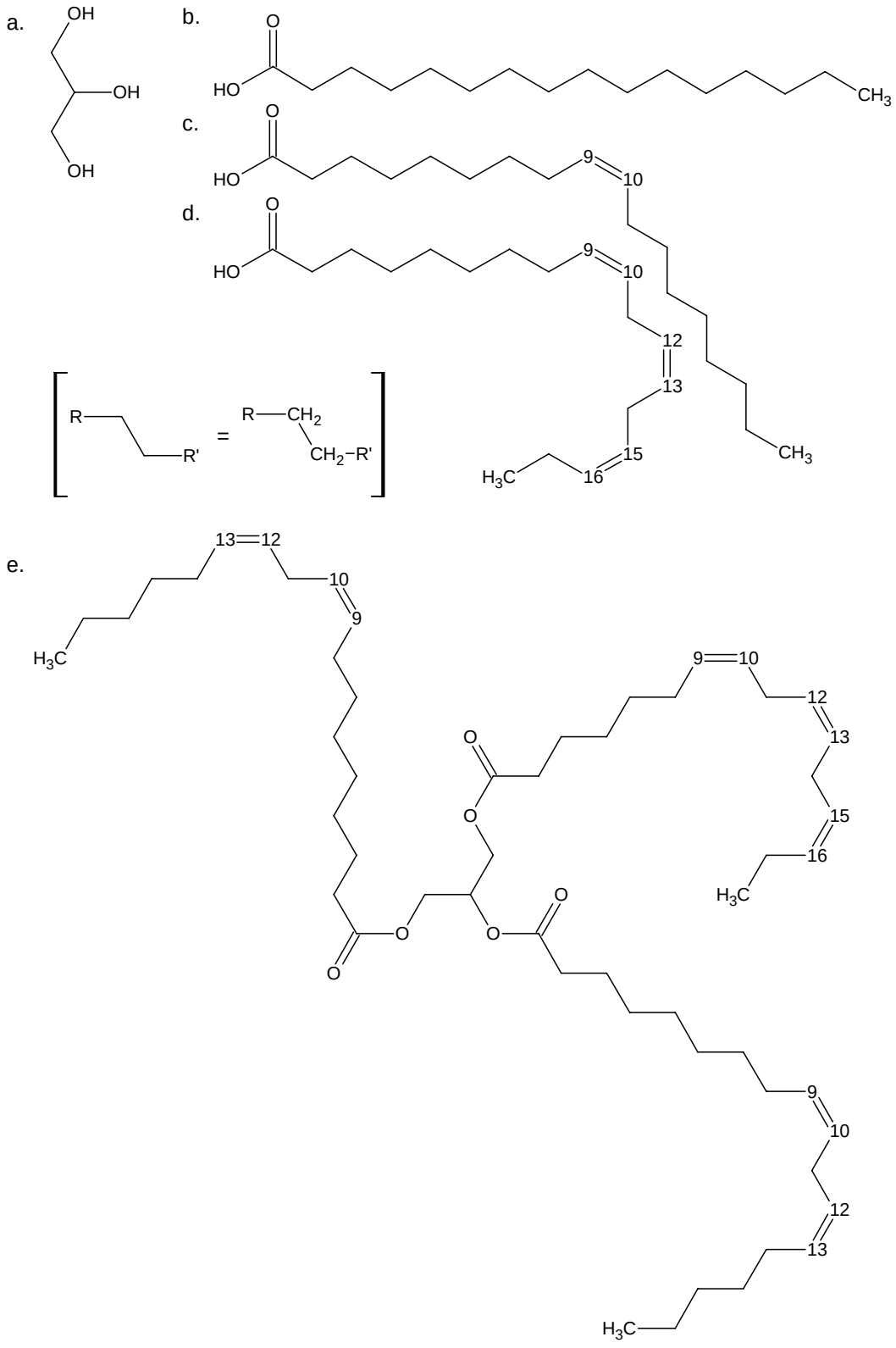


Figure 1. Constituents of a drying oil. (a) glycerol; (b) palmitic acid (C16); (c) oleic acid (C18:1); (d) linolenic acid (C18:3), and (e) a triacylglycerol with C18:2-C18:2-C18:3 fatty acid moieties (TAG).

Another way to classify the oil according to their composition is based on the iodine value (IV). This is the number of grams of iodine required to saturate the double bonds of 100 g of oil. Some authors define oils in the following categories [39]:

drying oils: IV > 140

semi-drying oils: IV=124-140

non-drying oils: IV < 125.

The iodine value can also be calculated, using the formula: 1.16IV=3.04(%linolenic acid) + 2.02(% linoleic acid) + (% oleic acid).

Table 3. Relative composition (%) of triacylglycerols from linseed oils

TAG[a]	CGC [36]	RP-HPLC [36]	DCI-MS [36]	DI-MS [37]	Number of double bonds	MW
PLnLn	5.2	4.2	4.3	8.5	6	850.7
PLLn	4.6	3.1	3.2	5.3	5	852.7
PLL	5.6	0.6	0.7	4.8	4	854.7
POL	10.7	0.8	1.0	1.1	3	856.8
PSLn	n.r[b]	n.r				
POO/PSL[c]	n.r.	n.r	n.r.	0.7	2	858.8
PSO	n.r.	n.r.	n.r.	0.5	1	860.8
LnLnLn	8.6	50.7	49.3	14.2	9	872.7
LLnLn	5.8	19.2	18.9	11.9	8	874.7
LLLn	5.9	3.6	13.0	20.0	7	876.7
OLnLn	12.9	9.2				
LLL	4.8	2.4	5.6	14.9	6	878.7
OLLn	7.6	3.2				
OLL	8.1	1.4	2.9	10.6	5	880.7
SLLn	4.2	0.8				
OOL	5.6	0.5	0.8	3.7	4	882.8
OOO	10.4	0.3	0.3	1.6	3	884.8
SOO/SSL	n.r	n.r	n.r	0.6	2	886.8
SSO	n.r	n.r	n.r	0.4	1	888.8

[a] Coding system used to indicate the three fatty acyl groups of TAGs: P=palmitic, Ln=linolenic, L=linoleic, O=oleic, and S =stearic acid

[b] n.r. = not reported

[c] These TAGs have the same molecular weight (MW) and cannot be differentiated with the method of analysis.

However, these values can only serve as a general specification of the quality and are actually misleading as an oil classification tool. A “fresh” linseed oil that has been oxidised to a certain extent upon standing or during processing will give lower IV values due to reduction of the double bonds, and hence could be classified wrongly.

TAGs are the main components of the oil, but there are much smaller quantities of other materials present. Some of these can have a marked effect on the drying properties of the oil [34, 35, 40]. Free fatty acids are always present, with an average composition reflecting the constituents of the TAGs. These are naturally found in the seeds or formed upon hydrolysis of the original TAG ester bonds. The proportion is commonly ranging from 0.5- 2 % of the total weight, but it may be much higher, depending on the identity of the oil, how it was obtained, and its history [41].

Water present naturally in seeds will also dissolve in the oil, although only in a small portion, usually about 0.1-0.2 %. The presence of phosphatides and mucilaginous materials is typical for all vegetable oils. The best-known species is lecithin, a phospholipid known for its ability to function as an emulgator. The oils contain a varying amount of this material, normally up to about 1% [40].

Other classes of compounds that are found in freshly pressed oils are included in the non-saponifiable fraction. This fraction includes colouring materials like beta-carotene or chlorophyl and several sterols. For linseed oil around 0.2-0.4 % of phytosterols are present of which the two major compounds are brassicasterol ($C_{28}H_{46}O$) and stigmasterol ($C_{29}H_{48}O$). Part of the colouring agents is thought to be oxidised and cause the more brownish colour of the oil. Besides these species, trace amounts (0.01-0.1%) of normal and branched paraffins, waxes and triterpenic alcohol (0.15 %) have been identified as well, with squalene as one of the major compounds. An important class of constituents found is the tocopherols (around 0.1 %). These are the natural protectors of the vegetable oil because of their anti-oxidising properties. Their presence therefore will have a noticeable influence on the drying process. The same applies for traces of numerous metals that can be found in the crude drying oil [42], and which are usually bound to organic compounds. Especially iron, copper, manganese and lead are to be mentioned in this respect since these metals are potent oxidation catalysts. Zinc and cadmium are other metals that have been found.

2.3 Refining and heat processing of linseed oil

Once the oil has been obtained it has to be purified in order to prevent drying problems later on. This requirement was already recognised by the craftsmen and a number of different recipes have been documented that were used to remove protein rich material. At the same time different recipes were available to obtain clearer oils [30, 43]. All materials that have a negative influence on the

additional processing should be removed as well. This is of importance in particular when a linseed oil is to be heated. A hazy suspension usually coagulates upon steadily heating of the oil at a temperature between 200 and 260 ˚C. This cannot be completely redissolved in the oil even after prolonged heating. When the insoluble material starts to separate, the oil is said to break and the material is called the break. The break consists of the phosphatides and other mucilaginous material and is thought to settle in combination with the action of water present in the oil. When a vegetable oil is to be heated above 150 ˚C the break has to be removed [30, 44, 45].

Just as was the case for the production process, the methods of refining have not dramatically changed over the centuries [45, 46]. The easiest method is washing with water, which leads to the precipitation of the proteinaceous material and free fatty acids, a method already known in the 16th century. Besides the removal of the unwanted material, the oil also became lighter in colour, which was considered advantageous and essential. Lightening of the oil is also ascribed to the effect of sunlight that was often not excluded from the oil during refining. A second method involves cooling the oil by frost in combination with water or snow. Besides precipitation of mucilaginous material, waxes and the more saturated TAGs or FAs were “frozen” out as a consequence of the cold. Several other varieties of washing principles are known as well in literature. The most important one is the simultaneous addition of a slightly acidic liquid like vinegar or sulphuric acid, comparable to acidic refining of oils nowadays [45]. This destroys the phosphatides and a large proportion of the colouring matter. A disadvantage is that free fatty acids can be formed, but these can be removed by water washing afterwards. A similar refining process is known using alkali like caustic soda or sodium hydroxide, which will also remove most of the break. Part of the fatty acids will be lost as well due to formation of soaps. In general the amount of free fatty acids will be lower compared to acid refined oils. Therefore, acid refined oils have much better pigment dispersion properties, as the free carboxylic acids are thought to react with the pigment surface [47-49].

The bleaching of the oil already has been mentioned. At present this is done with Fuller’s earth or activated carbon, which leads to the adsorption of the coloured pigments [39, 46]. In the early days of oil manufacturing standing in direct sunlight for a prolonged period was the most common practice, either alone or in combination with the addition of an absorbing material like breadcrumbs. It was also anticipated that the drying process was accelerated upon sun irradiation as a result of the increased uptake of oxygen. In order to prevent skin formation the oil had to be shaken regularly. This also meant that the oil thickened, which was not always an advantage. Standing under exclusion from air was therefore more commonly applied starting from the 17th century [30].

There are a lot more refinery recipes known from the literature but most of these are more or less based on the same principles. It may be worth mentioning that the effect of certain metal containing materials on the drying rate of the oil was already know at least since the 15th century. Recipes speak of oils that are allowed to stand in bowls made of zinc, copper or bronze during bleaching, which could have facilitated the drying of the oil later on [30]. At the same time the addition of

materials like lead white (basic lead carbonate, Pb_2CO_3 $Pb(OH)_2$), yellow- and red lead oxide (PbO and Pb_3O_4, respectively), calcium oxide (CaO), zinc sulphate ($ZnSO_4$) or umber (containing iron- and manganese oxides) have been reported to have a positive effect on the drying rate [30].

Apart from refining, linseed oil can be processed to obtain oils with different properties. In general this was done to increase the viscosity of the oil (pre-polymerisation) and/or to shorten the drying time. Two methods are applied: simple heating and air blowing. It suffices here to mention only the most common methods [39, 48, 49]. Unfortunately, the exact conditions of the more traditional heat treatments are often not known, other than that oxygen was excluded or introduced intentionally or that indications of (end) temperatures are given, e.g. the oil should boil gently, should be heated over a slow fire or should be of such a temperature that a piece of bread, onion or garlic turns brown [30]. Reconstructions of these old recipes, however, have given us a better insight into the oil production process and the relevant parameters. In the older heat bodying processes, the oil was heated in open, directly fired kettles which led to a considerable degree of oxidation, and accumulation of acidic materials by thermal cracking. Furthermore, the composition of the kettle wall could also have a major effect on the final product. Nowadays the heating process is completely controlled [48]. The present vocabulary that is being used may give rise to some confusion, especially since the historical terminology used before the 20th century is different. The types of oil that will be described in the next paragraphs are at present known as blown-, boiled-, and stand oil.

2.3.1 Blown linseed oil

This type of oil is produced by blowing air through the oil. The main characteristic of this oil is that no driers are added to the oil. Therefore the blowing usually has to be continued for a longer period until there is a pronounced rise in the viscosity. At the same time the oil is mildly heated to a temperature in the range of 40 to 150 ºC. The exact changes that occur during blowing are complex and depend on various factors including temperature, exposure time, amount of air passed and so on. These factors will vary from one manufacturer to another. The oil usually is a clear brown or light brown fluid with a typical “oxidized” smell.

2.3.2 Boiled linseed oil

The term “boiled linseed oil” is generally understood to be an oil to which metal salts of organic acids are added, apart from the deliberate introduction of air. Originally, the raw linseed oil was heated to a temperature of 150 ºC with metal oxides, carbonates or acetates, with or without the bubbling of air through the oil. The evolution of vapours of water, carbon dioxide or acetic acid led to the term

"boiled oil", because of the similarity in appearance to boiled water. During the treatment of the oil the viscosity increases slightly as does the acidity.

2.3.3 Stand oil

Heating to higher temperatures to increase the viscosity of linseed oil produces stand oil. The temperatures involved usually vary between 270 and 310 ^{o}C. The consequence of the high temperature is that a rather large amount of volatile and partially inflammable decomposition products will be formed. In open pot stand oils a current of air is directed over the surface to remove these fumes. On the other hand closed pot stand oils also are made. In this case the access of oxygen from the outside atmosphere is prevented. The fumes that arise may condense on the lid and drip back into the oil and so give it a high acid value. The open pot stand oils are usually slightly darker compared to closed pot oils due to increased levels of oxidation. The conversion of linseed oil into stand oil causes important chemical changes, although the product is still a drying oil.

These are the basic oil processing methods in use since the beginning of the fifteenth century. Pure oil may not always have been used but mixtures of oils are also expected to have been processed. In more recent production processes other types of driers are being employed, such as lead or cobalt salts of naphtenic acid (naphtenates; a mixture of acids of high molecular weight obtained from petroleum crudes) or 2-ethyl hexanoic acid (octoates). Furthermore, materials like sulphur dioxide or anthraquinone and some of its derivatives have been added until recently to increase the rate of bodying [48]. The increase in the degree of conjugation of the oil is thought to be sufficient to explain their effect. Another range of materials that can be mixed with drying oils are resins to produce oil-varnishes. The effect of this addition is that the drying rate of the oil will increase. Both natural resins like dammar, copal or kauri resins, and synthetic resins such as ester gum (glycerol esters of rosin) and phenolic resins have been used for this purpose [50].

It can be concluded that many of the principles of traditional oil refining and heat processing methods are still used today. It is fascinating that craftsmen were able to develop suitables oil for painting by trial and observation. It would be interesting to see whether analytical research on fresh and aged oil paintings can help identifying how craftsmen and artists treated their oil prior to paint manufacturing. It is expected that a number of the previously described processes will give rise to specific types of materials within the fresh oil paint due to chemical processes. This will be discussed in the next part of this chapter. The question remains, however, whether chemical changes within the oil upon processing still will be recognisable after curing and ageing of the actual oil paint.

2.4 Autoxidation

The most important chemical reaction taking place during the production and processing of drying oil, and the curing and ageing of the oil paint is a process in which oxygen from the atmosphere reacts spontaneously with the unsaturated (esterified) fatty acids of the oil. This process is generally referred to as autoxidation. The overall effect of this reaction on the liquid TAGs of drying oils is that they will start to cross-link to form higher molecular weight material. Ironically, degradation resulting in smaller molecules will take place at the same time.

The basic overall reaction is the incorporation of molecular oxygen into the unsaturated fatty acid: $RH + O_2 \rightarrow ROOH$, with RH being the substrate, an unsaturated fatty acid (R) with a removable hydrogen (H) and ROOH the newly formed hydroperoxide [51, 52]. An important characteristic of autoxidation is that it is autocatalytic. Once the process has started the rate is to increase as the reaction progresses. At the start the rate is often so slow that there seems to be an induction period, because the rate is too small to be quantified. This behaviour is typical for radical-chain mechanisms. Comparable to other radical chain reactions the autoxidation can be divided into three separate steps: initiation, propagation and termination, as shown here:

Initiation:	$In\bullet + RH \rightarrow R\bullet + InH$	(1)
Propagation:	$R\bullet + O_2 \rightarrow RO_2\bullet$	(2)
	$RO_2\bullet + RH \rightarrow ROOH + R\bullet$	(3)
Termination:	$RO_2\bullet + RO_2\bullet \rightarrow ROOOOR$	(4)
	$RO_2\bullet + R\bullet \rightarrow ROOR$	(5)
	$R\bullet + R\bullet \rightarrow RR$	(6)

Autoxidation of unsaturated lipids is affected by many factors, some of which increase and some of which decrease the autoxidation rate. The effect of any given factor depends on the reaction conditions, so that no factor can be classified exclusively as anti-oxidative or pro-oxidative. In general, the rate of autoxidation increases with increasing reactivity of the autoxidizing material, with increasing concentrations of the reactants (the number of active sites and concentration of oxygen), and as a result of physical factors like an increase in temperature or irradiation, but especially by increasing the rate of the initiation reactions. This is mainly done by factors that increase free radical concentrations, such as UV light or transition metals. On the other hand, the reaction rate can be suppressed by such factors as a decrease of the number of reactive sites, the decrease of partial oxygen pressure, or lower temperatures. The most important, however, is the reduction of the initiation rate by a decrease of the number of free radicals capable of chain initiation. This is done with so-called anti-oxidants.

2.4.1 Initiation reactions

The initiation, which is the key event of the whole process, is difficult to define, because of the very small quantities of radicals involved. These can be formed by thermal or photochemical homolytic cleavage of the RH bond or by hydrogen abstraction by an initiator free radical. The direct reaction of oxygen with the substrate is not likely to be very important for the initiation, since the activation energy of such a process is thought to be too large.

The production of the radical R from the substrate RH can take place by process (7).

$$RH + M^{N} \rightarrow R\bullet + H^{+} + M^{N-1} \quad (7)$$

The higher oxidation state of the metal ions is depicted as M^{N} and the lower oxidation state as M^{N-1}. The oxidation state of iron, copper, manganese, nickel and cobalt are known to change through this single electron transfer [53]. Furthermore, lead with oxidation state IV can react stoichiometrically with RH to give lead II species [54]. Whether these reactions are important for the initiation depends on the bond dissociation energy of R-H. The categories of reactions that involve ROOH as the initiator include those that consist of ROOH only (8) and those that employ a metal-ion catalyst (9,10).

$$2ROOH \rightarrow RO\bullet + ROO\bullet + H_2O \quad (8)$$

However, there is some doubt about the validity of this pathway (8) as the initiation process. Whatever the exact mechanism may be, it is clear from kinetic studies that two molecules of ROOH are involved. Competing processes catalysed by metal ions make it very difficult to study the initiation in lipid autoxidation studies. Metal ions are usually present in these systems, which causes the processes to have lower activation energies. Two radical producing reactions are possible which involve metal ions and ROOH. In both reactions the starting metal ions are in a different oxidation state. Their relative importance varies with the metal and other factors such as substrate, or coordination chemistry of the metal, but in general reaction (9) is much faster than (10). The metals that are most effective (cobalt, lead, and manganese) all have in common that the higher valency is the least stable, apart from the fact that they can exist in at least two valency states [55, 56].

$$ROOH + M^{N-1} \rightarrow RO\bullet + OH^{-} + M^{N} \quad (9)$$

$$ROOH + M^{N} \rightarrow ROO\bullet + H^{+} + M^{N-1} \quad (10)$$

These two reactions are interactive so the overall effect of the metal ion will be to produce radicals ROO•, a peroxyl radical, and RO•, an alkoxy radical, from ROOH. This metal-catalysed process is autocatalytic in the sense that the product is involved in the initiation of new reactions via a feedback mechanism. Soluble metals salts are most useful for this form of catalysis. Three different classes of drier catalysts are known: primary, secondary and auxiliary driers. The primary driers act during the oxidation. Co^{2+}, Mn^{2+}, and Fe^{2+} for example belong to this class. Pb^{2+}, Zr^{4+}, and Al^{3+} are secondary driers and active for polymerisation. Ca^{2+}, K^{+}, and Zn^{2+} are auxiliary because they modify the activity of the primary driers [57, 58].

All these mechanisms have been derived from studies conducted in relatively simple chemical model systems. The metal catalysis of the autoxidation of natural oils, of which the composition already is difficult to reproduce and control precisely, will not be as uncomplicated as the classical mechanisms suggest. Both valence forms of metals, for instance, have a catalytic activity, and the factors affecting the balance between direct initiation or “reinititation” by hydroperoxide decomposition are poorly understood. In summary, our present knowledge on the autoxidation of unsaturated lipids, can be described as a metal-catalysed reaction, which consists of an unspecified, but most probably metal-catalysed, initiation reaction (minor) followed by an autocatalytic phase (rapid).

2.4.2 Propagation reactions

The propagation steps (2,3) observed in the autoxidation process might be rather complicated and not as simple as depicted. In general, the next steps in the free radical chain process include radical coupling with oxygen, atom or group transfer, fragmentation, rearrangement, and cyclization [59]. Molecular oxygen is a ground state triplet with two unpaired electrons; consequently, molecular oxygen is a biradical and the reaction with an organic free radical is essentially a radical-radical coupling (2). The abstraction of hydrogen by the peroxyl radicals from the organic substrate can be classified as an atom transfer reaction (3). The third type of process that occurs in the autoxidation sequence is the -fragmentation of the peroxyl radicals, which is basically a reversal of the oxygen coupling to the free radical. This event is known to occur when R is a stabilised radical. The rearrangement mechanism as depicted in Figure 2a is also thought to start with the -fragmentation of the peroxyl radical to give an allyl radical-dioxygen caged pair [60] .The last reaction is essentially an intramolecular ring addition and yields 5- or 6-membered rings as is shown in Figure 2b. This peroxyl radical cyclisation is an important process in the autoxidation of FAs or their esters with at least three double bonds.

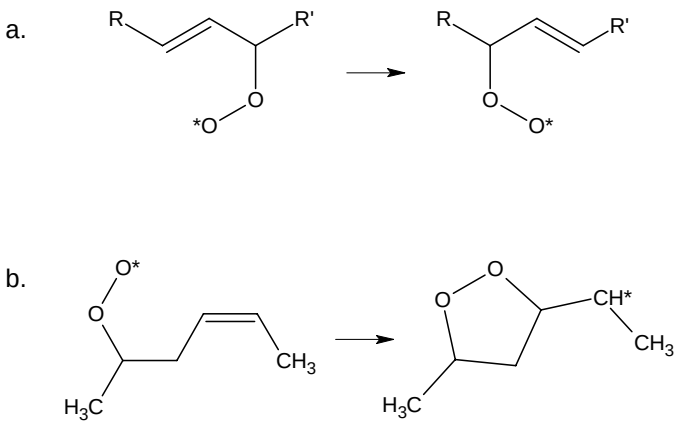


Figure 2. Rearrangement of a (a) hydroperoxide radical, and (b) a hydroperoxide cyclisation reaction.

2.4.3 Termination reactions

Just as was the case for the initiation process, termination reactions can also be divided in those involving organic free radicals only, and those in which metal ions play a role. Initially, when the autoxidation has just started, the most likely recombination under normal conditions is that of two ROO• peroxyl radicals to give an intermediate tetroxide, ROOOOR. This is not likely to be a very stable structure and it was shown to give rise to a ketone, a secondary alcohol and molecular oxygen [61, 62]. However, other recombinations will occur in time as well and give rise to different types of links between the acylglycerols and/or fatty acids. Initially, this will lead to the formation of dimers of TAGs and/or their hydrolysis products. Since almost all TAGs of linseed oil contain more than one unsaturated FA, oxygen can be incorporated on more than one position. As a consequence, when autoxidation proceeds, higher oligomers are formed. This will lead to an increase of the average molecular weight and viscosity of the oil, and a dried film will be the result.

Chain termination by metal ions is thought to be of equal importance as discussed by Ingold and co-workers [63-65]. They propose the reaction of a transition metal in its lower oxidation state and a peroxyl radical (11) to form stable end-products.

$$ROO\bullet + M^{N-1} \; : ROO\text{-}M^{N} \qquad (11)$$

These inhibitory effects are thought to result from oxidation and reduction of free radicals by iron and copper or from metal complexation of free radicals by cobalt. High concentrations of metal ions are in certain cases expected to be responsible for a more pronounced induction period. Inhibition will take place at a

concentration that will depend on factors such as the metal, its coordination chemistry, and the relative concentration of unsaturated fatty acids and oxygen.

Another reaction (12), which is not to be excluded also, has been reported by Kochi and was shown to be very fast [66]. It involves the higher oxidation state of copper, Cu^{II}.

$$RCH•CH3 + Cu^{II} \rightarrow RCH=CH_2 + H^+ + Cu^{I} \quad (12)$$

This implies that Cu is capable of competing with oxygen for R•, preferably at low oxygen concentrations, and can act as an effective chain terminator.

2.4.4 Anti-oxidants

Another class of species which was shown to have inhibitory effects on the rate of autoxidation are -. (and -) tocopherol, and -carotene, both components found in fresh vegetable oils. The first compound donates hydrogen of one of its OH groups to the peroxyl radical, ROO•. The rate of this transfer is four orders of magnitude faster than the propagation rate for hydrogen transfer from hydrocarbons to peroxyl radicals. The products of such a transfer are a tocopherol radical, Toc• and a lipid hydroperoxide (13). This Toc• can subsequently react with another peroxyl radical leading to chain termination (14) [67, 68], which makes tocopherols such good anti-oxidants.

$$ROO• + TocH \rightarrow ROOH + Toc• \quad (13)$$

$$ROO• + Toc• \rightarrow \text{non-radical products} \quad (14)$$

Natural phenolics such as flavonoids, which are used as organic pigments in oil paints can influence the drying process. Organic pigments that have been shown to inhibit the autoxidation included quercitin, and myricetin [69, 70]. The -carotene, Car, has been shown to inhibit oxidation *via* a singlet oxygen-quenching mechanism (15) [71, 72].

$$Car + {}^1O_2 \rightarrow {}^3Car + {}^3O_2 \quad (15)$$

2.4.5 Primary oxidation products of drying oils

2.4.5.1 Monoene lipids

It was postulated already in 1943 that 4 different hydroperoxides of oleic acid, a monounsaturated FA, would be formed upon autoxidation. There would be an equal oxidation probability for the 8, 9, 10, and 11 position, and the double bond would remain at the original position (9-10) or to shift to the two adjacent positions (8-9 and 10-11, respectively) [73]. The hydrogenated trimethylsilylated derivatives of these hydroperoxides really were characterised for the first time in 1977 by Frankel et al. [74] using gas chromatography in combination with mass spectrometry. The 8- and 11-hydroxy isomers were consistently present in higher amounts. It was also found in this study that the temperature at which autoxidation takes place, is an important factor in determining the relative ratio of the different isomers. A few years later, in 1984, ^{13}C-nuclear magnetic resonance spectroscopy (^{13}C-NMR) was used to determine the conformation of the double bond after oxidation of methyl oleate at 25 ˚C. The isomers were quantified in combination with GC/MS. The 8-OOH isomer was present in a percentage of 26.4% (ratio *cis/trans*=14.1:12.3), the 11-OOH in 26.6% (13.7:12.9), the 9-OOH in 24.2% (1.1:23.1), and the 10-OOH was found in 22.8% (1.1:21.7) [75]. Almost similar numbers were found by Porter [76] in a later study. Based on these results a mechanism was proposed to account for all products observed, which is depicted in Figure 3. In order to form the 11-*trans* and 8-*trans* compounds, a (2,3)-peroxyl rearrangement of the 9-*trans* peroxyl and 10-*trans* peroxyl group, respectively, has to occur. In the case that a good hydrogen donor is present with a C-H bond of sufficiently low dissociation energy the so-called “kinetic” allyl peroxyl radicals would be trapped before this rearrangement. Therefore, no “thermodynamic” products (11- and 8-*trans*) are being formed. The free radical nature of the hydroperoxide rearrangement is supported by the fact that it’s catalyzed by free radical initiators or light. Furthermore, the rearrangement has been shown to be inhibited by phenolic antioxidants [77].

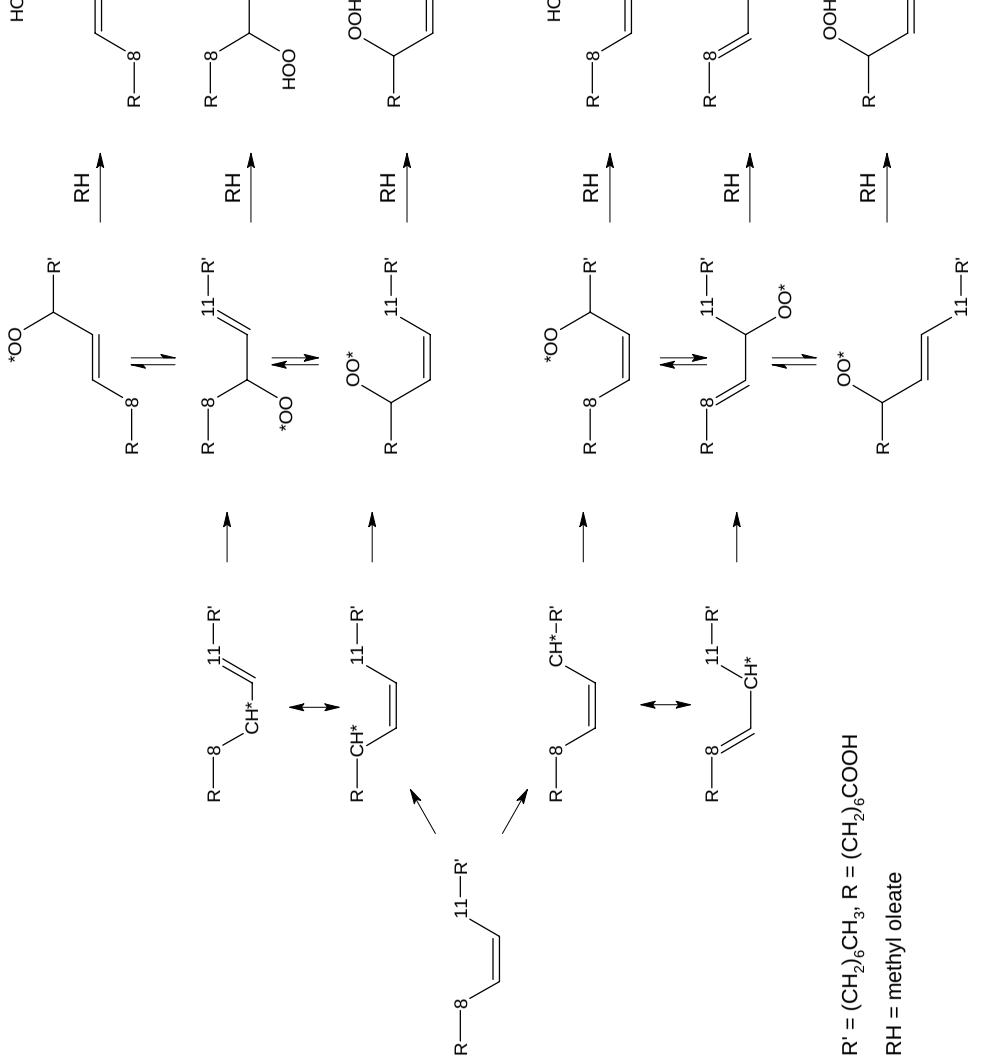


Figure 3. Formation scheme of the primary oxidation products of oleic acid.

2.4.5.2 Diene lipids

The autoxidation of doubly non-conjugated unsaturated FAs is by far the most studied lipid system. The four major primary products of the autoxidation of methyl linoleate are depicted in Figure 4 [78]. These conjugated dienes are formed after hydrogen abstraction from the bisallylic 11-position, followed by isomerisation and incorporation of oxygen on the 9- or 13-position (See Figure 5) [60, 79-81]. Two of the products have a *cis, trans* conjugated diene system while the other two display *trans, trans* geometry. The abstraction of the bisallylic hydrogen at the 11-position is much more favoured compared to the abstraction at the 8- or 14-position since a resonance stabilized pentadienyl radical is formed, as indicated by the rippled bonds in Figure 5. However, trace amounts (1-1.3%) of 8- and 14-hydroperoxides have also been observed upon autoxidation of linoleic acid [82, 83]. The relative rate of oxygenation of linoleic acid is about 25-fold higher compared to the autoxidation of the monounsaturated FA, but it should be noted that the final product distribution depends on both the temperature and the concentration of linoleic acid. At higher temperatures, -fragmentation pathways will compete with H-atom transfer reactions and more *trans, trans* products will be formed (thermodynamic conditions). The increasing concentration of the H-donor will favour the formation of the *trans, cis* species (kinetic conditions). The product distributions found vary between 1 and 42% for the four isomers [84].

In a recent study by Brash [85], also up to 10% of 11-hydroperoxide could be identified, but only when the autoxidation was carried out in the presence of -. tocopherol. The relatively unstable bisallylic peroxyl radical, normally formed under autoxidation conditions, is not transferred to the more stable conjugated diene peroxyls, which would produce the 9- and 13-hydroperoxide products, but are trapped to give a stable bisallylic hydroperoxide.

2.4.5.3 Triene lipids

The formation of hydroperoxides from fatty acids with more than one methylene-interrupted double bond system, such as linolenic acid, starts basically in the same way as for the doubly unsaturated lipids. However, the relative autoxidation rate of linolenic acid is about 3 times that of linoleic acid. Once more, the abstraction of reactive bisallylic hydrogen is the first step. Two positions are available: the 11- and 14-position. In a similar way as described in the previous section, the autoxidation will proceed and eight major primary oxidation products are formed: the 9-, 13-, 12- and 16-OOH species each present as *cis, trans* and *trans, trans* isomers. However, the outer isomers, 9- and 16-OOH are formed in larger quantities than the inner isomers, 12- and 13-OOH (31, 46, 11, and 12%, respectively) [86]. The explanation for this phenomenon is that new types of oxidised products are formed upon cyclisation and further oxygenation of the 12- and 13-OO• compounds. The 9- and 16-OOH are isolated in higher quantities because their peroxyl radicals are less reactive in these type of reactions [87]. Some examples of these reaction products will be given in the next paragraph.

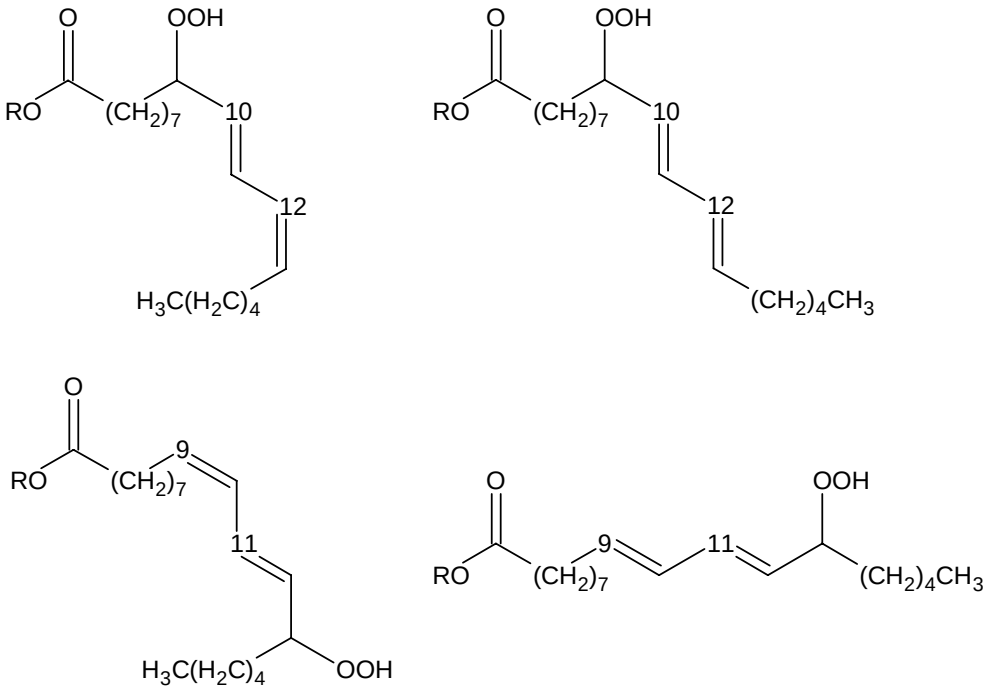


Figure 4. Primary oxidation products of linoleic acid.

2.4.6 Secondary oxidation products

Once an unsaturated hydroperoxide FA is formed it can react with a second oxygen molecule to produce either dihydroperoxides [88-90] or monocyclic hydroperoxides [91-93]. It is not necessary to discuss the mechanism of formation of these products here, since the basic principle is the same and has been presented already in the previous paragraphs. Some examples of multiple hydroperoxides that have been identified are depicted in Figure 6. Other products that will be formed from these secondary autoxidation products after cleavage of the peroxides include unsaturated species with one or more epoxy, oxo or hydroxy functional groups [82, 93, 94].

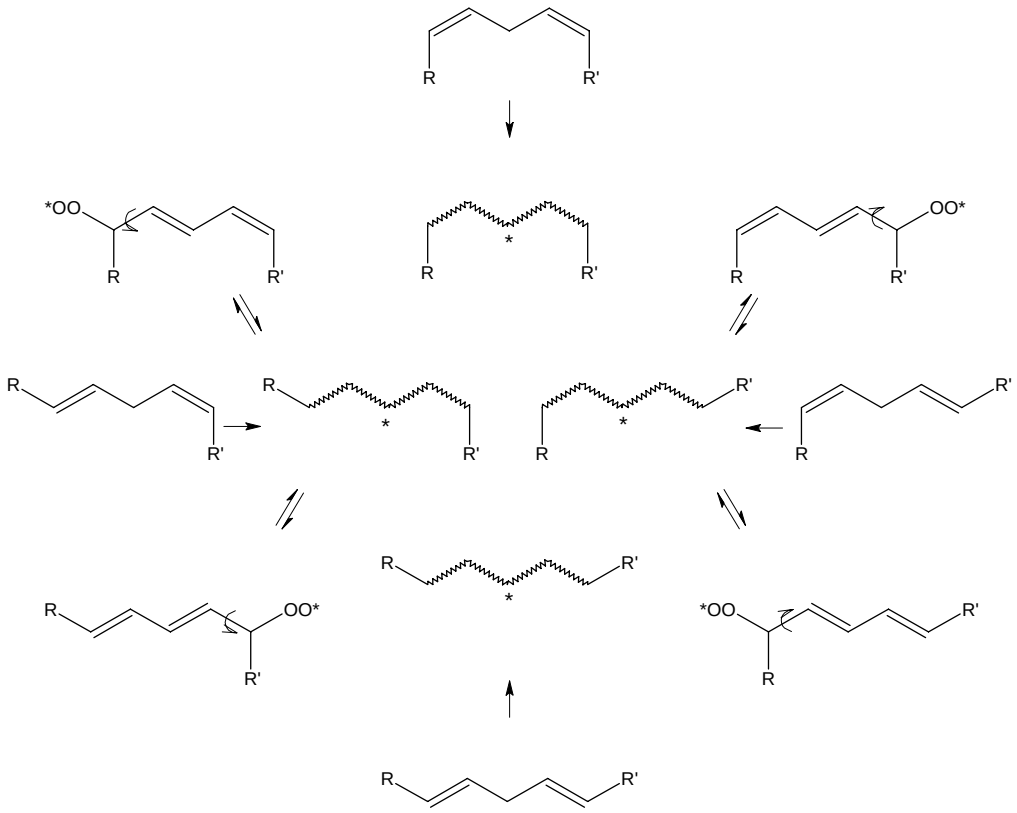


Figure 5. Formation of *trans,trans* and *trans,cis* isomers upon autoxidation of linoleic acid.

The situation is different for monohydroperoxide TAGs since these species can still have unreacted fatty acyl moieties. It has been shown for both trioleoyl and trilinoleyl that after the incorporation of the first hydroperoxide at low peroxide values (18<PV<28), the second and third hydroperoxide are introduced into the two remaining fatty acyl moieties giving rise to bis- and trishydroperoxides. No hydroperoxide was added to the same fatty acyl chain after formation of the first hydroperoxide. Furthermore, it has been shown that no preferential autoxidation occurs between the 1(3)- and 2-triacylglycerol position [95, 96]. Trilinolenoylglycerol behaves differently since at low peroxide values the most important secondary oxidation product is an epidioxy group, which is introduced at the same fatty acyl moiety where the initial hydroperoxide is present. Small amounts of bis- and trishydroperoxides were detected only at higher peroxide values (PV>31). At even higher values (PV>75) minor secondary products like hydroperoxy bicyclo endoperoxides and mono-dihydroperoxides are formed [97].

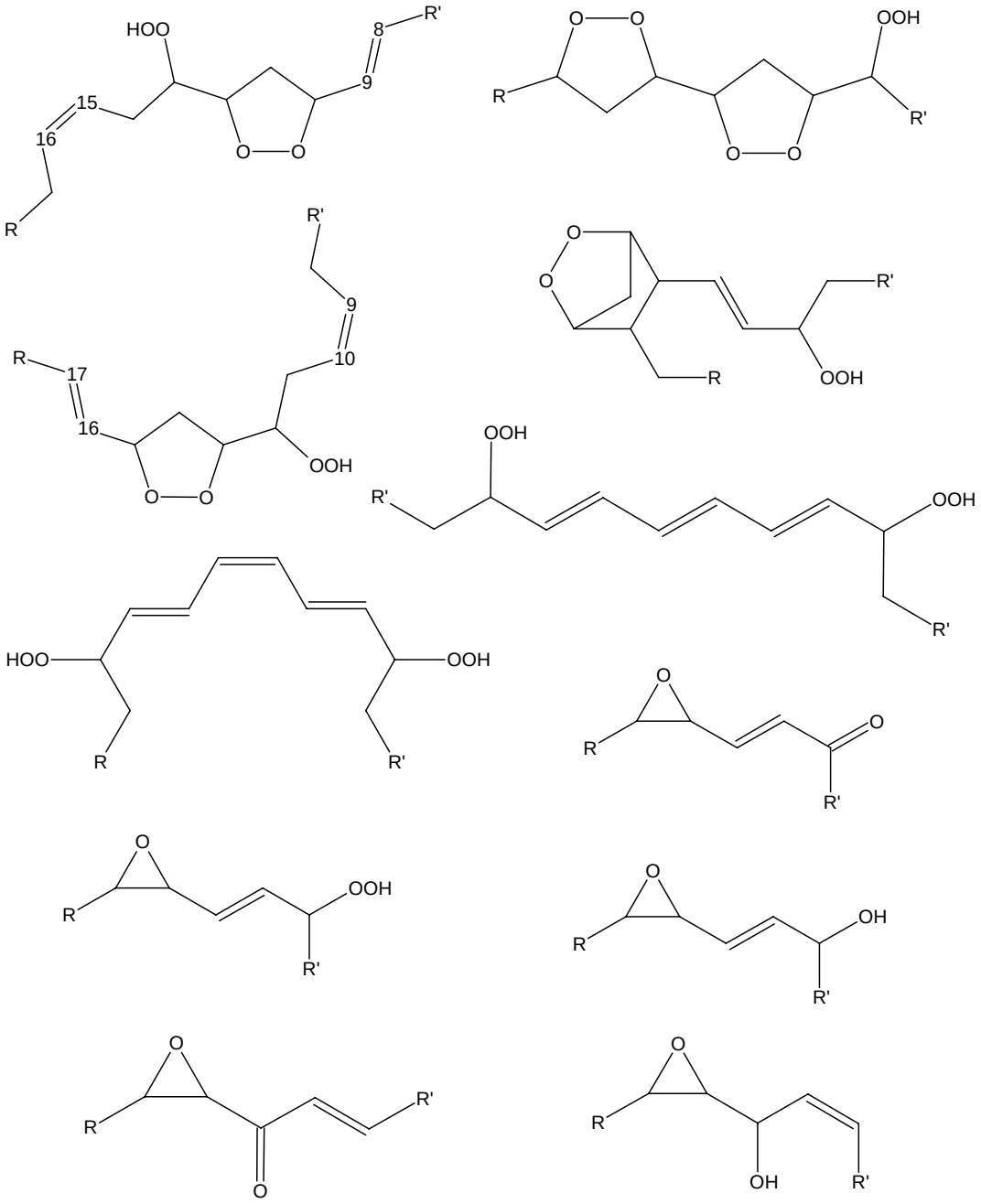


Figure 6. Various secondary oxidation products of unsaturated fatty acids.

The primary and secondary oxidation products of pure model FAs and TAGs can be separated and characterised relatively easy with a combination of wet chemical pretreatments and different analytical techniques like gas- and high performance liquid chromatography, mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy [98-102]. However, mixtures of oxygenated natural TAGs are difficult to analyse, especially when oxidation has proceeded beyond secondary oxidation products. A rather large variety of homologues and of regio- and geometric isomers is formed, which tend to overlap with each other and with unoxidized analogues. In principle, it should be possible

to identify these overlapping species by combining a chromatographic technique with mass spectrometry. In order to prevent the decomposition of oxidation products like hydroperoxides a mild ionization method like electrospray should be used. Several examples of this analytical method have been published for the oxidation products of relatively simple model TAGs [103-105]. The results show that chromatograms are already very complex but that identification is possible based on single ion monitoring. The analysis of (oxidised) TAGs of oils processed according to traditional recipes will be described in Chapter 3 of this thesis.

2.5 Photoxidation

Photochemical processes can also initiate the oxidation process. Although the mechanism of production of radicals and the subsequent reaction with oxygen is somewhat different, once the process is initiated and hydroperoxides have formed, it follows much the same pattern as (metal-catalysed) autoxidation reactions. Once hydroperoxides are formed, they actually can compete in the initiation process with the processes described for autoxidation.

In order to understand the mechanism of photoxidation a more precise picture of oxygen is needed. Ground state oxygen is a triplet $^{3}{}_{g}^{-}$ state ($^{3}O_2$), with two unpaired electrons with parallel spins. The direct reaction of triplet oxygen with singlet organic molecules is a spin forbidden process. The first excited state $^{1}{}_{g}$ has anti-parallel electrons paired and is not a free radical. The energy difference between the ground state and the first excited state is 94,7 kJ. This last state has a vacant molecular orbital and can therefore accept electrons, i.e. electrophilic, so it can react with the double bonds of the unsaturated FAs. A second excited state also exists, $^{1}{}_{g}^{+}$, with unpaired anti-parallel electrons and a higher energy level (158 kJ) [106].

When discussing singlet oxygen usually the first excited state is meant, also indicated as $^{1}O_2$. For this activation to occur, so-called sensitiser molecules must be present such as dyes like methylene blue, rose bengal, etc, or colouring materials like chlorophyl or porphyrin [107]. Upon absorption of light energy, these molecules are converted into an excited singlet state (^{1}Sens*). Now the sensitiser can emit fluorescent light and return to the ground state or it can react by a process called Intersystem Crossing to form ^{3}Sens*. This triplet sensitiser can react via two mechanisms: Type I and II. The first pathway involves the formation of a free radical and gives identical products compared to the normal autoxidation. However, in case of the latter mechanism, ^{3}Sens* reacts with $^{3}O_2$ to give singlet oxygen which can directly attach to unsaturated compounds [72]. Note that in this latter mechanism free radical intermediates are not involved and hence, inhibition of initial reactions by anti-oxidants is not possible.

For the simplest unsaturated fatty acid, oleic acid, this photoxidation mechanism implies that only two positional hydroperoxide isomers are formed, 9- and 10-OOH, compared to four isomers in the case of autoxidation [108]. For the polyunsaturated fatty acids, the situation is reversed and a larger number of photo-

oxygenated species can be found [109]. An important difference between the photoxidation and autoxidation is the rate of reaction. For the three most investigated unsaturated fatty acids, oleic-, linoleic-, and linolenic acid, the approximate relative rates are depicted in Table 4. Other numbers have been published in the past that show much larger differences between the relative reactivity, e.g. 18:1-18:2-18:3 = 1:120:330 [110].

Table 4. Relative rates of oxidation of unsaturated fatty acids [34]

	18:1	18:2	18:3
Autoxidation	1	27	77
Photoxidation	30000	40000	70000

2.6 Condensation reactions

Hydroperoxides are not stable under the conditions encountered in an oil paint and therefore they will homolyse to give ROO•, RO• and R• radicals. A spontaneous homolysis is not likely, however. The removal of an H-radical requires a rather large bond-dissociation energy of 90 kcal/mol. Therefore the H-radical is almost always removed by reaction with another radical to form a higher energy bond. The dissociation of a hydroperoxide to form an alkoxy- and hydroxyl radical is half as large, but nonetheless facilitated by either a catalyst or conditions such as light or heat. The formation of alkoxy (RO•) radicals is essentially an irreversible process and once formed, the alkoxy radicals are transformed into end-products. In this way, the alkoxy radicals differ from the peroxyl radicals, which can become hydroperoxides again by H-abstraction or participation in reversible -scission processes.

Radicals can recombine to form cross-linked compounds. One of the mechanisms already encountered is the Russell mechanism, which describes the recombination of two peroxy radicals. Part of the formed tetroxides is thought to decompose to give dimers linked by a peroxide bond as was proposed by Schieberle [82].

$$2\ ROO\bullet \;:\; RO\bullet O_2\bullet OR \;:\; ROOR + O_2 \qquad (16)$$

However, an alternative pathway to explain the dimer formation may go via -scission and radical recombination (17) or the direct recombination of R• and ROO• (18) or 2RO• (19). A definite answer to this question has not been given yet and more research is required before the importance of these reactions can be established.

$$ROO\bullet \;:\; -O_2 \;:\; R\bullet \;:\; +ROO\bullet \;:\; ROOR \qquad (17)$$

$$R\bullet + ROO\bullet \;:\; ROOR \qquad (18)$$

$$RO\bullet + RO\bullet \;:\; ROOR \qquad (19)$$

Direct intermolecular addition of free radicals to double bonds is another important route to cross-linked material. A high degree of polyunsaturation, the presence of O_2, and the lack of anti-oxidants promote this reaction. The addition leads to the formation of a mixture of dimers, trimers, and higher oligomers, However, the structural details of these compounds are mostly unknown.

A number of authors have presented evidence for the formation of dimeric- and even trimeric materials in autoxidizing lipids [111-117], but the exact nature of the type of cross-link always has been a point of discussion [118, 119]. Peroxide cross-linked material was formed upon low-temperature autoxidation of unsaturated fatty acids [118]. The cross-linking of methyl oleate in an anaerobic environment gave ten different carbon-carbon linked dimers as expected from free radical chain termination theory [120]. Upon autoxidation of methyl linoleate the formation of peroxide cross-linked dimers during the initial stage of autoxidation has been proven by Miyashita and co-workers [121, 122]. The dimers were cross-linked via the 9,9'-, 9,13'- or 13, 13'-carbons, and in some of these compounds an additional hydroperoxide or hydroxy group was incorporated. It was discovered in the aforementioned studies that the polymerisation didn't progress beyond the stage of dimers and trimers. Recent studies on the cross-linking mechanism of ethyl linoleate, however, not only demonstrated the formation of oligomers up to pentamers but also identified the cross-linking mechanism [123-125]. It was clearly shown that recombination of two R•'s led to oligomeric material. Oxygenated oligomers were also identified although it was not clear whether the oxygen was included in the cross-link or accommodated on the chain. Evidence for both carbon-carbon (minor) and oxygen cross-linked material however was presented. The exact geometry of two dimers was revealed in a mechanistic study on both (*Z,Z*)- and (*E,E*)-3,6-nonadiene as model compound for linoleic acid. Both an ether- and peroxide linked dimer was positively identified [93]. Although a single and pure compound was used in this study, to limit the large number of possible isomers with very similar physical properties, the isolation of these dimers already required considerable effort. Studies by the same authors on the cross-linking of conjugated fatty acids revealed another cross-linking mechanism for these type of compounds, namely the addition of radicals to the double bonds [124].

2.7 Formation of low molecular weight material

Not only high molecular weight material is formed upon autoxidation but a multiplicity of low-molecular weight volatile compounds is formed at the same

time. These compounds have a low odour and taste threshold and therefore are easily detected as a rancid flavour. The typical acrid after smell of curing drying oil paint is an example of this phenomenon. Alkoxy radicals, RO•, are formed due to the homolytic cleavage of the hydroperoxides. These evolve by fragmentation of adjacent C-C and C-H bonds to produce aldehydes, ketones, alkyl and vinyl radicals which in turn react with H• and OH•, and produce aldehydes or (un)saturated hydrocarbons. This is depicted in Figure 7. This scheme has been proposed on the basis of work by a large number of authors that studied the (thermal) breakdown of primary and secondary oxidation products of lipid material [89, 126-131]. An extensive review of this work has been given by Labuza [132], Nawar and Witchwoot [133], Frankel [86, 134], and Grosch [135]. The qualitative and quantitative differences that were found for the different lipid model systems investigated are to a large extent the result of differences in the reaction conditions. Furthermore, the simultaneous presence of still intact monohydroperoxides that can degrade to form volatiles upon analysis leads to variation in the results. To circumvent this problem, autoxidations have been carried out at high temperatures, under conditions where labile peroxides are converted to more stable substances [136]. Even today, the off-flavours formed upon (thermal) autoxidation of lipid materials in food is still a field of great interest and a lot of questions are still open due to the complexity of these systems [137-140]. The effect of different metals, or anti-oxidants on the formation of volatiles shows a dependence on the type and amounts of products formed [141-143].

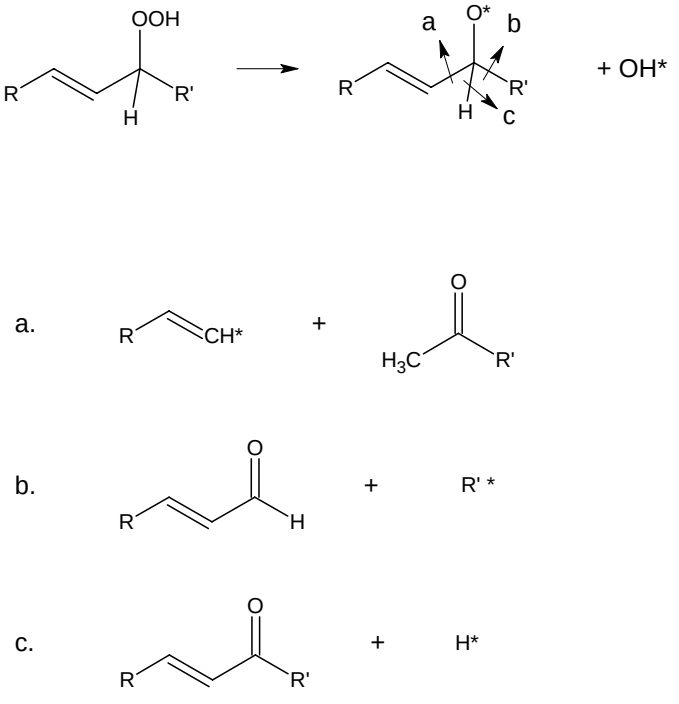


Figure 7. Schematic drawing of the decomposition pathway of a primary hydroperoxide.

An overview of almost all volatiles detected in the different model studies performed at different temperatures is given by Grosch [135]. The most important ones are summarised in Table 5.

Once the volatile compounds are formed most of these will be lost from the autoxidizing material by evaporation. However, the more polar compounds like the short chain oxo-fatty acids can be retained within the oxidizing material. These can turn into a carboxylic acid upon further oxidation of their aldehyde group to form a so-called diacid (20). These are relatively stable end-products of the autoxidation [144].

$$2HOOC\text{-}R\text{-}CH{=}O + O_2 \;:\; 2HOOC\text{-}R\text{-}COOH \qquad (20)$$

Table 5. Main volatile compounds of different lipid materials (see [135] for literature references)

Lipid material	Compounds
methyl oleate, oleic acid, trioleyl glycerol	octanal, nonanal, 2-decanal, 2-undecenal, heptane, octane, methyl 9-oxononanoate, methyl 10-oxodecanoate
methyl linoleate, linoleic acid, trilinoleyl glycerol	hexanal, 2-heptenal, 2-octenal, 2-nonenal, 2,4-decadienal, pentane, methyl octanoate, methyl 8-oxooctanoate, methyl 9-oxononanoate,
Methyl linolenate, linolenic acids, trilinolenyl glycerol	propanal, 2-hexenal, 2,4-heptadienal, 2,4,7-decatrienal, methyl 9-oxononaoate

In case that proteins are present, as can be the case when egg based materials have been added to the drying oil, part of the (non-)volatile aldehydes or hydroperoxides can react with amino groups, especially lysine, to form a Schiff base (21) [145-148].

$$R\text{-}CH{=}O + R'\text{-}NH2 \;:\; R\text{-}CH{=}N\text{-}R' \qquad (21)$$

However, it has been shown by Braddock [149] that this type of reaction only occurs after the oxygen uptake had ceased. Prior to that, radical formation and amino acid destruction occurs. It was concluded that radicals are the major cause of reactions with proteins and not the aldehydes. As a consequence free radicals may be induced in the proteinaceous material, which may in turn results in

dimerization or polymerization. Another typical volatile oxidation product of unsaturated lipids known to react with proteins is malondialdehyde. This reaction (22) also can lead to cross-linking of the proteins.

$$O{=}CH\text{-}CH_2\text{-}CH{=}O + 2R\text{-}NH_2 \;:\; R\text{-}NH\text{-}CH{=}CH\text{-}CH{=}NR \qquad (22)$$

Other products of the reaction of malondialdehyde with proteins have also been described [150].

2.8 Heat-induced chemical changes in drying oils

Apart from oxidation, heating is another important factor leading to alteration of the TAGs and their physicochemical properties. This is in particular important when the oil has been heat treated during refining or processing. There are three main processes that can change to fatty acyl moieties of non-oxidized unsaturated lipids upon heating: hydrolysis, *cis-trans* isomerisation, and cyclisation.

The first process is hydrolysis of the glycerol ester bonds and the formation of di-, and monoacylglycerols, glycerol and free fatty acids [133, 151-153]. The higher the content of water in the oil, the more severe this process will be [154, 155]. The formation of these free fatty acids not only alters the acid value of the oil but also enhances the rate of oxidation as has been shown experimentally [156-158]. It is thought that the free carboxylic acid group of the FAs catalyzes the decomposition of a small amount of the hydroperoxides formed in the initial stage of autoxidation although a precise mechanism has not been proposed.

The second process that occurs is *cis-trans* isomerisation of the double bond systems. The natural lipids have double bonds that are non-conjugated with a *cis* configuration. The *cis*-configuration, however, is easily formed into a *trans*-configuration, especially in the presence of free radicals. Upon heating an equilibrium is obtained between both forms. For example, the equilibrium mixture for a C18:1 fatty acid, oleic acid, contains at least 2/3 of the *trans* configuration (elaidic acid) [159]. *Trans* linoleic- and linolenic lipids can be similarly prepared although this process is complicated by the ease of conjugation (transformation of the 1,4-diene to a 1,3-diene system), especially at elevated temperatures. This is nicely shown by Martin and co-workers who identified eight isomers in trilinolenin after heating to 240 ˚C [160]. The *cis-trans* isomerisation is in this case accompanied with a migration of the double bonds.

The conjugation leads furthermore to a series of other transformations: intermolecular cyclization, giving rise to cyclic fatty acids or Diels-Alder additions. This last type of reaction leads to either intramolecular cyclic compounds or dimerization due to intermolecular reactions. Examples of the

intramolecular cyclization of unsaturated lipids have been given by a large number of authors [161-171]. A good and comprehensive review is available from Sebedio and Grandgirard [172].

These studies show there are two major classes of cyclic fatty acids formed upon heating of both 18:2 and 18:3 containing lipid material. Cyclic C18 FA compounds have been identified containing saturated or unsaturated cyclic rings of five or six atoms, as depicted in Figure 8. The same major cyclic FAs were found under low and high temperatures. Differences were only observed in their relative proportions and amounts in the heated oils. This is mainly determined by the nature of the polyunsaturated FAs present in the original oil. The 5-carbon-membered ring is mostly found in oils with relatively high amounts of linoleic acid, whereas the 6-carbon-membered ring is only present in trace amounts. These fatty acids contain one residual double bond, either in the ring or on the chain. Heating linolenic acid rich oils gives an equal amount of both 5- and 6-cyclic FAs. Two of the original double bonds are retained in this case. Surprisingly, hardly any reports are available in which the formation of aromatic C18 fatty acids is shown, although their formation can be expected, especially when acid or base catalysed processes can occur [166, 173]. Compound e in Figure 8 is an example of such a product.

The primary reactions underlying the thermal polymerisation of TAGs, the bodying of the oil, consists of the formation of a conjugated system in either linoleic- or linolenic acid groups [174], followed by a Diels-Alder addition to form unsaturated six-membered rings. In conventional terminology, this is the 1,4-addition of a diene with a double bond. Such a reaction may take place between two fatty acyl groups belonging to different TAGs, which lead to dimerisation of the TAGs. This process is depicted in Figure 9a. On the other hand, the Diels-Alder addition may occur between two fatty acyl groups that are part of the same TAG. This obviously will not lead to polymerisation (Figure 9b). However, in a next step the remaining fatty acyl chain of the TAG may undergo a Diels-Alder addition with a fatty acyl chain from another TAG, which does lead to the formation of polymeric material. The formation of dimers and higher oligomers upon heating of unsaturated fats and oils has been investigated extensively and a large number of different reaction products has been identified [152, 153, 175-181]. An extensive literature overview on the different reaction mechanisms and reaction products of the thermal dimerisation processes of the unsaturated fatty acid esters has been given by Figge [182].

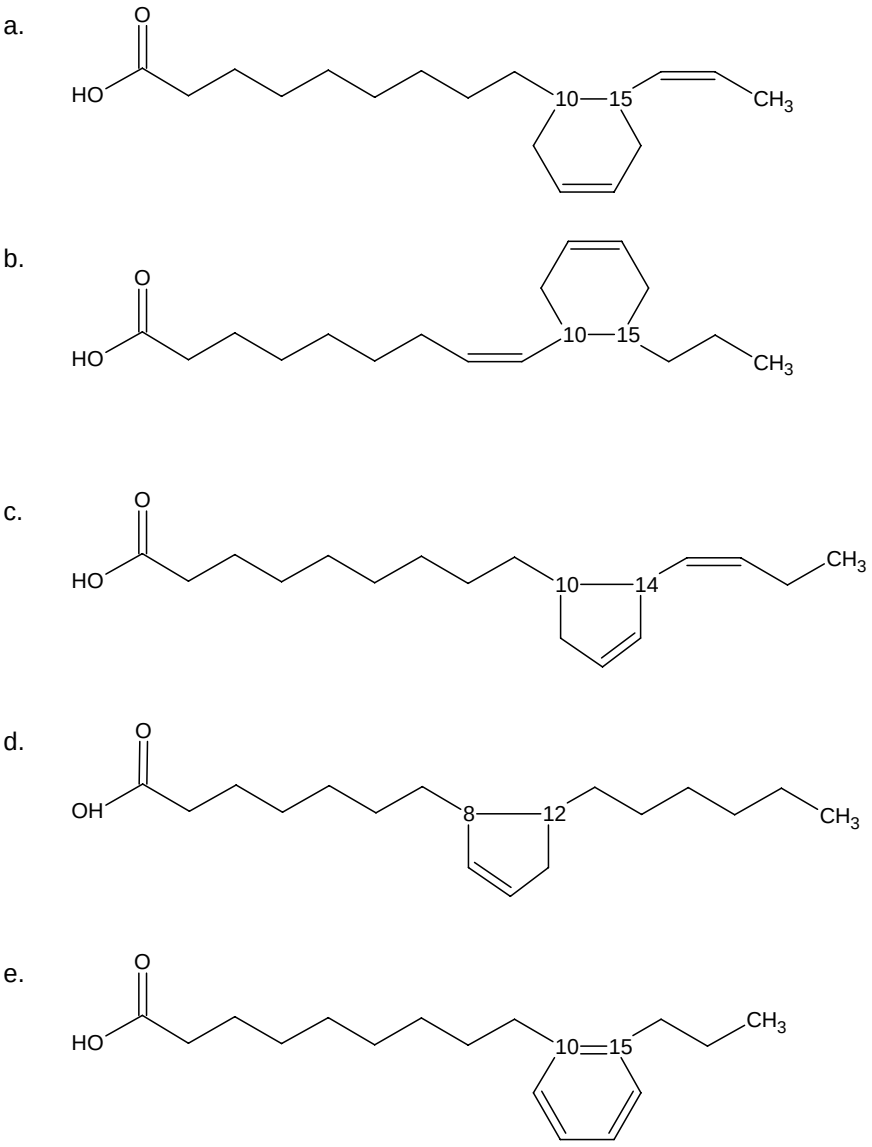


Figure 8. Typical cyclic fatty acids formed upon heating of unsaturated fatty acids.

Besides the formation of dimeric products, other substances can be formed due to thermal breakdown of acyl chains on both sides of the double bonds. Due to the fragmentation, free radicals will be formed that pick up hydrogen and part of the products already encountered in the breakdown of the hydroperoxides will be formed: alkanes, alkenes and short chain (unsaturated) fatty acids [133, 155]. Formation of these compounds is accompanied by the formation of TAGs of lower molecular weight relative to the starting material. On the other hand free radicals may recombine and new species can be formed with increased molecular weights.

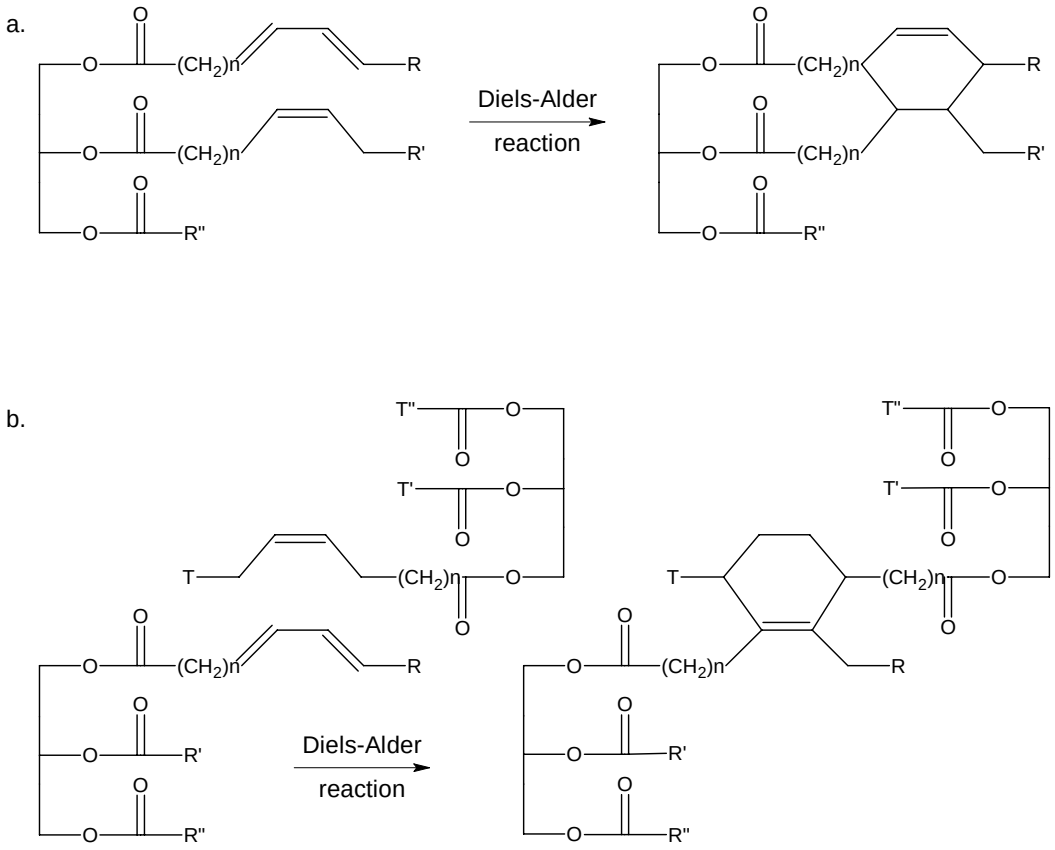


Figure 9. Diels-Alder cyclisation of (a) two fatty acyl moieties within one TAG. and (b) dimerisation of two TAGs via a Diels-Alder cyclisation.

The previously described processes are all occurring when an oil is heated under exclusion of oxygen. The presence of atmospheric or dissolved oxygen during the heating process is responsible for an oxidative alteration as well. Again this will give rise to free radicals, followed by the formation of hydroperoxides. The kinetics of the autoxidation process will be enhanced due to the increased temperature, which speeds up the hydroperoxide decomposition and subsequently leads to a large number of reactive free radicals. This will lead to more polar (oligomeric) material, with hydroperoxide, epidioxide, hydroxide, epoxide and carbonyl groups as well as ether and peroxide cross-links [153, 182-187]. Above a temperature of 100 ˚C, the hydroperoxides are not stable and mostly ether linked or other oxygenated polar compounds will be formed [184]. The formation and breakdown of the different radicals leads to similar decomposition products as observed for the normal autoxidation process [133, 188, 189]. The saturated lipids present in the oil are considerably more stable than the unsaturated analogues under normal conditions. However, when heated to temperatures above 150 ˚C in the presence of oxygen, they can also undergo oxidation giving rise to a range of decomposition products including homologous series of fatty acids, 2-alkanones, alkanals, alkanes and 1-alkenes [133, 151, 190]. The types of products formed are the same when different temperatures are compared. However, higher amounts of

decomposition products are formed at higher temperatures. When mixtures of saturated and unsaturated lipids are heated, the decomposition products observed arise from the unsaturated material. Rather than attacking the methylene groups of a saturated chain, the alkoxy radicals are more likely to abstract the hydrogen from the (bis)allylic systems. The unsaturated material therefore acts as an antioxidant inhibiting the oxidation of the saturated chains. Non-oxidative reactions however, will not be significantly influenced by the unsaturated lipids.

The question remains to what extent thermal reactions of unsaturated lipids in an inert atmosphere are influenced by traces of oxygen or by hydroperoxides already present or whether the lower level of oxygen in the heated lipids will lead to a change of the free radical mechanisms. Both the thermal and oxidative thermal reactions can be of a radical nature, which will proceed simultaneously in the oxidative-thermal treatment. If only limited amounts of oxygen or hydroperoxides are present in the reaction system, it is likely that the autoxidation processes will come to a standstill. The formation of apolar dimeric and oligomeric products will prevail as soon as oxygen-containing species are consumed and have been incorporated into unreactive autoxidation products [176, 191].

The quantitative differences observed for lipid mixtures at different temperatures of heating are not easy to explain. Hydroperoxide decomposition and secondary oxidation reactions occur at extremely rapid rates and at a given time during processing there will be a net balance between a number of factors. Hydroperoxide structure, temperature, degree of autoxidation, and the stability of the reaction products themselves surely will influence the final reaction products.

Summary and relevance for paintings

It is clear from the previous descriptions of the different processes involved in (thermal) autoxidation of lipid materials, even when they are pure reference materials, that the actual course and end-products of the reactions are very complex and depend on a variety of factors, including temperature, light, oxygen pressure, the balance of anti- and proxidative compounds, and the presence and amount of catalysts like (transition) metals. The fact that there are many puzzling and often contradictory claims in literature underlines the difficulty of analysis and complete understanding the autoxidation process. To illustrate the basic processes Gardner [192] made a hypothetical figure (Figure 10), displaying the autoxidation of a polyunsaturated lipid as function of time. This figure is a good representation of the complexity of the autoxidation event. A number of processes are proceeding at the same time and some of these processes will be synergetic, whereas others will inhibit each other. This will lead to complex kinetics and a large number of different low and high molecular weight oxidation products.

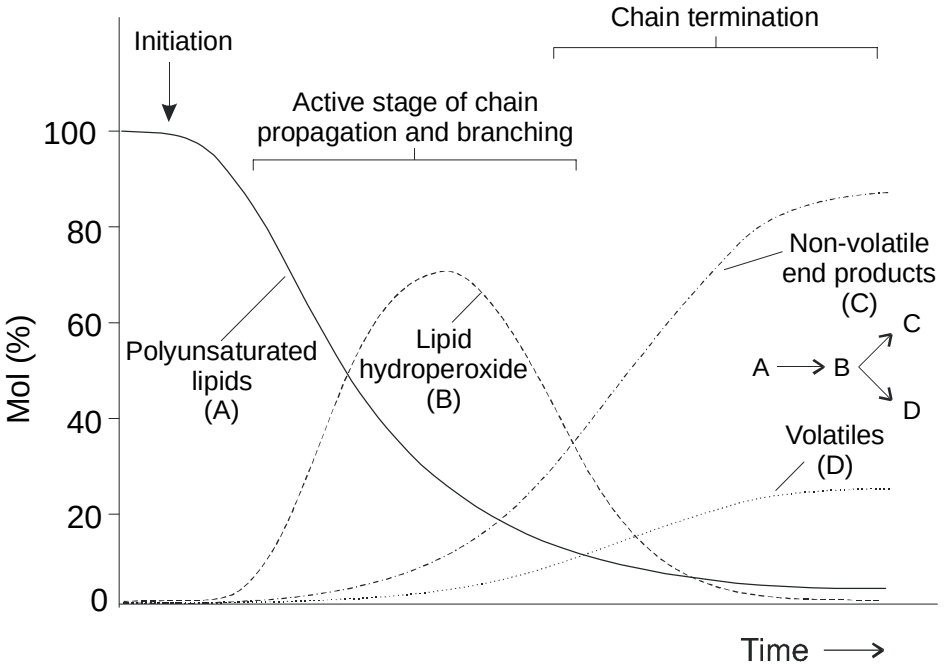


Figure 10. Schematic time course of the autoxidation of a polyunsaturated lipid showing the various stages in the reaction [192].

This figure also can serve as a starting point for the description of the temporal evolution of a drying oil paint. However, in addition to the processes already described, new factors have to be taken in account that are thought to affect the development of an oil paint system. The most important one is the addition of an inorganic pigment to the paint, which has been shown to have a major impact on the chemistry in the paint system. A second and less defined contribution can be expected from the environmental conditions imposed on the oil paint, especially during the initial stage of drying.

2.9 Oil paint

The changes in chemical and physical properties of oil paints can be briefly divided in four categories: (1) those which occur during the preparation of the paint (2.9.1.); (2) those which occur in the period between the application of paint and the “dry” stage (2.9.2.); (3) those which lead to the ageing of the paint (2.9.3.); and (4) those which lead to the degradation of the paint (2.9.4.). The first two categories have been subject of a large number of studies, whereas the chemistry involved in the ageing and degradation has hardly been addressed. Traditional oil paints were made by grinding pigment in oil on a stone plate, in such a ratio that a workable paint was obtained. Other materials could be added, such as thinner or siccatives to modify the flow characteristics and the drying properties. In the following paragraphs a literature overview will be given of both the chemical and physical changes that occur in the lifetime of an oil paint. Most of the studies

published relate to simple oil or alkyd paints without any addition of modern paint related materials, e.g. fillers and driers, unless otherwise noted. The oil itself, however, is obtained with modern refining and processing techniques, which will give the oil slightly different properties compared to traditional oil paints. The production processes of modern synthetic pigments have changed as well, e.g. the surface of modern pigments is modified and the size of modern pigment particles has decreased. This will influence the interaction between pigment and binding medium.

2.9.1 Preparation of paint

The type and amount of pigment added to a drying oil is an important factor, which will influence both the properties of the paint, including viscosity, flow characteristics and dispersion stability, as well as the overall stability of the paint after it has cured. The nature of the pigment-medium interaction when dealing with a fresh, non-oxidised oil is an important parameter. When a pigment is introduced into the oil, the interaction can be both physical and chemical in nature depending on the type, the particle size, and the surface properties of the pigments. Impurities present in the paint system may also play an important role in these processes with water being one of the important factors [193]. The large variety in the nature of the pigments [194] does not make it easy to give a general description of these interactions. Artists' pigments [195, 196] may be inorganic solids like lead white (basic lead carbonate; $2PbCO_3 \cdot Pb(OH)_2$), Naples yellow ($Pb_3(SbO_4)_2$), azurite (basic copper carbonate; $2\ CuCO_3 \cdot Cu(OH)_2$), vermilion (HgS), verdigris, (copper acetate; $Cu(OCOCH_3)_2 \cdot 2Cu(OH)_2$), malachite ($CuCO_3 \cdot Cu(OH)_2$), umbers, red and yellow ochres (mainly $Fe_2O_3 \cdot H2O$ or Fe_2O_3), the more recent materials zinc oxide (ZnO) and cobalt blue ($CoO \cdot Al_2O_3$), or more complex inorganic mixtures like smalt (cobalt potash glass) or the natural zeolithe ultramarine ($3Na_2O_3 \cdot 3Al_2O_3 \cdot 6SiO_2 \cdot 2Na_2S$). Examples of traditional organic pigments are indigo, indian yellow (Mg or Ca salts of euxanthin acid), alizarin, madder and several yellow colouring flavonoids, including luteolin and quercetin [12, 197]. Nowadays, a vast number of synthetic colorants is available which will not be discussed in this thesis [198]. Complex mixtures of inorganic and organic compounds have also been used as pigment. Van Dyck brown (lignitic coals or undefined mixtures of "humic" acids, iron oxide and sand), asphalt [199] (cross-linked aromatic and aliphatic hydrocarbons) and a number of thermally produced products like ivory-black (char bone), lamp-black (soot) and vine black (charcoal), which mostly consist of carbon.

The interaction of oil with these pigments has not been investigated. Only the interactions of a few, mostly modern, inorganic pigments, e.g. TiO_2, Fe_2O_3 or Al_2O_3 with oil, have been studied in detail. It is clear from these studies that the surface area of the pigment plays an important role. Another important factor is the surface free energy, which determines whether the medium is capable of displacing the air or water surrounding the pigment particle. This is called the wetting process. Wetting requires the surface tension of the medium be lower than that of

the pigment. This usually is the case in the dispersion of pigment in oil, because both the inorganic and most organic pigments have higher surface tensions than the medium [200]. In order to form a stable paint system each particle in the pigment – oil suspension must be stabilised by the constituents of the binding medium, anchored to its surface by intermolecular interactions. In this way, the agglomeration of pigment particles is prevented.

In a number of experiments performed by Crowl [201] alkyds were allowed to interact with different pigments by a simple technique in which a dilute alkyd solution was allowed to pass down a column full of the pigment. The results showed that the low molecular highly polar material was mainly adsorbed, although only the results for the titanium oxide anatase were described in detail. In another series of experiments pigments were brought into contact with a mixture of water and oil in order to investigate whether the pigment passed from the water phase to the oil phase. In the presence of "free acids" in the oil, as was the case for the linseed oil used, the following tested pigments went into the oil phase: ZnO, ZnS, $BaCO_3$, carbon black, phtalocyanine blue and antimony oxide (Sb_2O_3) [201]. The nature of the pigment is crucial in this process. Unfortunately, not much information is obtained on the actual mechanism of the adsorption process.

Adsorption studies with infrared spectroscopy (IR) on TiO_2 and stearic acid show a rapid decrease in the signal of the free hydroxyl groups present on the surface of the pigment [193]. Furthermore, the strength of the acid/pigment interaction was found to be related to the strength of the acid. A stronger acid rapidly replaced stearic acid, with little evidence that this process is reversible. IR experiments have shown that the stearic acid can be adsorped on the TiO_2 surface by two mechanisms: (1) the formation of a carboxylate ion (an ionic interaction); and (2) adsorption of the free acid by hydrogen bonding (an acidic interaction). These two processes could be differentiated easily by looking at the carboxyl band of the fatty acid, which is replaced by two new bands at lower frequencies. The exact frequencies of these bands are dependent upon the metal with which the ion is associated. The decrease in intensity of the far-infrared band in the 660-690 cm^{-1} region of the TiO_2 on adsorption of stearic acid indicated that a metal-oxygen band near or at the surface was influenced by the adsorption process.

Water was shown to have an effect on the type of interaction. Increasing the water content of the pigment led to an increase in the ionic nature of the interaction. Traces of zinc, when present in the pigment were shown to dominate the adsorption process. Experiments with oleic acid and methylated stearate have shown that these fatty acids had a lower affinity and no affinity at all, respectively, for the pigment surface. The latter finding indicates that pigment wetting is facilitated by the presence of free fatty acids. The type of bond and structure of complexes of carboxylic acids with different metals, including spectral properties, have been reviewed by Oldham [202] and Mehrotra [203]. A number of IR studies on metal salts of fatty acids have been published which showed the difference in the stretching frequencies of the various metal carboxylates [204-209].

Apart from these ionic interactions, adsorption of a polyester on an inorganic oxide has been studied in which a large number of carbonyl groups are attached to the hydroxy groups of the surface and sequences of methylene and ester

oxygen atoms form protruding loop-like structures [210]. The same phenomenon was observed for Al_2O_3, TiO_2, and Fe_2O_3, although slightly different adsorption mechanisms were thought to play a role in these cases.

The chemical reactivity of some of the pigments can make them unsuitable for certain purposes. Zinc oxide is such an example, because soap formation will occur when zinc is used in an oil medium with a high proportion of acid groups. Because zinc is a divalent metal the oil will tend to cross-link, causing excessive viscosity increase upon storage [211]. Lead driers were also shown to be separated from a paint that contained a measurable amount of free fatty acids due to the formation of insoluble lead soaps upon preparation [212]. Another example in this respect is the selective reaction of the pigment surface with cobalt driers upon storage of the paint, which leads to a loss of drying power [213]. Despite these experiences little research has been published about efforts to understand and control these problems.

The pigment-medium interaction described so far mostly involves the carboxylic acid groups. However, interaction with the double bond systems is not to be excluded, since the complex formation of metals with unsaturated organic compounds has been known for a long time [214]. A literature search on the formation of such complexes with specific metals or pigments, however, did not result in a large number of articles on this subject [215].

The above examples are showing that reaction between the pigment surface and the oil or its hydrolysis products are expected for a number of typical pigments. It is inferred that a number of the more traditional pigments will behave in a similar way. If such processes take place, a decrease is expected in the catalytic power of the pigment surface, leading to different drying mechanisms and reaction rates within the paint film. At the same time, pigment particles can “dissolve” in the oil as a consequence of the modification of the polarity of surface.

2.9.2 Curing

The available literature on the curing process of films consisting of mixtures of unsaturated FAs and pure TAGs, drying oils, traditional oil paints or alkyd paints, which has been investigated extensively for a long time by a large number of authors, is extensive. Most of these studies have been performed on relatively simple systems that were kept under different light, temperature and humidity conditions, with or without the addition of pigments. This tremendous amount of work has lead to a good understanding of the processes that occur [144]. Initially there is a viscous liquid, composed of a complex mixture of highly unsaturated non-cross-linked TAGs as schematically depicted in Figure 11a. After an induction period hydroperoxides form due to autoxidation, accompanied by a shift of the non-conjugated *cis* double bonds to a system containing conjugated *trans-cis* double bonds as can be easily seen with attenuated total reflectrance (ATR), and (photoacoustic-)FTIR [217-222]. At the same time, the iodine value, which is an indication for the number of double bonds, drops. This has also been

observed with NMR [125, 216]. The incorporation of oxygen, which has been shown to occur at the paint surface initially [217, 218], leads to an increase in the mass of the system [219]. In the beginning the peroxide value increases rapidly but after some time the value starts to decrease again [156, 220]. The hydroperoxides are subsequently decomposed and a high concentration of free radicals will be built up, leading to cross-linking of the TAGs and formation of a more viscous, gel like material. The nature of the cross-links has been investigated using (solid state) NMR [93, 221], and GC/MS, with or without selective breakdown of hydroperoxides using stannous chloride [122, 125]. The linking was found to consist of C-O-O-C, C-O-C, and C-C bonds. Due to the cross-linking process high molecular weight material is formed. This stage is depicted in Figure 11b. At the same time, a variety of (non-)volatile decomposition products will be formed, giving the paint its typical smell. These compounds have been thoroughly characterised [135, 138, 141, 142, 222]. The evolution of this volatile fraction leads to a slowing down of the mass increase due to oxygen uptake and eventually the mass of the system will decrease [219]. Both the carboxyl and hydroxyl values have become higher and their signals are broadened in the IR spectra. The acid value of the paint is increasing during the curing of the film and has been ascribed to the formation of breakdown products and not to a rapid increase in hydrolytic processes. Whereas the fresh oil is soluble in solvents like white spirit or hexane, when dried it is hardly soluble anymore and therefore of a quite different nature. The soluble fraction has been analysed using size-exclusion chromatography (SEC) and the formation of both higher molecular weight material up to pentamers, as well as lower molecular weight products have been detected [8, 117, 124].

This scheme of curing applies not only for films made of drying oils or drying oil-based materials but also is thought to be a valid model for oil-based paints in general. However, the type of pigmentation is known to have a large effect on the rates and type of curing reactions. A number of scientists investigated the effect of either the pigment or the drier on the autoxidative drying of linseed oil paints. From early investigations it is known that certain pigments speed up drying, e.g. lead and zinc containing pigments, whereas others will reduce the drying rate, e.g. black pigments like ivory black, or lamp black [223]. The effects of some common pigments on the auto- and photoxidation were studied by Rasti & Scott [224]. This was done by investigation of a number of parameters: oxygen absorption, weight loss by looking at the decrease of the CH_2 stretching vibration, the formation of hydroperoxides by monitoring the UV absorbance at 235 nm, and GC determination of the composition of the hydrolyzed paint. Upon UV radiation or exposure to diffuse daylight paint made with vermilion (HgS) shows the highest oxidation rate and was more prone to oxidation compared to the unpigmented oil. Both lead white ($PbCO_3 \cdot Pb(OH)_2$), and verdigris ($Cu(OCOCH_3)_2 \cdot Cu(OH)_2$) pigmented paint were stabilised compared to the unpigmented oil. Light red (Fe_2O_3) pigmented paint showed a strange behaviour: of all films it had the lowest weight loss by the evaporation of volatiles, whereas the amount of azelaic acid, one of the relatively stable end-products of autoxidation, had almost the highest relative concentration. The latter suggests that a relatively large amount of volatile

compounds should have been formed. This contradiction however wasn't discussed.

The same authors reported on the stabilising effect of verdigris on linseed oil oxidation [225]. In addition oleic acid was reacted with verdigris and the brown mass was investigated using IR and UV absorbance. It is proposed that upon oxidation of oleic acid a multiply conjugated system is formed. This has never been reported in the vast amount of literature on the oxidation of oleic acid and seems therefore very unlikely. Furthermore, in autoxidised lipid systems of reasonable age these type of compounds are hardly present anymore due their high reactivity.

The influence of various types of cobalt blue ($CoO \cdot Al_2O_3$) pigments on the autoxidation of linseed oil was investigated by studying the weight changes in time. It was shown that the pigments possessed different catalytic activities. This was ascribed to the chemical composition, shape, particle size, adsorption ability and solubility of the pigment. It was found that a higher solubility of the cobalt cation in the organic phase as well as a better adsorptive ability led to an increased activity [226]. Three different copper (II) salts (acetate, abietate, and basic carbonate (malachite) have been investigated and those of abietate and acetate significantly increased hydroperoxide breakdown and incorporation of oxygen compared to an unpigmented oil. A reduction of the weight loss of the coper salt pigmented films compared to the unpigmented oil was also observed. Investigation with GC/MS of the transesterified fraction shows lower amounts of oleic acid and increased amounts of azelaic acid, indicative for a higher rate of oxidation. The basic copper carbonate shows intermediate behaviour compared to the unpigmented film [227]. It is clear that the results of Rasti & Scott previously described do not match these latter data. It indicates once more how difficult it is to understand the overall result of the drying processes of linseed oil based paint, in particular when results are contradictory.

There are numerous studies on the action of different driers or mixtures of driers on the drying behaviour of alkyd and oil paints [56, 58, 228-231]. These studies are in general directed to finding an optimal combination of primary and secondary driers. Other studies focus on the influence of the different acid groups like naphtenates, stearates, and 2-ethylhexanoates that have been used to dissolve the metal catalysts into the oil. It has been shown that, the choice of acid is inconsequential for the activity of the driers provided that the solubility of the drier in the coating remains satisfactory [56], although later studies on several cobalt drier complexes have indicated that the catalytic activity may differ greatly [232].This can be understood if the metallic cations are seen as complexes and not as free ions. They are always surrounded by binding medium molecules or by other ligand groups, so in order to oxidize or reduce the transition metal an electron has to extracted from, or added to the *d* shell of the metal via the surrounding materials [232].

Environmental influences on the curing process also have been investigated. The parameters investigated include temperature, humidity, UV light and gaseous pollutants like SO_2 or ozone. High levels of humidity as well as high concentrations of SO_2 slow the initial reactions down [233, 234]. Low levels of

SO_2, however, do lead to an increase in drying rate. The interaction of SO_2 with the drying oil was thoroughly investigated by Simendinger and Balk [235, 236]. Compared to an air-dried film the FTIR spectra show additional changes, which were ascribed to the formation of sulphate ester cross-links between the drying oil molecules. The fatty acids that are most reactive in the normal autoxidation reaction were shown to have the highest reactivity with SO_2 as well. The temperature effect was shown to be inversely proportional to the time required for a through drying [237]. The same phenomenon was observed for experiments done with both dry and humid air during the drying stage [233].

Ozone, O_3, is a well-known industrial reagent for the oxidative cleavage of double bonds. Exposure to ozone results in an increased rate of oxidation and the subsequent formation of low molecular weight breakdown products, with azelaic acid as the main reaction product [238]. The same catalytic effect has been shown for UV-light in the initial stage of drying. Even in the presence of nitrogen and without the presence of oxygen the oil shows an increased viscosity, acid number and molecular weight, and a decrease in the iodine number, although slower than linseed oil dried in the presence of air or oxygen. The longer the oils were illuminated the more absorbing they became, and hence the greater the effect of the light was [239]. The refractive index and density of the individual unsaturated fatty acids and their glyceryl esters, when exposed to UV light, has been shown to increase as a consequence of the increasing number of conjugated double bonds [240, 241].

2.9.3 Maturing and ageing

The natural ageing process is the least understood of all the stages in the life cycle of oil paint. This can be due to several factors. First, the long time that is required for the study of this phenomenon makes it difficult for researchers to perform these investigations as most of the industrial paint manufacturers are not interested in the long term changes as will take place in paintings. Accelerated ageing with elevated levels of light, humidity, salts or temperature, with different exposure cycles and times has been used to investigate the accelerated ageing and degradation of oil or alkyd paints [242]. Whether the outcome of such studies resembles the reality remains to be seen. Harsher conditions may drive reactions that do not occur under normal storage conditions. A uniform acceleration of the numerous competing and simultaneously occurring reactions involved in the ageing of a material as complex as oil paint would be essential. At the moment no method is known that faithfully predict the long-term behaviour of oil paints. However, worldwide a number of oil paint collections of different age and compositions are available that have been made with traditional pigments. Some of these test sets were made with the intention to study the longer-term process of natural ageing. The samples are reasonably well documented with respect to the composition or the materials used. Unfortunately, it is often not exactly known under what (environmental) conditions these paint collections have been stored. Furthermore, the origin and quality of pigments and oils, and the pigment to

volume concentration is not always known either. This makes it difficult to compare the analytical results.

A second problem of ageing experiments is the complexity of the material formed. Due to the insoluble nature of the dried film the analytical techniques are essentially limited to the measurement of changes in weight, density, number of double bonds, oxygen uptake and oxygen content.Volatile degradation products of the autoxidation [127, 130, 131, 138, 141, 142, 188, 190, 222] and low molecular weight material can be studied using GC/MS, after extraction or chemical and thermal cleavage of the cross-linked system [10, 11, 14, 15, 26, 27, 29, 123-125, 243-249]. Analytical methods that can be applied to the bulk of the oil paint, the oil networks, include analysis like NMR [216, 221, 250, 251], IR [220, 252-254], and attenuated total reflection (ATR) spectroscopy [255-257], although the amount of information that can be obtained on the structure of the cross-linked systems is rather limited. The results of all these studies indicate that the ageing can be seen as a continuation of the processes initiated in the curing stage. The paint is becoming more polar as a consequence of the incorporation of additional oxygen in the form of ether, carbonyl- , epoxy-, and hydroxyl-groups. It's weight however is now decreasing due to the higher loss rates of volatile materials like aldehydes, carbon dioxide and lower acids. The total number of aldehydes is reduced together with the hydroperoxides that are being degraded continuously [241]. The oil paint film shrinks steadily upon ageing and an increase in density is observed [258]. When subjected to water relatively high amounts of water-soluble material can be washed away, mostly acids of high acid value and oxygen content, whereas a substantial amount of lower acid value can be extracted with acetone [259]. The high content of extractable acidic material and the increased acid value indicate that hydrolytic processes occur.

One would expect that the hydrolysis process might be accompanied by a gradual weakening of the paint film. However, another process, the interaction of (basic) pigments, with the various types of acidic material, namely high molecular weight acidic cross-linked systems, long chain fatty (di)acids (metal soaps) or low molecular weight acids, such as formic acid may occur. With the first group and probably the second, this interaction is thought to reinforce the paint film and enhance some of its properties [260]. The type of material that is formed resembles a specific class of compounds known in the field of synthetic polymers as ionomers [261-264]. These materials consist for the major part of an organic fraction, (met)acrylic or dicarboxylic acids, linked by an lower percentage of inorganic element, e.g. Cs, Li, Na, Ca, Mg, Cu, Zn or Pb. The studies on these systems have all shown that upon the neutralisation of the acid groups (the anion), a structural reorganisation occurs to ionomers with salt groups that tend to aggregate into domains, which are separated from the hydrocarbon matrix. The size and exact nature of such domains is not clear [262]. A schematic representation of such a system as it is thought to exist in the oil paint in the mature stage is drawn in Figure 11c. The properties of such ionomeric systems will largely depend on the number of metal salts vs. free acid groups, hence the degree of neutralisation, the type of metal cation (monovalent or multivalent), and the nature of the organic matrix. In general, the incorporation of higher amounts of metals into the polymer leads to an increased connectivity of the material and therefore

the stiffness will increase. The divalent ions are in this respect more effective compared to monovalent ions. Just like the initial ester bonds from which the ionic bonds are formed, the linkage doesn't have unlimited hydrolytic stability. From the information available in literature, it can be concluded that ionic polymers cover the whole range of hydrolytic stabilities, from those who disintegrate in water, to those who swell but remain intact. Water, for instance, was shown to have a considerable effect on the stability of divalent metal salts of dicarboxylic acids [265]. On the other hand, the metal dicarboxylates are insoluble in organic solvents with the exception of zinc dimerates, which are soluble in certain amines. As little as 5% of butyl amine is enough to solubilise these compounds in non-solvents [265]. They are also soluble in drying oils at 300 °C, but they tend to separate upon cooling. The transition from a single metal carboxylate into the polymeric structure is referred to as a halatopolymeric transition. These halatopolymers are very brittle due to the strong dipole interactions of the polymer chains [265].

An interesting example of the effect of the metal salt formation is an ethylene-methacrylic acid copolymer, originally opaque, which turns transparent upon metal salt linking [266]. Occasionally, similar observations are seen on lead white pigmented paints. These have become transparent upon ageing [267]. The exact mechanism is still unknown, but it is thought that the crystallinity of the sample and the accompanying refractive index plays a role. It is clear from this and other observations that there are still a lot of unanswered questions on the structures of ionomeric materials and how the metal-polymer interactions effect the polymer properties.

A number of authors have studied the reactivity of pigments with linseed oil during ageing. Basic pigments like lead white and zinc oxide show appreciable reactivity [217], as was also deduced from the rapid formation of a hard and sometimes brittle film, especially when using zinc oxide as pigment [268]. Lead white reacts at a somewhat slower rate so that the paint obtains the right type of plasticity for a good stabilisation. The use of zinc oxide reduces the volume decrease compared to an unpigmented film, which suggests some kind of stabilisation of the three-dimensional oil network. The reactivity of the pigments in the presence of water is enhanced, as was seen from the condition of pigment particles, when cross sections of zinc oxide or calcium plumbate ($PbO_2 \cdot 2CaO$) paints were treated with water. These cross sections show a "corona" that spread out from many pigment particles, though not all, suggesting dissolution and subsequent diffusion of pigment into the surrounding medium [269]. The difference in reactivity of pigments with materials from the mature oil paint is most likely caused by differences in surface properties. Investigations of the extractable fraction of mature paints indicate a gradient in the metal salt formation: appreciable reaction is taking place with the basic pigments, less with the carbonates and only a little with the inert oxide pigments. Furthermore, it has been found that a major part of the salts were formates [269]. More recent experiments, which focussed on the FTIR investigation of the non-extractable fraction of a large number of pigmented films indicated the capability of most of the lead, cobalt, zinc and copper containing pigments to form metal carboxylates [270]. The formation of metal carboxylates is also reflected in the reduced extractability of the fatty acids and the lower swelling of pigmented paint films as a result of exposure to

solvents [271-273]. Aged lead white and calcium carbonate pigmented films are relatively stable and show less amounts of leachable material, compared to raw sienna and vermilion pigmented paints [24]. Stolow [22] noticed a strong influence of pigments on the extractable amount of material from pigmented oil paint films. Lead white pigmented paints of different age only show around 20 % of solvent leachable materials whereas iron oxide, titanium dioxide or barium sulphate pigmented systems gave values above 50%.

Another study worth mentioning in this context is the examination of the impact of citrates as cleaning agent on pigmented linseed oil films. These compounds are well known for their excellent metal binding capacity and therefore are thought to interfere with the metal carboxylate links present. Closer inspection of the surface layer of paint films treated with ammonium citrate confirms this idea, as the top layer had completely lost its morphology, most likely due to the loss of metal ions [274]. In line with this, White and Roy published results that indicate that upon normal solvent cleaning of aged paint samples hardly any material is removed and that the surface structure is retained [27]. Sutherland [8] came to similar findings when studying the amount of materials extracted upon solvent cleaning of oil paintings.

The fact that a reasonable number of fifteenth to seventeenth century old masters paintings still are in very good condition is a good indication of the strong interaction of certain pigments with the binding medium. Without this internal strengthening most likely materials would have been lost in the course of time, either by evaporation or cleaning procedures. The presence of fatty acids in these old paintings suggests that most of them are chemically bound instead of being in a free state. The concept of reinforcement nowadays is also exploited in the paint and glass industry. A number of studies on ceramer coatings made with a mix of metal oxides and drying oil via the sol-gel process (polymerisation of metal oxides in organic polymer matrices) show an increased hardness and cross-link density as a function of an increased concentration of the metal oxides [275-278]. These studies also indicate that a covalent bonding exist between the two phases. Another example is the use of aluminium compounds, especially complexes of alcohols, which tend to exchange with carboxylic acid groups of alkyd paints. Eventually, these very stable Al-carboxylate bonds may determine the properties of the film [279, 280].

Although not directly related to pigment-medium interactions, a final remark should be made here on the interaction of linseed oil paint with a metal substrate. The chemical interaction of the paint medium with the metal could lead to a very high level of interaction when it is occurring between reactive sites, such as carboxyl groups, on the main polymeric networks. If, however reactions occur with free fatty acids or smaller acids produced upon autoxidation, the reaction products may leave the surface and diffuse into the medium, leading to a reduced adhesion. This has been observed when soluble metal soaps were formed after application of drying oil media on zinc or cadmium surfaces. The metal content of linseed oil films after drying on different metal substrates has been determined [217] and gave a general impression of the reactivity of the metal. The following percentages were reported: lead 5.2; copper 4.1; cadmium 3.6; zinc 1.3 and iron

0.1. Corrosion inhibition of such metal surfaces by addition of neutralising pigments to the paint is another way of intelligently making use of the reactivity of pigments. The acidic breakdown products of the oil or alkyd paint are chemically trapped and the corrosion is inhibited [281-284].

2.9.4 Degradation

Theoretically speaking, the formation of the volatile materials discussed in the two previous stages of drying oil paint development already can be seen as the first steps in the degradation of the paint. However, the paint film itself stays relatively intact and no typical degradation phenomena like chalking, blistering, brittleness, cracking, and flaking are observed at that time. These phenomena are normally only first observed when the oil paint is exposed to harsh condition in weathering tests using high temperature, humidity, ozonization, or high doses of ultraviolet (UV) light [6, 242, 285]. The thinner the tested paint film is, the more dramatic the changes and loss of material will be.

The overall effect of strong doses of light on the paint film results in its decomposition forming carbon dioxide, carbon monoxide, water, and low molecular weight volatiles and (di)acids [286-288]. In case of pigmented films the extreme breakdown of the thin surface layer leads to chalking [289]. This is especially associated with certain pigments which are activated by UV radiation of which the most active wavelengths lie between 290 and 350 nm, such as lead white or lithopone ($ZnS + BaSO_4$). Oxides of iron and carbon black are pigments that are resistant to chalking. These pigments are thought to absorb the radiation turning light energy into heat. Lower layers of pigmented paints, however, are also affected because of the penetrating power of UV radiation [290]. The changes in density are not similar for the top and lower layers. As a result of unequal contraction, stress and strain is built up in the paint system, which can lead to failure of the oil paint in the form of cracking or flaking. Another factor of importance in this process is the non-homogeneity of the different layers due to formation of metal salts within particular layers [258].

Heat is thought to be a less complex factor compared to other parameters involved in the degradation of oil paint. High temperatures are responsible for the alteration of the kinetics of the different processes like autoxidation, photoxidation, and hydrolysis, and furthermore volatile compounds will be lost faster from the paint film.

The effect of high levels of humidity already has been partially discussed above. Water absorption in the paint leads to the hydrolytic breakdown of ester bonds of drying oil and alkyd paints. The overall effect is a gradual weakening of the film, so that the mechanical effects of alternate swelling and shrinking [291], or of movements of the substrate, should become more destructive with time. On the other hand, water can be absorbed and may collect at the interface of substrate and paint or different paint layers, leading to loss of adhesion and peeling. Adhesion may be restored on drying, but eventually this cycle will produce an irreversible

loss of adhesion. Blooming, the formation of exudation on the surface on part or the whole of the surface, is occasionally observed under high levels of humidity as well [6]. This last example can be seen as a change in the equilibrium between the three types of fatty acids present in the oil paint: esterified-, metal bound-, and free fatty acids. The increased rate of hydrolysis leads to an excess of the free fatty acids within the paint, which are thought to migrate eventually to the surface of the painting when not trapped by the reaction with pigments. Examples of two studies on paintings suffering from blooming are given in Chapter 7.

2.10. Summary and relevance for paintings

Traditional linseed oil paint consists of a viscous mixture of finely divided (in)organic pigment and oil. Initial chemical reactions involved in the curing process of the fresh oil paint after application are mainly a result of autoxidation. This very rapid process involves formation of hydroperoxide-substituted fatty acids, breakdown of the hydroperoxides, giving rise to highly reactive radicals and subsequent cross-linking. The breakdown process is accelerated (catalysed) by the presence of (transition) metals from pigments or driers. The resulting cross-links are covalent ethers, peroxides and carbon-carbon bonds. In solidifying paint a three-dimensional network is formed because there is more than one reactive site on the unsaturated FAs, hence more than one point to create a cross-link. This is schematically drawn in Fig. 11. Figure 11a presents a spatial distribution of reactive oil molecules, which forms 3D network by cross-linking (Fig. 11B).

Apart from cross-linking reactions, also degradation reactions occur which transform the triacylglycerol radicals into volatile low molecular weight (un)saturated aldehydes, ketones, alcohols, acids and hydrocarbons. Most of these smaller volatile molecules arise from the hydrocarbon chain of highly unsaturated fatty acid moieties. These compoounds will leave the paint film by evaporation, although short chain FAs (e.g. C7-C11) for example may be trapped within the paint film for a longer period of time.

Due to incorporation of oxygen into the paint film the weight increases and the film becomes more polar. At this stage the paint is very sensitive to organic solvents due to the open structure of the network, the high amount of extractables and the flexibility of the paint system.

After the paint film has become a solid but elastic film, oxidation processes do not stop but proceed leading an increasing relative amount of acidic oxidation products. Hydrolysis of the initial triglyceride ester bonds occurs leaving a residual cross-linked fraction with free carboxylic acid groups, free FAs and diacids, and glycerol. Glycerol and some of the lower molecular weight FAs will evaporate.

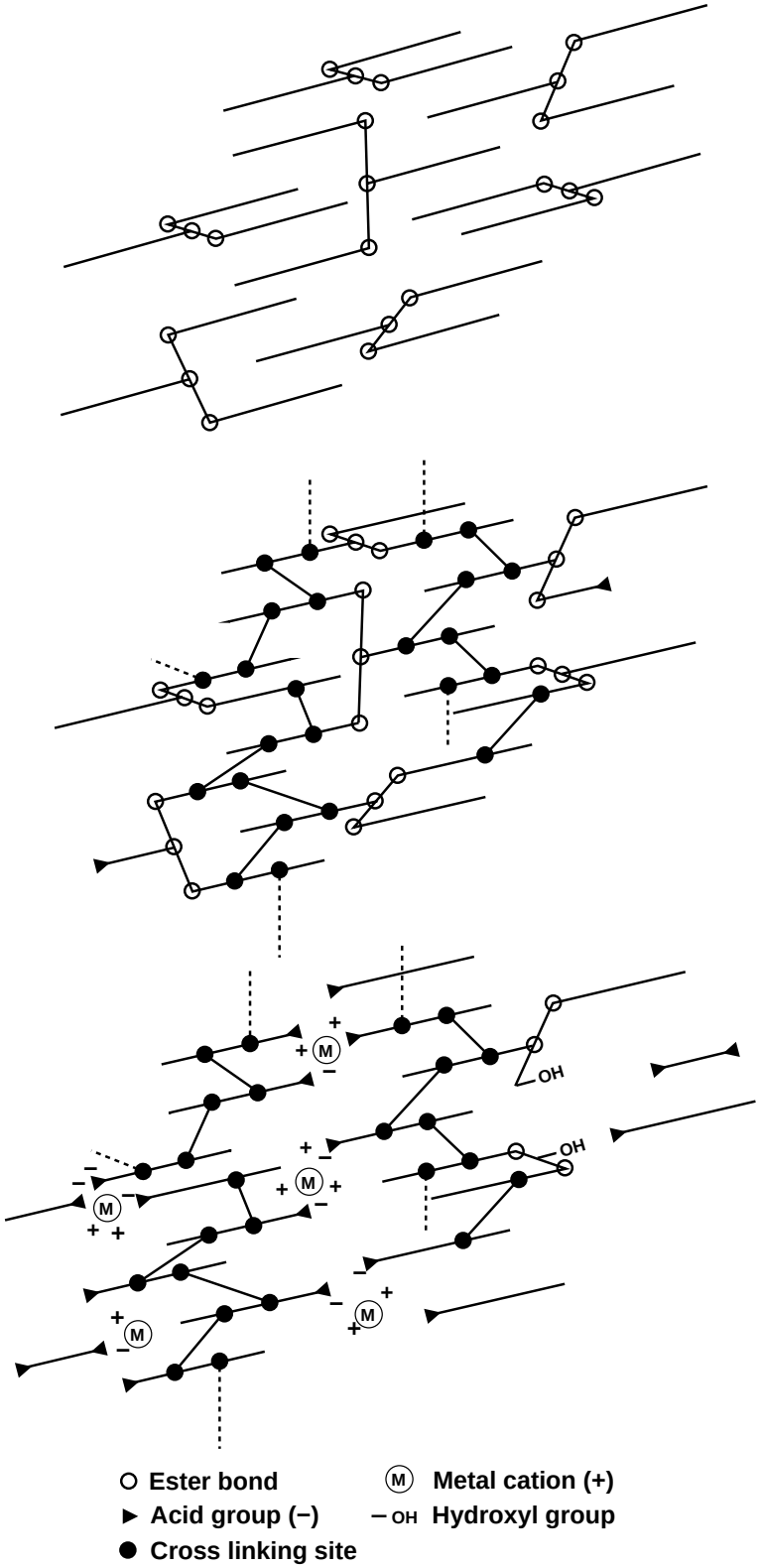


Figure 11. Schematic model depicting different stages in the development of oil paints (a) fresh oil; (b) dried oil after curing (plastic stage), and (c) mature aged oil paint (lithification stage).

The liberated acid groups of both the cross-linked networks and free FAs are immobilised by reactions with metal ions to metal carboxylates, leading to a so-called ionomer. Metal ions at the surface of pigment particles as well as dissolved ions (from pigments or driers) can act as an anchor point for the carboxylic acid groups. Multivalent interactions lead to complex coordination chemical relationships, which form linkages between different parts of the cross-linked networks. Fig. 11c summarises the result of the maturation process of the non-hydrolysed cross-linked networks to the polyanionic ionomer stage of the oil paint.

The physical changes caused by the transition from cured to mature oil paint are very drastic. The paint film loses weight because more volatile compounds will evaporate from the paint than oxygen is incorporated. As a result the paintfilm shrinks.

The solvent sensitivity in a truly ionomeric oil paint film will be strongly decreased because non-cross-linked FAs in the paint film are chemically trapped in the form of solvent stable salt. On the other hand the sensitivity for acid, base and ionogenic detergents is greater because they affect the coordination chemistry of the paint system.

Without stabilisation of the hydrolysed paint by multivalent metal ions, the paint film will be more vulnerable towards swelling. In this case liberated FAs can appear at the surface (blooming), evaporate (precipitation on the protecting glass) or become extracted under restoration.

Chapter 3

The effects of traditional processing methods of linseed oil on the composition of its triacylglycerols

Different oil processing methods were performed, which included washing with water and treatment with lead-based driers, with and without heating to different temperatures, giving a set of 7 oils to be investigated. The effects of the traditional processing methods of linseed oil on its triacylglycerol (TAG) composition were studied, using the following analytical methods: high performance size exclusion chromatography (HPSEC), Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography - atmospheric pressure chemical ionisation – mass spectrometry (HPLC-APCI-MS), direct temperature resolved mass spectrometry (DTMS), matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionisation fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). A decrease of the initial cis-double bonds and the formation of trans-double bonds upon heating of the oils was observed. Heating a lead and oil mixture to 150 ºC, or heating the oil alone to 300 ºC led to the highest degree of oxidation. A difference was observed for the oxidation patterns for oils with and without the addition of lead. Furthermore, levels of oxygen incorporation were higher when lead was added to the oil. High temperature treatment of the oils resulted in an increased average molecular weight.

The changes in the initial conformation of the double bond systems observed with FTIR, were supported by HPLC-APCI-MS measurements that showed the formation of a number of new isomeric TAGs in the heated oil compared to freshly pressed, untreated oil. Oligomerisation up to hexamers was observed with HPSEC, and MALDI-TOF-MS. The formation of oligomers up to trimers only, however, was observed with ESI-FTICR-MS. Incorporation of oxygen was mainly observed with MALDI-TOF-MS and ESI-FTICR-MS whereas with DTMS and FTIR hardly any evidence was found for this.

3.1 Introduction

Drying oils are one of the oldest binders used in paints for both decorative and protective purposes. Today they are still in use as raw materials in modern coating systems such as alkyd paint or polyester resins. Traditionally, the oil was pressed from the seeds of plants like flax, walnut or poppy. After removal of all unwanted material like foreign seeds or chaff by processes such as blowing with air or sieving, the seeds were ground to a fine meal and pressed to extract the oil. The oils obtained are mixtures of triesters of glycerol (also called triacylglycerols or TAGs) with a high content of double and triple unsaturated fatty acids (see Table 1). It is important to note that the number of different TAGs is much larger than the number of fatty acids. Theoretically, for the 5 main fatty acids 75 different TAGs can be obtained when positions 1 and 3 of the glycerol backbone are considered to be unequal. Aside from TAGs, the oil can contain other substances, some of which can have a marked effect on the (drying) properties. Free fatty acids are always present in a relative proportion of about 0.5 tot 2 %. Furthermore, about 0.1 - 0.2 % water can be dissolved in the oils, without any effect on their clarity. Other substances present are phosphatides, of which lecithin is the best known, carotenoid pigments and sterols [35].

Table 1. Fatty acid composition of freshly prepared linseed oil[a] [292].

Fatty Acid[b,c]	Freshly pressed oil (F) [d]	% of total FA			
		Europe	Canada	Argentina	India
16:0 (palmitic acid)	5.9	4-6	5-6	4-5	9-10
18:3 (linolenic acid)	57.6	56-71	54-61	45-53	50-61
18:2 (linoleic acid)	16.4	12-18	14-16	15-24	13-15
18:1 (oleic acid)	17	10-22	19-20	19-21	10-21
18:0 (stearic acid)	3.1	2-3	3-4	5-6	7-8

[a] The relative amounts largely depend on climate and the variety of *Linum usitatissimum* of the seeds [33].
[b] 18:1 indicates the carbon chain length : number of double bonds.
[c] Trace amounts of 16:1, 20:0, 20:1, and C22 fatty acids have also been reported.
[d] Provided by Dr. Nimal Ratnayake, Nutrition Research Division, Health Canada Banting Research Centre, Ottawa, Canada

In principal, freshly pressed oil can be used for paint formulation although normally the water-soluble component or mucilage (phosphatides and other non-TAG matter), which is present after expression, is removed. Additional processing is undertaken to give the paint specific properties desired by the painter. Traditionally, processing included the removal of mucilage by washing or settling, and various procedures to enhance drying, such as heating and/or treatment with driers (metallic compounds). Oils were also treated by sun or chemical bleaching to reduce their initial colour [30, 43].

Some of these processes lead to changes in the composition of the original highly unsaturated triacylglycerols (TAGs). Hydrolysis can occur giving rise to di- and monoacylglycerols [151, 152]. Incorporation of oxygen into the unsaturated fatty acid strands leads to oxidised TAGs [52, 96, 293] and low molecular weight (volatile) breakdown products [135, 141]. The presence of drier during processing increases the rate of formation of active radicals due to the catalysed breakdown of hydroperoxides [232, 294]. As a result of the breakdown of the various oxidised products a variety of radicals are formed that can recombine to form cross-linked material with an increased molecular weight. This cross-linked material will consist of the different oxidised and hydrolysed species [115, 121, 122, 139, 295].

As a result of heating during oil processing trans isomerisation of linoleic and linolenic fatty acids will occur leading to an increase in the number of isomeric TAGs and their oxidation products [160]. At the same time, heat-induced Diels-Alder addition reactions of fatty acids will result in intermolecular cross-linked TAGs, intramolecular coupled species [113, 170, 178, 182, 296] and cyclic FAs [163-165, 169, 171]. However, the latter two types of compounds will not lead to an increase of the average molecular weight of the oil since no cross-linking between TAGs is occurring.

3.1.1. Analytical Techniques

In order to study the alterations of the oil due to processing a broad range of analytical techniques can be used, each with its own advantages and disadvantages. To elucidate the qualitative composition of a TAG mixture based on chromatographic retention times, it is necessary to synthesise a large variety of pure oxidised TAG standards. However, this still does not result in a definitive structure for the unknown compounds investigated [104]. These mixtures of oxygenated TAGs are difficult to analyse because of the presence of a large variety of homologs and stereoisomers, which overlap with each other and with analogues of unoxidised TAGs when chromatographic methods are used [105, 297, 298]. In theory it should be possible to structurally identify these overlapping species by a combination of chromatographic techniques and mass spectrometry or nuclear magnetic resonance. The established method for the separation of triacylglycerols (TAGs) is non-aqueous reversed-phase high-performance liquid chromatography (HPLC) with solvents like methanol - 2-propanol - hexane [299], dichloromethane - acetonitrile [298, 300], dichloromethane - acetonitrile - propionitrile [301, 302], pure propionitrile [303], acetone or chloroform with acetonitrile [304], or methanol - 2-propanol [305]. The retention of particular acylglycerol species increases with increasing equivalent carbon number (ECN) defined as ECN=CN-2*DB, where CN is the carbon number in all acyl chains and DB is the number of double bonds. Under optimised chromatographic conditions, most of TAGs with the same ECN value also can be separated by the number of double bonds, in addition to the separation according to ECN. Four HPLC detection techniques are most frequently used for TAGs detection:

a. UV detection at 200-205 nm,

b. evaporative light-scattering detection (ELSD),

c. positive-ion atmospheric pressure chemical ionization (APCI)

d. positive-ion electrospray ionization (ESI) mass spectrometric detection.

Mass spectrometric techniques provide structural information and good sensitivity. ESI mass spectra show only $[M+Na]^+$ or $[M+NH_4]^+$ ions [306] without the fragmentation or adducts with other added cation like $[M+Li]^+$ [297]. Information on acyl chains can be obtained by tandem mass spectrometry (MS/MS) analysis [306] which opens up the possibility to determine double bond position(s), e.g. in case of MS/MS of $[M+Li]^+$ ions [297]. APCI yields both molecular weight and individual acyl moiety information, but unfortunately the ionisation efficiencies of individual TAGs and DAGs are dependent on the number of double bonds [299] (similarly to ESI [306]), which causes problems with direct determination of relative amounts of the acylglycerol species.

There are a number of other mass spectrometric techniques that could be applied to the analysis of (oxidised) TAGs as well. In order to be satisfactory, an ionisation method should be choosen that produces MS information on both the molecular weight as well as the characteristic fragment ions to facilitate the identification of both the location and the nature of functional groups formed upon oxidation. Ionisation techniques like (low voltage) electron ionisation [307] and (atmospheric pressure) chemical ionisation [297, 302, 308] are therefore thought to be more suitable than electrospray (ESI) [103, 104, 309, 310] unless MS/MS is performed. More recently, the coupling of matrix assisted laser desorption/ionisation (MALDI) or ESI with Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS) has proven suitable for the analysis of oxygenated TAGs without prior chromatographic separation [311, 312]. However, this technique involves structural identification by MS/MS, which is time-consuming.

All of the aforementioned analytical techniques require that the analyte has to be brought into the gas phase, preferentially intact. This is more difficult to achieve for the higher molecular weight material that is formed upon cross-linking, especially since the polarity will be high as well due to the presence of oxygen containing functional groups. Besides, the nature of the oxidation process in oils is such that a broad envelope of molecular masses will be formed with a lot of isomeric structures. Therefore, in order to study the properties of both the high- and low molecular weight material formed, the following techniques provide additional information: size exclusion chromatography (SEC) [113-115], Fourier transform infrared spectroscopy (FTIR) [253, 254, 256] and nuclear magnetic resonance (NMR) [95, 189, 216, 313, 314]. SEC gives an approximation of the molecular weight distribution of the sample. The ratio of the relative amounts of free fatty acids, triacylglycerols, oligomers and higher molecular weight material, which is a result of processes such as oxidation and/or heat induced cross-linking

and hydrolysis and/or degradation, can be determined. FTIR provides information on the presence of free and esterified fatty acids, specific functional groups, and the double bond conformation. NMR techniques provide information especially on the presence of specific functional groups. The advantage of FTIR and solid state NMR techniques is that the intact sample can be analysed and does not need to be dissolved. Most of the techniques previously described have been used to analyse the set of seven oils, except NMR.

3.1.2. Experimental Design

Artists' oil recipes were recreated using historical recipes. Hand-ground oil paint was produced with authentic materials as they have been described in historical sources [30, 43, 315] in order to investigate the effects of traditional oil processing on the linseed oil triacylglycerols and the physical properties of the paint. The processes that were monitored include water-washing, treatment of the oil with heat alone, treatment with and without heat in the presence of lead(II)monoxide as drier. Chemical changes induced by these processing methods were evaluated relative to the fresh pressed linseed oil. The oils obtained were analysed by: SEC, FTIR, MALDI-time-of-flight mass spectrometry (MALDI-TOF-MS), direct temperature resolved mass spectrometry (DTMS) and ESI-Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICRMS). The freshly pressed oil, and a heated oil were also investigated using HPLC coupled to an atmospheric pressure chemical ionisation (APCI)-MS system.

3.2. Materials and Methods

3.2.1. Chemicals and Reagents

Ammonium acetate (NH_4Ac) was purchased from Jansen Chimica, Geel, Belgium. Ethanol (EtOH), methanol (MeOH) (both p.a), acetonitrile and dichloromethane (DCM) (HPLC grade) were obtained from Merck, Darmstadt, Germany. Lead (II) oxide (99,9%), tristearoyl glycerol (approximately 99 %), and 2,5-dihydroxy benzoic acid (DHB) are products of Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands. Hexane (99,5%) was bought from Fluka Chemika, Buchs, Switzerland. Tetrahydrofuran (THF) was purchased from Biosolve Ltd., Valkenswaard, The Netherlands. Linseeds were supplied by MACOS B.V., Swifterbant, The Netherlands. Umber (Umbra gebrannt Cyprisch bräunlich), lead white (Cremnitz lead white) and vine black (Rebschwarz reines Pflanzen) pigment were purchased from Kremer-Pigmente, Aichstetten, Germany. A second type of

lead white pigment ($2PbCO_3 \cdot Pb(OH)_2$) was made according the traditional Dutch process by a private manufacturer, currently out of business.

3.2.2. Oil pressing

The oils were prepared by L. Carlyle. Flaxseed (Linseed) was first cleaned by blowing plant matter away with pressurised air and shaking the seed through a metal sieve. The cleaned seeds were subsequently ground in an electric coffee grinder. The ground meal was then placed in a custom-built stainless steel oil press [316]. Oil was expressed under a pressure of 200 to 350 kg/cm^2. The oil yield from the ground meal was approximately 22 to 23% [315].

3.2.3. Oil processing methods

Various historical recipes for oil processing were followed. Full details, including the recipes followed are included in a report by Carlyle [315]. Samples of the oils were stored in closed 3 ml vials in the dark at room temperature (18-20 ºC) which had been flushed with nitrogen.

3.2.3.1. Linseed oil (F)

Freshly pressed oil was collected in a glass pipette then transferred to clear glass bottles, which were kept stoppered until use. Oil was pressed regularly throughout the 14 weeks of the project, so that the fresh oil used for processing and paint making was never more than a maximum of a few weeks old.

3.2.3.2. Water-washed oil (W)

Following a recipe from the *Handbook of Young Artists and Amateurs in Oil Painting* [317], oil was mixed with two times its volume of water, and placed in a glass separation funnel. The oil and water were shaken together three times a day, five days a week, over a period of three weeks. The water was replaced with fresh water twice a week. At the end of three weeks, the water (plus water-soluble material which had separated from the oil) was poured off, and the oil filtered three times through cotton filter cloths.

3.2.3.3. Oil treated with drier at room temperature (D)

Lead (II) oxide was added to oil in a clear glass jar in the same ratio used for the lead-treated heated oil above (2:1 oil to drier w/v). Oil and drier were shaken together by hand, 3 times a day for one week. After two days of rest, the oil was decanted to a new container.

3.2.3.4. Heated oils (F150, F300 and D150)

Oil was heated in a round-bottom flask to an end temperature of 150 ºC and 300 ºC using a heat mantle. Maximum temperatures were reached after 15 minutes (150 ºC) and 57 minutes (300 ºC). A third oil sample was prepared by heating to 150 ºC in the same apparatus with the addition of the traditional drier, lead (II) oxide (litharge), in a ratio of 1 part drier to 2 parts oil, weight/volume. With drier added, the end temperature was reached in 18 minutes. During preparation of the oil treated with drier at elevated temperature (D150), a precipitate of drier and oil was formed in the container. This precipitate (D150Precipitate) was separated from the oil for further analysis.

Observations on the paint rheology and colour were recorded during hand grinding of paint prepared with the oils, and during application to various substrates [315]. Paints were prepared with four pigments: two different lead whites, raw umber, and vegetable black. Paint samples are being monitored with a Minolta Spectrophotometer CM2002 for colour changes during alternating periods of dark storage (yellowing) and light exposure (bleaching). The formation of film-forming defects is being recorded with macro-photography. A selection of the paints has been subjected to both natural and artificial ageing and has been analysed for chemical changes. These findings, however, will not be discussed in this chapter.

3.2.4. Analytical methods

3.2.4.1. HPSEC

The formation of dimers and higher oligomers in each oil was monitored using HPSEC. Oil samples were dissolved in THF (10 mg/µl), centrifuged and 10 µl was analysed on a Shimadzu HPSEC system, consisting of a SCL-10AD *vp* control panel, a LC-10AD *vp* pump, a DGU-14A degasser, a SIL-10AD *vp* autoinjector, a CTO-10AS column oven and a FRC-10A fraction collector (Shimadzu Benelux, ‘s-Hertogenbosch, The Netherlands). Separation was achieved on a PLGEL 5 µm 1000 Å column (300 x 7.5 mm) of Polymer Laboratories, Heerlen, The Netherlands. Three different detectors, connected in

series, were used for detection: a SPD-10A *vp* UV/VIS detector operated at 240 nm, a RID-10A refractive index detector (both Shimadzu) and a Waters 996 photo diode array (PDA) detector (Waters Chromatography B.V., Etten Leur, The Netherlands), in combination with Class *vp* 5.03 (Shimadzu) and Millenium 32 (Waters) software, respectively. The system was operated at a temperature of 40 ºC with a flow rate of 1 ml/min. Calibration was performed with polystyrene standards (Polymer Laboratories, Heerlen, The Netherlands) with an average mass ranging from 580 to 370,000 in combination with tristearoyl glycerol (m/z 890).

3.2.4.2. FTIR

Initial chemical changes in the freshly prepared oils were investigated using a FTS-6000 Bio-Rad FTIR imaging system (Bio-Rad, Cambridge, MA, USA), consisting of a Michelson interferometer (Bio-Rad FTS-6000), an IR microscope (Bio-Rad UMA-500) and a MCT narrow band detector. A small droplet of oil was applied onto a Graseby Specac PN 2550 diamond cell (Graceby Specac, Orpington, Kent, UK) and analysed in transmission mode at a resolution of 4cm^{-1}. Data were processed using Win-IR Pro 2.5 software of Bio-Rad.

3.2.4.3. HPLC/APCI-MS

Oil samples F and F300 were dissolved in acetonitrile - 2-propanol - hexane (2:2:1) yielding 3 % solutions. HPLC separation subsequently was performed using a Waters 616 liquid chromatograph equipped with a Model 996 photodiode-array (PDA) detector and a Model 717 autosampler (all from Waters, Milford, MA, USA). The separation conditions were as follows: column NovaPack C_{18} (150×4.6 mm I.D., Waters), flow rate of 1 ml/min, injection volume of 15 µl for fresh oil (F) and of 30 µl for heated oil (F300), UV detection at 205 nm, gradient of the mobile phase: 0 min - 90% acetonitrile/ethanol, 90 min - 18% acetonitrile/ethanol (i.e. 0.8%/min). The HPLC system was coupled to a VG Platform quadrupole mass analyser (Micromass, UK) using positive-ion atmospheric pressure chemical ionization (APCI). Mass spectra were obtained in the mass range m/z=35-1200, with an ion source temperature of 100 ºC, APCI heater temperature 400 ºC and cone voltage of 20 V.

3.2.4.4. DTMS

For the analysis of the freshly processed material 0.5 µl oil was dissolved in 3 ml hexane. Aliquots of about 3 µl were applied onto the analytical filament and dried *in vacuo*. The analyses were performed on a Jeol SX-102 double focussing mass spectrometer (B/E) (Jeol-Europe, Schiphol-Rijk, The Netherlands) using a direct insertion probe equipped with a Pt/Rh (9/1) filament (100 micron diameter). The probe filament was temperature programmed at a rate of 0.5 A/min to an end temperature of about 800 °C. Compounds were ionised at 16 eV under electron

ionisation conditions in an ionisation chamber kept at 180 °C, mass analysed over the range *m/z* 20-1000, with a 3 seconds cycle time. Data was processed using a JEOL MP-7000 data system. A mass defect of 0 mu at 20 amu to 0.5 mu at 1000 amu was subtracted from decimal masses to give nominal masses.

3.2.4.5. MALDI-TOF-MS

All MALDI-TOF-MS spectra were obtained using a Bruker Biflex MALDI-TOF mass spectrometer (Bruker-Franzen Analytik GMBH, Bremen, Germany). The instrument used a nitrogen laser at 337 nm (OEM VSL-337i, Laser Science, Inc., Newton, MA, USA) attenuated to about 75% of its maximum power (250 μJ), with a 3 ns pulse width for desorption/ionisation. Positive MALDI spectra were obtained by averaging 50 individual spectra, recorded using delayed extraction, the reflector mode and an accelerating voltage of 19.6 kV, unless otherwise stated. Mellitin, isatin, and polyethylene glycol mixtures with an average molecular weight of 400 and 1000, respectively, were used for internal and external calibration. A saturated solution of 2,5-dihydroxybenzoic acid was used as matrix. An amount of 3-4 μl of this solution was mixed with 0.2-0.5 μl of a 100-fold diluted oil sample with DCM. Data was processed using XMASS 5.0 software (Bruker Daltonik GmbH, Bremen, Germany).

3.2.4.6. ESI-FTICR-MS

A modified APEX 7.0e FTICR-MS instrument (Bruker-Spectrospin, Fällanden, Switzerland) equipped with a 7–Tesla superconducting magnet and an in-house designed external ion source was used for ESI-FTICR-MS measurements of the oil sampless. Details of instrumental parameters have been described before [318, 319]. The ICR cell used was a home-built open cell. The fresh oils were dissolved in DCM: EtOH (7:3, v/v) (1/100 μl) containing 10mM NH_4Ac. Ions were generated using an atmospheric pressure electrospray ionisation interface, maintained at 3-4 kV, and were subsequently trapped in the ICR cell at ~5 x 10-9 mbar. Obtained data were processed using XMASS 5.0 software (Bruker Daltonik GmbH, Bremen, Germany).

3.3. Results

3.3.1. Observations during preparation of the oils and paints

3.3.1.1 During oil processing

After the lead-treated oil was heated to 150 ^{o}C (D150) it was decanted to a fresh glass jar. Approximately 15 days after preparation the oil appeared cloudy with a whitish-grey precipitate forming at the bottom of the jar. After a month there was a large white cloud in the oil, which, when shaken, became distributed throughout causing the oil to be uniformly cloudy. Prior to shaking, a sample of the whitish-grey precipitate was taken for analysis (D150Precipitate).

3.3.1.2 During paint preparation

In some cases the oil processing method had a strong effect on the rheology of the paint produced during hand-grinding. For a full discussion of all the methods used and their effect on the paint see [315], the results below are limited to the set of oils being reported on in this chapter. Two common terms to describe paint handling characteristics (rheology) are used to describe the general characteristics of the paint: short and long. Short paint has a buttery or smooth consistency, and long paint is more fluid [320]. These terms were modified with additional adjectives, such as stringy, sticky, stiff or dense (see Table 2).

The greatest effect on paint rheology was observed with lead white ground in oil heated to 300 ^{o}C (F300, Table 2). The resulting paint was long, stringy, sticky, dense and extremely difficult to manage during application. It also had very poor covering power [321]. When the oil was only heated to 150 ^{o}C (F150), the paint was short, dense and sticky, but much more manageable. Adding drier (lead (II) oxide) and heating to 150 ^{o}C (D150) made the paint more fluid and manageable than when it was heated without drier, but it was still somewhat sticky. Lead white ground with untreated freshly pressed oil (F) was more buttery than paint made with water-washed oil (W), but the water-washed oil was the nicest of all to handle, it was creamy and pleasant to work, although slightly more viscous than lead white paint prepared with untreated oil.

With the lead white paints, the quantity of oil used did not correlate directly with paint rheology (see Table 2). For example the most fluid, that is “stringy” paint in the set, was that heated to 300 ^{o}C (F300) yet the percent oil by weight was only 13.8% compared to the least fluid paints which took 17.2% (D) and 20.6% (F150). Paints made with umber and vegetable black pigments did not exhibit the range of difference between long and short experienced with the lead white. Although there were some differences according to the oil processing method,

these paints generally tended to be short, none developed the fluidity and length seen in some of the lead whites. During grinding in the oil heated to 300 ºC (F300) and the lead-treated oil heated to 150 ºC (D150), the lead white pigment gave the impression that it was "dissolving" into the oil. This did not occur while grinding the umber and vegetable black.

Table 2. Oil processing and paint rheology.

Oil processing method[a]	Percent oil by weight to produce 25 g. Dutch Process lead white	Observations : Handling quality while brushing[b]
F	13.8%	Short
F150	20.6%	Short and sticky and dense
F300	13.8%	Long, sticky, stringy, not a viable paint
D	17.2%	Short and stiff
D150	13.8%	Long and sticky
W	19.3%	Short and very creamy (pleasant to work)

[a] Oil code summary
F = Freshly pressed oil (untreated) (Z in [315])
F150 = Freshly pressed oil heated to 150^{o} C. (ZH150 in [315])
F300 =Freshly pressed oil heated to 300^{o} C. (ZH300 in [315])
D = Oil treated with drier at room temperature (A-2 in [315])
D150 = Oil treated with drier while heating to 150^{o} C. (AH2 in [315])
W= Freshly pressed oil washed in water for 3 weeks. (X in [315])
For a detailed explanation of codes see experimental section
[b] Definitions of terms are given in text

3.3.2. HPSEC

HPSEC analysis was applied to obtain information on the formation of cross-linked materials in the processed oils and to monitor the degree of de-esterification and oxidation. The molecular weight distributions of the oils F, F150 and F300 are shown in Figure 1. The peak eluting at t_R=8.7 minutes can be ascribed to intact (unsaturated) TAGs based on standards establishing the elution times of a saturated tristearoyl glycerol and a free stearic acid which were determined to be 8.7 and 9.5 minutes, respectively. The material detected at longer retention times suggests the formation of low molecular weight breakdown products resulting from oxidation or hydrolysis of the ester bonds. The latter leads to the evolution of free fatty acids, unless the fatty acid had already formed a cross-link prior to hydrolysis. Breakdown products can consist of diacylglycerols (DAGs), dimeric fatty acids, monoacylglycerols (MAGs) or free fatty acids. Another possibility is that material is retained on the column upon elution, which leads to a tail in the chromatogram. Peaks with a decreased retention time

compared to the elution time of TAGs are ascribed to oligomerised TAGs or combinations of breakdown products with TAGs.

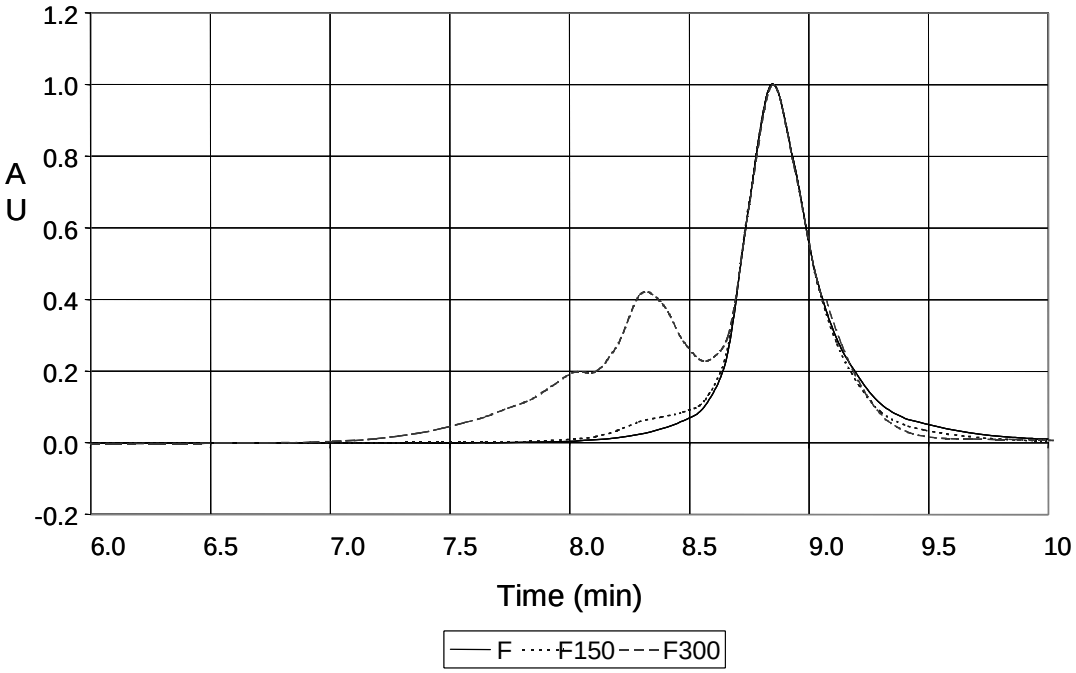


Figure 1. HPSEC chromatograms of oils F, F150 and F300.

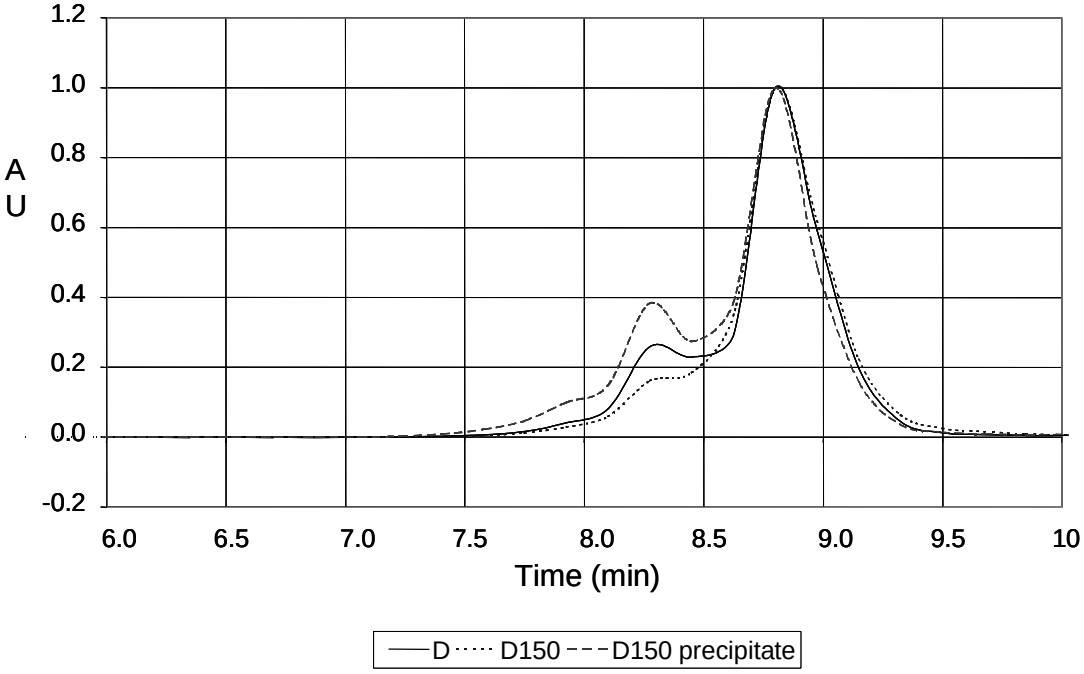


Figure 2. HPSEC chromatograms of oils D, D150 and the THF soluble part of D150Precipitate.

It is clear from a comparison of the molecular distributions that heating led largely to formation of higher molecular weight cross-linked TAGs (see Fig. 1). The effect of heating to 150 °C is less pronounced than to heating to 300 °C. Peaks eluting at 8.3 and 8.02 minutes are ascribed to dimeric and trimeric TAGs, respectively. This material can be formed by heat-induced Diels-Alder reactions [113, 178] or cross-linking upon autoxidation. An approximate molecular weight scale determined from calibration with the standard series (not shown) suggests that oligomers upto 6-mers (molecular weight ± 5400) are present in the oil heated to 300 °C. Relatively low amounts of low molecular weight products were detected in both of the heated oils (F150 and F300).

Water washing of the oil did not lead to great alterations as a chromatogram similar to the fresh oil was obtained (results not depicted). The addition of litharge as drier, however, led to increased amounts of dimeric material as can be seen in Figure 2 for oil D. This is likely due to lead catalysed oxidation leading to higher amounts of free radicals, and subsequent cross-linking. The same phenomenon was observed for the heated mixture of drier and oil (D150).

The results of HPSEC analysis of the THF soluble fraction of the precipitate, depicted in Figure 2, indicate that the whitish-grey precipitate (D150Precipitate) is enriched in high molecular weight material relative to the rest of the sample D150 and to the oil mixed with the drier alone (D). It is likely that it precipitated as the cross-linked material formed because its increased lipophobicity led to reduced solubility in the oil. The tendency of the cross-linked material to accumulate at the bottom of the jar means that oil in the uppermost part of the container will contain less cross-linked material than anticipated given the treatment carried out with heat and the drier.

Inspection of the photodiode array detector data (Table 3.) shows that there is a correlation between the extent of cross-linking and an increase in the maximum absorptivity wavelength. This was observed as early as 1926 by Stutz [239] who studied the light absorption of different commercial linseed oils. There is an increase by a few nm in the maximum wavelength observed in the monomeric material for the untreated oil F, and the heat-treated oils (F150 and F300) (Table 3), which indicates the formation of an absorbing conjugated double bond system. The value obtained for the oil heat-treated with drier (D150) is lower than the value for the oil treated with drier but without heat (D), and the highest value of all samples is found for the precipitate (D150Precipitate), which confirms that the number of chromophores is mostly in the higher molecular weight material. This is also reflected by the fact that the maximum wavelength is highest for the dimeric material in most oils. Interestingly, according to the data in Figure 1, the maximum wavelength for the water washed oil has increased despite its unchanged molecular weight. The higher value for the maximum wavelength appears to indicate water washing has some effect on the double bond system of the TAGs. *Cis-trans* isomerisation of the unsaturated fatty acids upon (phot)oxidation during the relatively long washing procedure may account for this since the oil was placed on the window-sill in a clear glass container.

Table 3. Absorption maximums of mono and dimeric TAG compounds (Retention times 8.7 and 8.15 minutes, respectively) in processed oils[a] as determined with photo diode array detection.

Oil Sample	Maximum (nm) (t_R =8.7)	Maximum (nm) (t_R =8.15)
F	219.5	-
F150	220.7	-
F300	234.8	234.8
D	234.8	237.1
D150	221.8	234.8
D150Precipitate	237.1	237.1
W	233.6	-

[a] For an explanation of codes used see experimental section.

3.3.3. FTIR

Analysis of the processed oils by FTIR is the means to obtain information on the degree of oxidation and hydrolysis and changes in double bond conformation. A number of characteristic spectral features can be seen in the FTIR spectra of the oils F and F300 (see Figure 3). There are 4 distinctive peaks in the 3000 cm^{-1} region that can be attributed to C-H stretching vibrations, which appear at 3010, 2956, 2927, and 2855cm^{-1}, respectively. An intense carbonyl band of the ester linkages of TAGs can be seen at 1746 cm^{-1} whereas no distinct signal indicative for the formation of free fatty acids is observed at 1700 cm^{-1}. Several bands from both the fatty acid chain and the carboxylic acid functional groups are seen in the region 1600-600 cm^{-1}. The assignments of the different bands are listed in Table 4. The spectra for both oils look very similar except for the absorption bands that are assigned to the presence of *cis* and *trans* unsaturations. A distinct band at 968 cm^{-1} emerges in the spectrum of the heat-bodied oil F300, on top of a broader distribution ranging from 920-1080 cm^{-1} while the bands at 3010 and 722 cm^{-1} have decreased. This implies that non-conjugated *cis* olefinic bonds have been mostly transformed to non-conjugated *trans* double bonds, and in minor quantities to conjugated *trans* double bonds (a closer inspection of the FTIR spectra revealed new bands of low intensity at 988 and 947 cm^{-1}). The ratios of the areas of the bands at 3010 and (2927,2855) cm^{-1} have been determined for the different oils and are depicted in Table 5. It is thought that during oxidation and/or cross-linking the number of CH_2 groups initially stays intact whereas the relative amount of cis-double bonds in the oil will decrease. It should be noted at this point that upon advanced oxidation a broadening and a lowering of the bands at 2927 and 2855 is expected [252]. It can be seen in Table 5 that the relative amounts of *cis* C=C bonds decreases upon heating (compare F vs F150 and F300). There is very little difference between the unheated lead-treated oil D relative to the untreated oil F, indicating that fewer double bonds are involved in the initial cross-linking process when the sample is not heated. Upon heating in the presence of a drier (D150), the

amount of consumed double bonds is slightly increased, in accordance with the observation for oil F150. The findings for D and F suggest that formation of oligomeric material is mainly due to radical-radical recombinations in the autoxidation process, in contrast to the more polymeric F300, which showed a much larger relative decrease in *cis*-double bonds. This suggests that Diels-Alder condensation takes place when the oil is heated as high as 300 ºC. The water-washed oil only shows a slight increase in the ratio of the bands at 3010 and (2927,2855) cm^{-1} compared to the untreated oil F, which is in contrast to the large change in its light absorption observed with PDA detection (HPSEC).

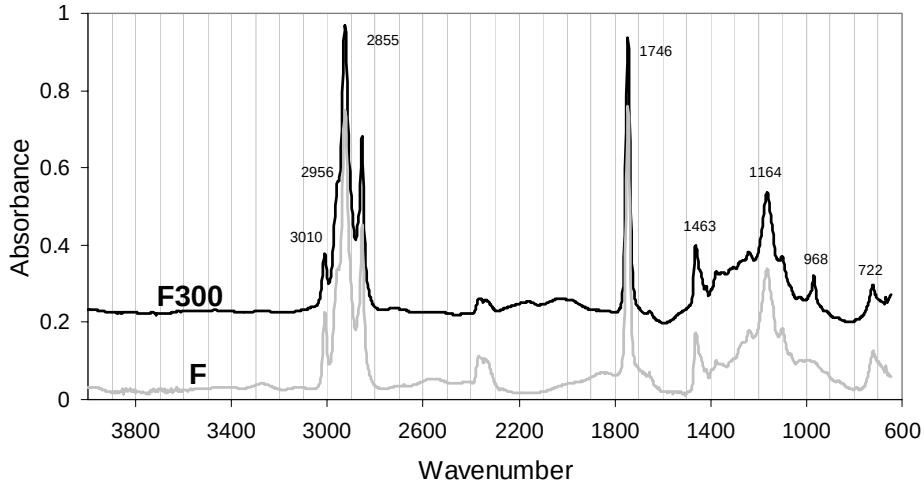


Figure 3. FTIR spectra of oils F and F300.

Table 4. Tentative absorption band assignments for the peaks observed in the processed oils.

Band (cm^{-1})	Assignment
3010	C-H stretching of aliphatic –CH=CH–
2956	C-H stretching of CH_3
2927	C-H stretching of CH_2
2855	C-H stretching of CH_2
1746	C=O stretching of ester
1652	C=C stretching of *cis* –CH=CH–

1463	C-H bending of CH_2, CH_3
1239	C-O stretching in ester
1164	C-O stretching
1101	C-O stretching
968	*trans* C-H out-of-plane deformation
722	*cis* C-H out-of-plane deformation

Table 5. Ratios of the FTIR absorption bands area of *cis* CH=CH (3010 cm^{-1}) and CH_2 (2927+2855 cm^{-1}) in FTIR data of processed oils [a].

Oil sample	CH2/*cis* CH=CH
F	18.90
F150	20.14
F300	30.31
D	19.69
D150	20.69
W	19.06

[a] For an explanation of codes used see experimental section.

3.3.4. HPLC APCI-MS

Changes in the processed oils due to oxidation and heat-induced isomerisation of double bonds can be investigated by HPLC-APCI-MS. Only the untreated freshly pressed oil F and the heated F300 were analysed in this study. The comparison of the chromatographic records with UV detection at 205 nm of fresh linseed oil (Fig. 4a) and of heated linseed oil (Fig. 4b) is shown. Only the part of chromatogram with TAGs is selected, because the greatest differences between these samples can be observed in this region. In the case of fresh oil, the identification of particular peaks is easy and unambiguous based on their positive-ion APCI mass spectra [299]. The molecular weights can be determined from protonated molecules $[M+H]^+$ and adducts with ammonium $[M+NH_4]^+$ or sodium ions $[M+Na]^+$. The characteristic fragment ions $[M+H-RCOOH]^+$ and $[RCO]^+$ enable the identification of all acyl chains, as indicated in Fig. 4a. The m/z values of observed ions are listed in Table 6. The identification of individual peaks in

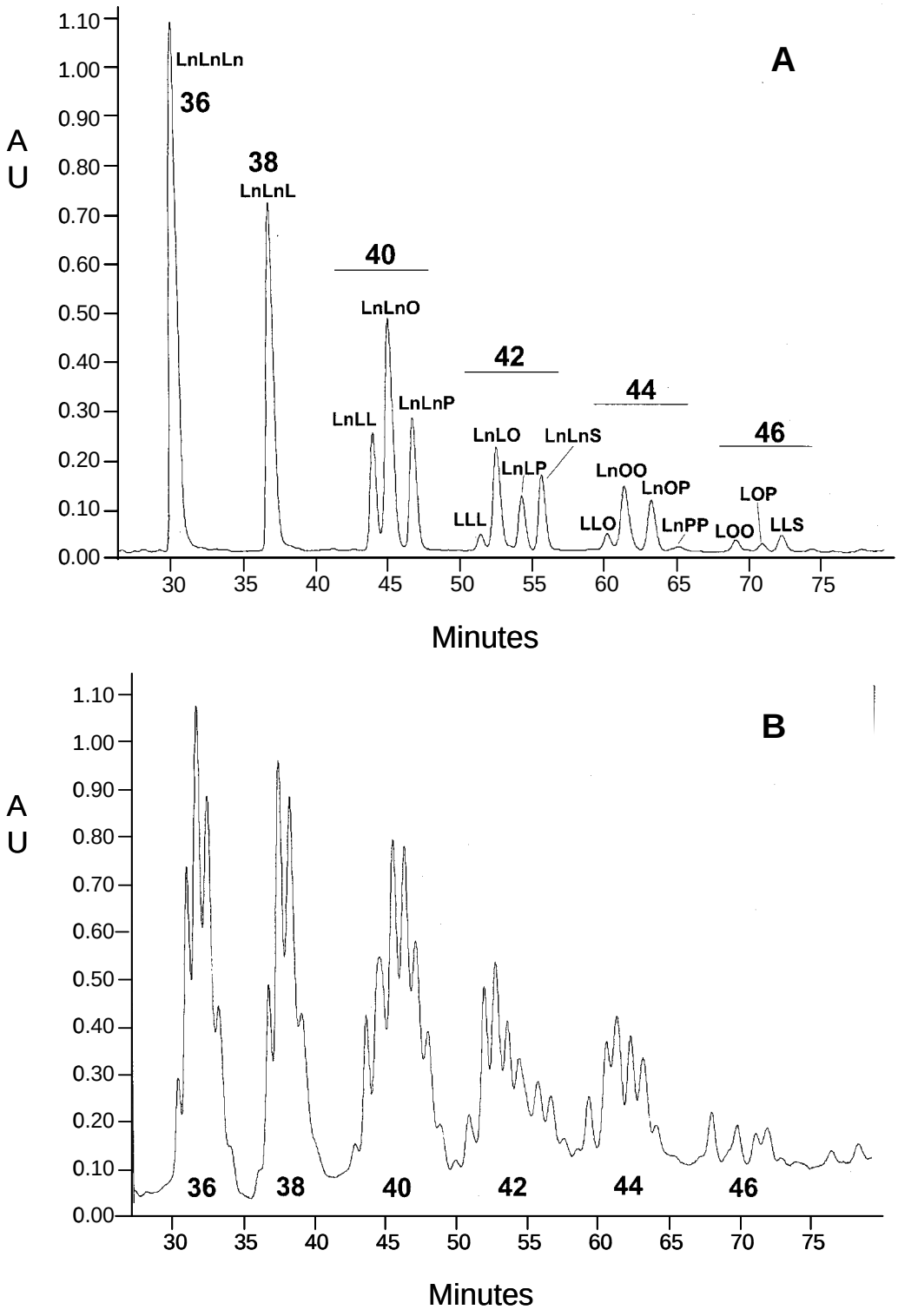


Figure 4. HPLC chromatograms obtained with UV detection at 205 nm of (a) fresh linseed oil (F), and (b) heated linseed oil (F300). HPLC conditions as in Experimental. Numbers correspond to equivalent carbon number (ECN).

heated linseed oil is much more difficult due to the coelution of chromatographic peaks. Another problem is the significantly decreased relative response despite doubled injection volume (30µl), because the total signal is divided among many peaks and the resulting response of individual peaks is therefore reduced. A decrease of UV-absorbing groups at 205 nm is another possibility, which explains diminished signal intensity. The highest UV response is 1.1 AU for unheated oil in comparison to 0.11 AU for heated oil (see Fig. 4a/b).

APCI mass spectra are identical for "single" peaks in the chromatogram of fresh oil (Fig. 4a) and for corresponding "multiplet" peaks with the same ECN in the chromatogram of heated oil (Fig. 4b), which confirms that the molecular weights and also masses of acyl chains are identical. If the total ion current is compared with the reconstructed ion currents of characteristic TAG ions (see Table 6) for LnLnLn in the time window 28 - 35 min for heated oil, the same time profiles are obtained and no additional ions are found in heated oil. Therefore new TAGs in heated oil can differ due to the *cis/trans* isomerisation, the migration of double bonds or the formation of cyclic fatty acids. These possibilities can be expected, as indicated by results obtained by FTIR and HPSEC.

The UV spectra in the range 195 - 500 nm were obtained for all chromatographic peaks using photo-diode array detection. The UV spectra of all peaks in the chromatogram of fresh oil (Fig. 4a) are identical with only one absorption maximum at 203 nm, as can be expected for TAGs. If the UV spectra of the group of peaks with the same ECN (Fig. 4b) is averaged, then a second minor band is observed in the region 225 - 255 nm for all groups of peaks. Attention was focused on five explicit peaks with ECN = 36 (the notation: No. 36a - the first peaks with t_R = 30.2 min, No. 36e - the last peak with t_R = 33.0 min). If the UV spectra are obtained only from the narrow time window at the top of peaks, then the maximums of the secondary UV bands can be determined: No. 36a - 254 nm, No. 36b - 252 nm, No. 36c - no secondary maximum, No. 36d - 233 nm and No. 36e - 231 nm. It clearly confirms the presence of compounds with conjugated double bonds in peaks referred as 36a, 36b, 36d and 36e, while the peak 36c probably corresponds to LnLnLn. A similar behaviour can be observed for the peak with ECN = 38, where the following UV maximums were determined (peak notation in the same way): No. 38a - 247 nm, Nos. 38b and 38c - no significant maximum, No. 38d - 232 nm. This approach cannot be applied to other peaks, because more than one compound corresponds to other ECN values. This result clearly confirms that the double bond migration occurs to some extent upon heating. Whether *cis/trans* isomerisation occurs cannot be deduced from the presented HPLC/MS data.

Wide and overlapping peaks with low abundances are observed in the time window 10 - 26 min (not shown) with characteristic ions differing by 16 m/z units (e.g. 871.7 and 887.7, 873.7 and 889.7, 875.7 and 891.7), which suggests the presence of oxidised TAGs. Each TAG can in principle lead to a large number of oxidised isomers differing in the position of oxidation, the number of oxygen atoms incorporated, and degree of *cis/trans* isomerisation. The compounds will have very similar but not identical chromatographic behaviour, which causes extensive peak splitting and broadening and makes the interpretation difficult. In

the same way as the non-oxidised TAGs, a decrease of sensitivity is observed due to splitting of the total signal among many peaks. There are two possible solutions: First, MS analysis without HPLC which would give better sensitivity, but unfortunately compounds with the same molecular weights and different structures cannot be distinguished. The second option is optimisation of the separation conditions [297], but this is time-consuming and complete separation of all positional isomers of oxidation products has not been achieved so far.

Table 6. Equivalent carbon numbers (ECNs) and m/z values of characteristic ions of identified triacylglycerols (TAGs) by HPLC/APCI-MS (for better clarity, the decimal places are not shown in the table. They are approximately XXX.7 for $[M+H]^+$, XXX.5 for $[M+H\text{-}RCOOH]^+$ and XXX.3 for $[RCO]^+$).

TAGS[a]	ECNS	$[M+H]^+$	$[M+H\text{-}RCOOH]^+$	$[RCO]^+$
LnLnLn	36	873	595	335
LnLnL	38	875	595, 597	335, 337
LnLL	40	877	595, 597	335, 337
LnLnO	40	877	595, 601	335, 339
LnLnP	40	851	573, 595	313, 335
LLL	42	879	599	337
LnLO	42	879	597, 599, 601	335, 337, 339
LnLP	42	853	573, 575, 597	313, 335, 337
LnLnS	42	879	595, 601	335, 341
LLO	44	881	599, 601	337, 339
LnOO	44	881	599, 603	335, 339
LnOP	44	855	573, 577, 599	313, 335, 339
LnPP	44	829	551, 573	313, 335
LOO	46	883	601, 603	337, 339
LOP	46	857	575, 577, 601	313, 337, 339
LLS	46	883	599, 603	337, 341

[a] TAG fatty acids: P=palmitic, S=stearic, O=oleic, L=linoleic and Ln=linolenic acid

HPLC/APCI-MS analysis of autoxidation products (incubation for 1 - 21 days at 50 – 60 ºC in the dark) [297] of pure standards of triolein, trilinolein or trilinolenin enabled the identification of main oxidation products, e.g. mono- and bishydroperoxides, mono- and diepoxides, hydroxy TAGs, epidioxides and

hydroperoxide epidioxides. The retention of identified oxidation products is in agreement with our findings. Two things should be pointed out: groups of broad and multiplet peaks of particular classes of oxidation products are observed (see Fig. 1 in discussed paper [297]), APCI mass spectra of particular compound classes yield many fragment- and adduct ions, which reduces S/N ratio. Compared to the oxidation of one pure TAG, the number of possible oxidation products in vegetable oil is much higher and this prevents a reasonable interpretation of the HPLC/MS data on the oxidation products in our study.

Small amounts of diacylglycerols (DAGs) were positively identified in the front part of the chromatogram (not shown): 1,3-LnLn (t_R = 6.46 min), 1,2-LnLn (6.87 min), 1,3-LnL (8.78 min) and 1,2-LnL (9.49 min). Cross-linked products of TAGs cannot be studied with the current HPLC/MS method, because such species are retained on the chromatographic column under the HPLC conditions used.

3.3.5. DTMS

When a complex mixture of materials is heated in the ion source, as is the case with DTMS, initially volatile, lower molecular weight material will evaporate and at higher temperature less volatile oxidised, i.e. more polar, and/or high molecular weight material will be brought into the gas phase. In cases where the sample cannot be evaporated pyrolysis of the organic material will take place by thermally assisted bond breaking. This will lead to a separation in the relative short time period of 2 minutes of the different materials present in the sample. In order to detect the gaseous compounds with mass spectrometry electron ionisation is applied which makes it possible to quickly determine the presence of free fatty acids, oxidation products and cross-linked material in processed oils. The analysis of TAGs using direct insertion mass spectrometry is well known [37, 322-324] but the 70 eV electron ionisation most frequently utilised causes extensive fragmentation and reduced intensities of molecular ions [307]. Therefore, 16 eV EI ionisation was applied in this study, which leads to less fragmentation and an increased abundance of the molecular ions. Furthermore, in the case of DTMS the sample is introduced into the ion source on a Pt/Rh (9/1) filament, which is resistively heated, instead of a fixed temperature that has been used in direct insertion mass spectrometric analysis of oils.

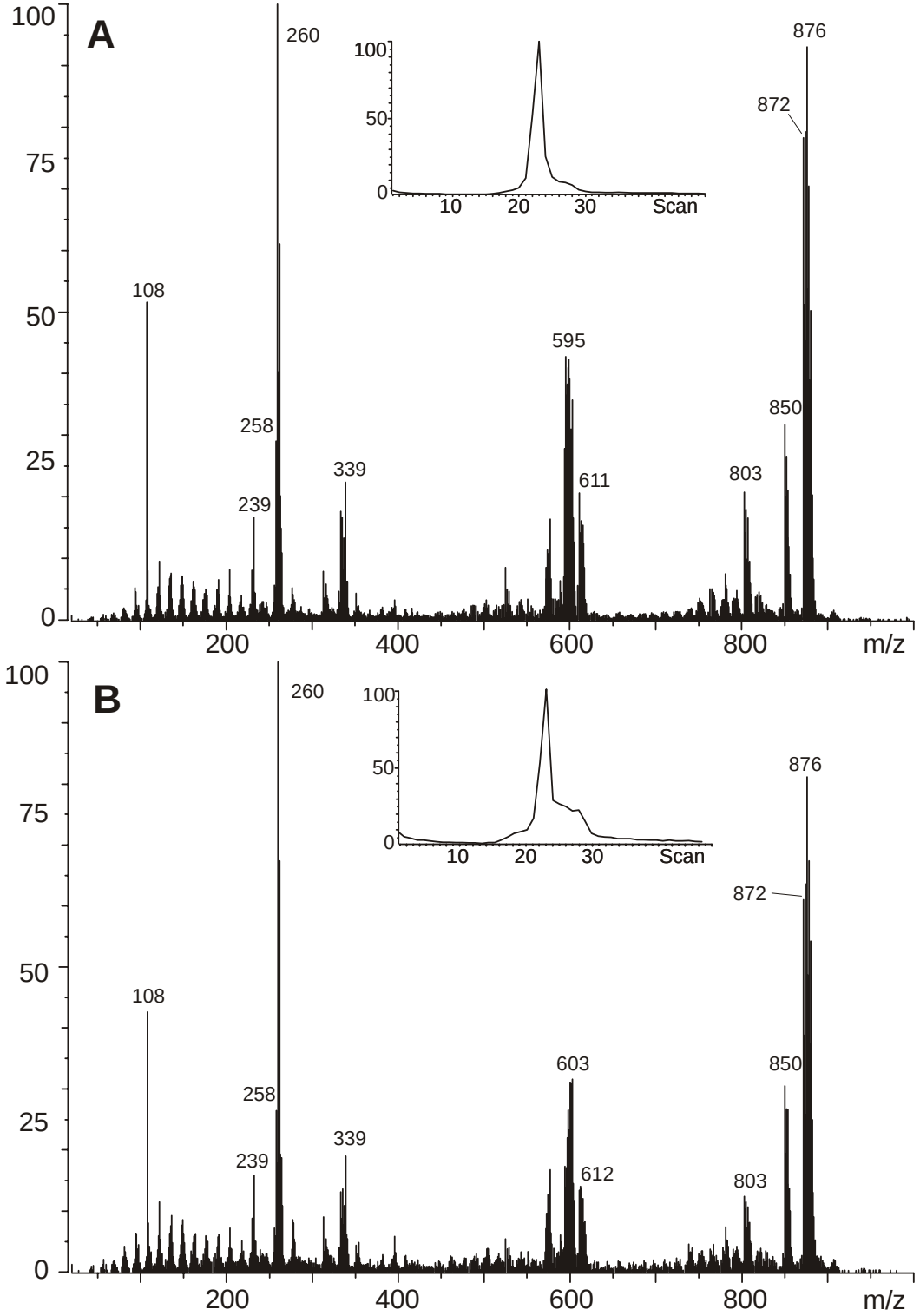


Figure 5. DTMS mass spectrum of (a) oil F, and (b) oil F300 (scan 23-25). Insert: Total Ion Chromatogram (TIC).

In the insert of Figure 5a, the total ion current (TIC) is depicted for oil F. It can be seen that most of the sample evaporates within a relatively small time domain, pointing towards a low degree of polymerisation. According to the TIC, free fatty acids, MAGs and DAGs are almost absent. These materials are expected to evaporate at lower temperature [28]. The mass spectrum (Fig. 5a) of the summed scans 23-25 (see insert Figure 5a) shows intact molecular ions (m/z 848-884), fragments due to the loss of one or two intact (un)saturated fatty acids (m/z 570-620 and 310-344, respectively) and the acylium ions of saturated C16 and (un)saturated C18 fatty acids (m/z 239, 258-267). Fragment ions at m/z 258 are ascribed to a triple unsaturated acylium ion that has lost two additional hydrogens [307]. The most important molecular ions and fragments are assigned in Table 7. Closer inspection of the mass region just above the molecular ions reveals two small clusters with an increased mass of 16 and 32, respectively, pointing to incorporation of 1, respectively 2, additional oxygen atoms.

Table 7. Most important (fragment) ions of TAGs observed upon 16 eV direct temperature resolved mass spectrometry analysis of linseed oil.

Mass(es)	(Fragment) Ion	Composition
872-888	M^+	$C_{57}H_{110-2n}O_6$ (n=1,2,3,…9)[a]
850-860	M^+	$C_{55}H_{106-2n}O_6$ (n=1,2,3,…6)
805-819	$[M-C_5H_9]^+$	$C_{52}H_{101-2n}O_6$ (n=1,2,3,…8)
783-791	$[M-C_5H_9]^+$	$C_{50}H_{97-2n}O_6$ (n=1,2,3,…5)
613, 614,…,619	$[M+O-RCOO(H)]^+$ [b]	$C_{39}H_{74-2n}O_5$ (n=1,2,3,4)
594, 596,…,604	$[M-RCOOH]^+$	$C_{39}H_{74-2n}O_4$ (n=1,2,3,…6)
595, 597,…,605	$[M-RCOO]^+$	$C_{39}H_{75-2n}O_4$ (n=1,2,3,…6)
572, 574,…,578	$[M-RCOOH]^+$	$C_{37}H_{70-2n}O_4$ (n=0,1,2,3)
573, 575,…,579	$[M-RCOO]^+$	$C_{37}H_{71-2n}O_4$ (n=0,1,2,3)
331, 335,…,341	$[RCO+74]^+$	$C_{21}H_{41-2n}O_3$ (n=0,1,2,3)
313	$[RCO+74]^+$	$C_{19}H_{37}O_3$
259, 261, 263, 265, 267	$[RCO]^+$	$C_{18}H_{35-2n}O$ (n=0,1,2,3,4)
258, 260, 262, 264	$[RCO-H]^+$	$C_{18}H_{34-2n}O$ (n=1,2,3,4)
230, 232, 234	$[R-H]^+$	$C_{17}H_{35-2n}$ (n=1,2,3)
108	$[R-C_9H_{17}]^+$	C_8H_{12}

[a] n denotes the number of double bonds present.
[b] R denotes the (unsaturated) carbon chain attached to the carboxylic acid.

On first inspection, the mass spectrum of summed scans 23-25 of the heated oil F300 (Figure 5b) looks very similar to the previous spectrum. However, when the lower masses of each cluster are examined it is immediately clear that heating results in the diminishing of the highly unsaturated molecules relatively to the less unsaturated species. This phenomenon confirms that upon heating and/or oxidation TAGs with double bonds are consumed to form oligomeric material, which is also suggested by the TIC (see insert) that shows there is relatively more material detectable at higher temperature indicative for the more polar and/or

polymeric nature of the material. Intact molecular ions of cross-linked TAGs are not detectable with the settings used.

The quantity of the different TAGs present in the oils was determined using the relative intensity of the molecular ion peaks. The contribution of the second isotope peak, which has an intensity of 20% relatively to its molecular ion for the all C18 TAG, was taken into account. Our analytical results on the composition of the fresh untreated oil F are in reasonable agreement with direct insertion measurement values reported for untreated linseed oil in literature [37]. Relative changes between the oils as a result of different processing methods can be detected easily (see Table 8) although absolute quantification is not achieved in this way because of differences in ionisation efficiency, fragmentation behaviour upon electron ionisation, and susceptibility towards oligomerisation and/or oxidation of the TAGs. Heating leads to a relative decrease of TAGs with triple unsaturated fatty acids (m/z 872-876) and therefore a relative increase of TAGs without linolenic acid groups. This is most clearly seen for oil sample F300. The addition of lead drier to the oil leads to a small decrease in the relative number of TAGs with the more highly unsaturated fatty acids. This becomes evident when comparing sample F and D. The presence of the drier enhances oxidation and as a consequence the decrease of more reactive triple unsaturated species will be faster [173]. The composition of the precipitated material, D150Precipitate, was clearly different from the oil above (D150) and a strong reduction of the triple unsaturated fatty acids is observed. The trends observed in Table 8, point to Diels-Alder type reactions that lead to oligomerisation, although loss of highly unsaturated fatty acids by oxidation cannot be excluded. Part of the oxidised species, especially the hydroperoxides are not stable and will fragment upon electron ionisation, which makes quantification of the oxidation process by DTMS difficult without prior derivatisation.

Table 8. Percentages of different TAGs present in the processed oils, as determined by DTMS[a].

TAG Mol. wt	F[b]	F150	F300	D	D150	D150 Precipitate	W	TAG name[c, d]
828	0.52	0.54	0.84	0.56	0.62	0.70	0.71	PPO
830	0.29	0.33	0.65	0.36	0.39	0.13	0.28	PPL
832	0.31	0.33	0.41	0.12	0.22	0.16	0.24	PPLn
850	7.88	7.86	7.93	7.67	7.98	8.24	8.81	PLnLn
852	5.06	5.08	5.42	4.97	5.49	5.68	5.37	PLLn
854	3.95	3.96	5.63	4.68	4.62	5.41	4.88	PLL
856	0.76	0.89	1.19	0.80	1.17	1.19	1.06	POL/PSLn
858	0.33	0.26	0.46	0.31	0.25	0.37	0.32	PSL

860	0.21	0.18	0.20	0.18	0.17	0.15	0.15	PSO
872	19.69	19.36	16.02	19.42	18.42	16.03	19.00	LnLnLn
874	15.99	15.76	13.54	15.43	15.12	14.13	14.96	LnLnL
876	19.40	19.08	18.05	18.76	18.54	17.77	18.17	LnLnO/ LnLL
878	12.99	12.74	13.47	12.94	12.55	13.49	12.39	LnLO/ LnLnS
880	9.05	9.23	10.72	9.21	9.84	10.76	9.45	LnOO/ LnLS/OOO
882	2.51	2.56	3.68	3.31	3.59	3.84	3.25	SOLn/LOO/ SLL
884	0.62	0.67	1.04	0.79	0.73	1.08	0.65	SOL/OOO/ SSLn
886	0.09	0.05	0.21	0.21	0.20	0.36	0.14	SOO/SSL
888	0.10	0.11	0.55	0.23	0.07	0.47	0.15	SSO

[a] See Materials and Methods section for DTMS conditions.
[b] See experimental section for explanation of codes
[c] TAG fatty acids: P=palmitic, S=stearic, O=oleic, L=linoleic and Ln=linolenic acid
[d] Different isomers are possible, e.g. 886

3.3.6. MALDI-TOF-MS

MALDI-TOF-MS was applied to study the oxidation and formation of oligomeric material in more detail. Analysis of the oils with MALDI-TOF-MS in both the linear and reflectron mode confirm that intact molecular TAG ions, cross-linked and/or oxidised products thereof and minor amounts of low molecular weight materials are present in all of the oils studied. A typical example of an analysis in both the linear and reflectron mode is given for oil F300 in Figures 6 and 7, respectively. Besides the two clusters of intact sodiated TAGs with 55 and 57 carbon atoms, respectively, small amounts of protonated diacylglycerol (DAG) fragment ions are observed for all the oil samples at m/z 573-579 and 595-605 [325, 326]. Only samples F300 and to a lesser extent D150Precipitate showed significantly higher signals for these fragments, although identical laser power was used. In addition, trace amounts of intact sodiated DAGs are observed ranging from m/z 635 to 643, in all samples except for the untreated oil. This indicates that hydrolysis is not a major process occurring during the treatment of the oils, which is in accordance with the results obtained for the other analytical techniques previously described.

Table 9. Overview of MALDI-TOF-MS measurements of processed oils.

Sample	Maximum number of oxygen atoms[a] incorporated into TAGs	Maximum number of cross-linked TAGs[b]
F	1	1
F150	2	2
F300	4	6
D	4	3
D150	4	2
D150Precipitate	6	4
W	4	2

[a] As obtained with reflectron mode measurements.
[b] As obtained with linear mode measurements.

Incorporation of oxygen into the TAGs is seen for all oil samples and in the case of highest oxidised oils for the DAGs as well. Oxidised products were already present before the freshly pressed oils underwent further processing. Different numbers of oxygen atoms are built-in depending on the processing method as can be seen in Table 9 and Figures 8a-e. In these figures a partial mass spectrum is depicted of the mass range of the monomeric TAGs and the oxidised species of some of the oils analysed. Insertion of oxygen is observed, in for instance m/z 896, $[LnLnLn+Na]^+$, that becomes m/z 912 (See Fig 8c for the details).

It is clear from these results that a rather large number of different species is formed upon (thermal-)oxidation of the linseed oil. Dimers were observed in the mass spectra recorded in the reflectron mode for samples F300 and D150Precipitate, next to the monomeric TAGs. The observed masses indicate that no oxygen is present in these dimers, which would imply they are formed by a Diels-Alder cyclisation reaction. Carbon-carbon links due to radical-radical combinations can be formed theoretically also but are less likely since closer inspection of these dimers reveals no hydrogens that seem to be consumed. The most unsaturated species that is detected contains an equivalent of 18 double bonds, hence two times 9. Theoretically it cannot be excluded that the “dimers” consist of non-covalently bound complexes that are formed during the MALDI analysis. The SEC results are more straightforward in this respect, and do confirm the presence of cross-linked material in the oils. Analysis of the oils using the linear mode shows (see Table 9) that most oils contain higher molecular weight material. The highest degree of cross-linking is observed in oil sample F300 (Figure 6), which contains traces of oligomeric TAGs up to hexamers. This is in agreement with the upper mass calculated from the HPSEC results. The HPSEC results of the remaining oils fit nicely with the linear MALDI-TOF-MS data.

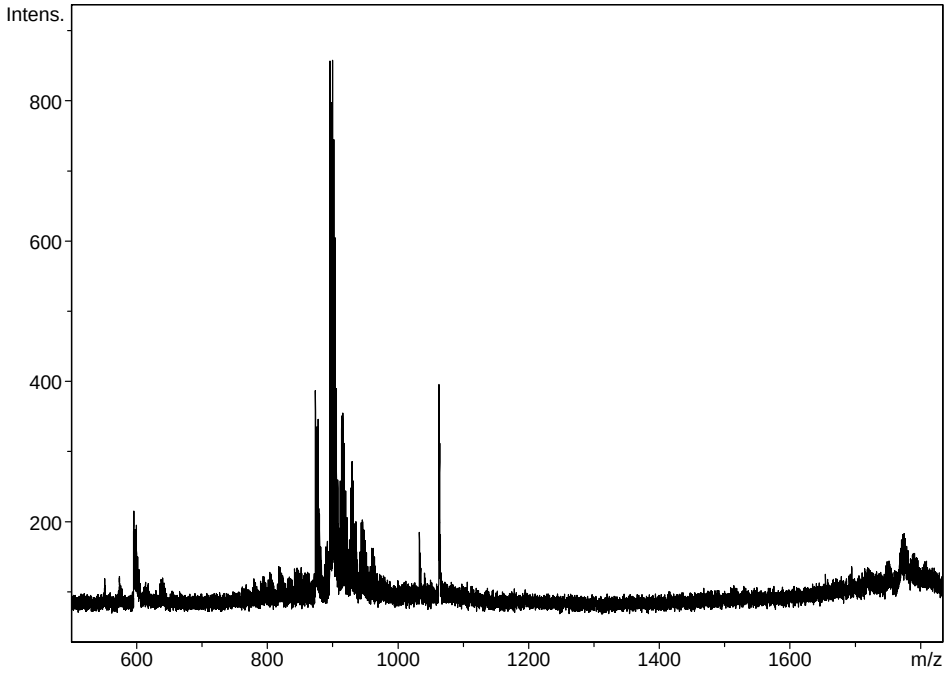


Figure 6. MALDI-TOF-MS spectrum of oil F300 (Reflectron mode)

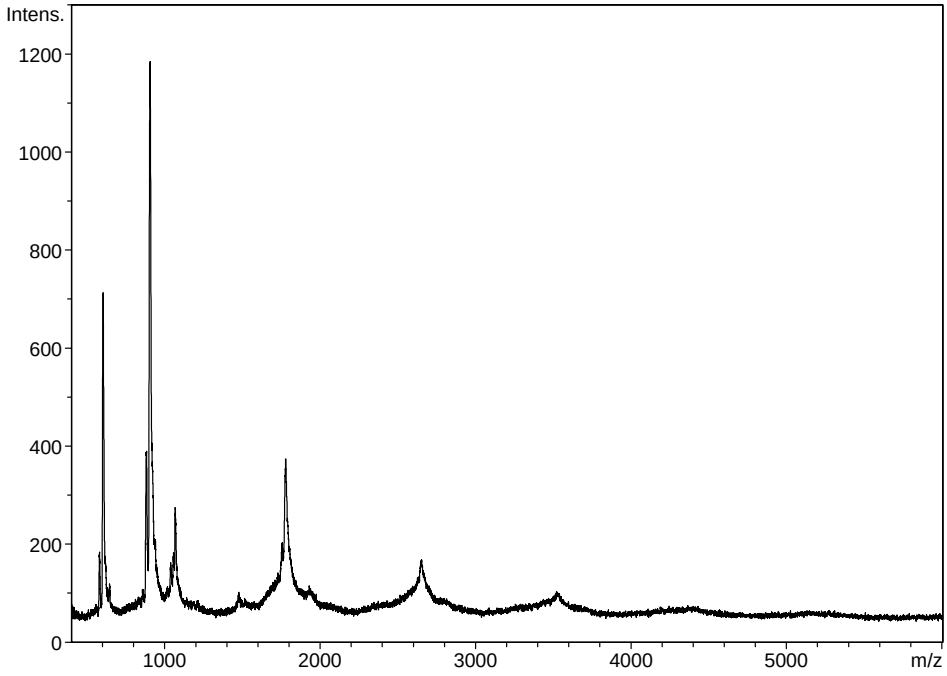


Figure 7. MALDI-TOF-MS spectrum of oil F300 (Linear mode).

3.3.7. ESI-FTICRMS

High resolution ESI-FTICRMS was used as well in order to determine the exact elemental composition of the TAGs in the oil and primarily, the oxidised compounds formed upon processing. Due to the softer nature of the electrospray ionisation technique compared to MALDI, less fragmentation of the TAGs is expected. Figure 9 shows the ESI-FTICR mass spectrum of oil F150. Ammonium cationised molecular ions are observed of both intact TAGs and trace amounts of DAGs. The exact masses obtained for the different TAG species, including the error in mass assignment, and their identities are depicted in Table 11. As can be seen the observed masses agree well with the true values. The loss of fatty acids, giving rise to protonated fragment ions as was seen with MALDI-TOF-MS, is not observed here. In the TAG mass window three major clusters are detected: a cluster of TAGs that consists of two (unsaturated) C18 and one C16 fatty acyl chain, a cluster with only (unsaturated) C18 fatty acyl chains and their monooxygenated derivative, as determined by the mass increment of m/z 15.993. The exact mass of oxygen (^{16}O), 15.9949, agrees well with this mass difference in the third cluster. In total, the incorporation of 4 additional oxygen atoms is observed for the monomeric TAGs. In the higher mass region three clusters are clearly visible. In the mass window between 1475 and 1525, low intensity ammonium cationised molecular ions can be found (see also Table 10), which are ascribed to a combination of TAGs and DAGs (see insert A, Fig. 9). No oxygen incorporation is observed. The two highest clusters in the dimer region (m/z 1710-1820, see insert B) are formed by the combination of two non-oxidised TAGs. Isolation of some of these dimeric ions, followed by collisionally induced dissociation (CID) using argon as collision gas, resulted in the formation of monomeric TAGs with a maximum number of 9 double bonds and a double bond distribution in accordance with the number of double bonds originally present in the dimer (results not shown). Although data on the monomeric TAGs showed the loss of one of the fatty acyl chains upon ester cleavage under the same MS/MS conditions, this was not observed for the dimeric material. These findings suggest that part of the dimers is not covalently bound but exist as a complex in the gas phase, formed during the ionisation process. Dimers with one or two oxygens and trimeric TAGs are observed as well (see insert of m/z 2590 – 2700, Fig. 9C). The signal of these clusters already starts to overlap and it is clear that upon further oxidation as the oils dry a rather complex envelope of peaks will be obtained. High-resolution FTICR-MS will be beneficial in this case since small mass differences will exist between the different species. The ESI-FTICR-MS results obtained for all of the oils are summarised in Table 11. These results and trends are the same as the previously described MALDI data (Table 9) with, however, two exceptions: the number of cross-linked TAGs for F300 is 3, whereas 6 was observed with MALDI (in the linear mode). This may be due to the poorer detection sensitivity of FTICR-MS especially at higher mass. Furthermore, the degree of oxygenation of sample F150 as detected with ESI is somewhat higher. A closer comparison of the results obtained with both MALDI-TOF-MS and ESI-FTICR-MS shows for almost all samples a strong resemblance in the TAG mass

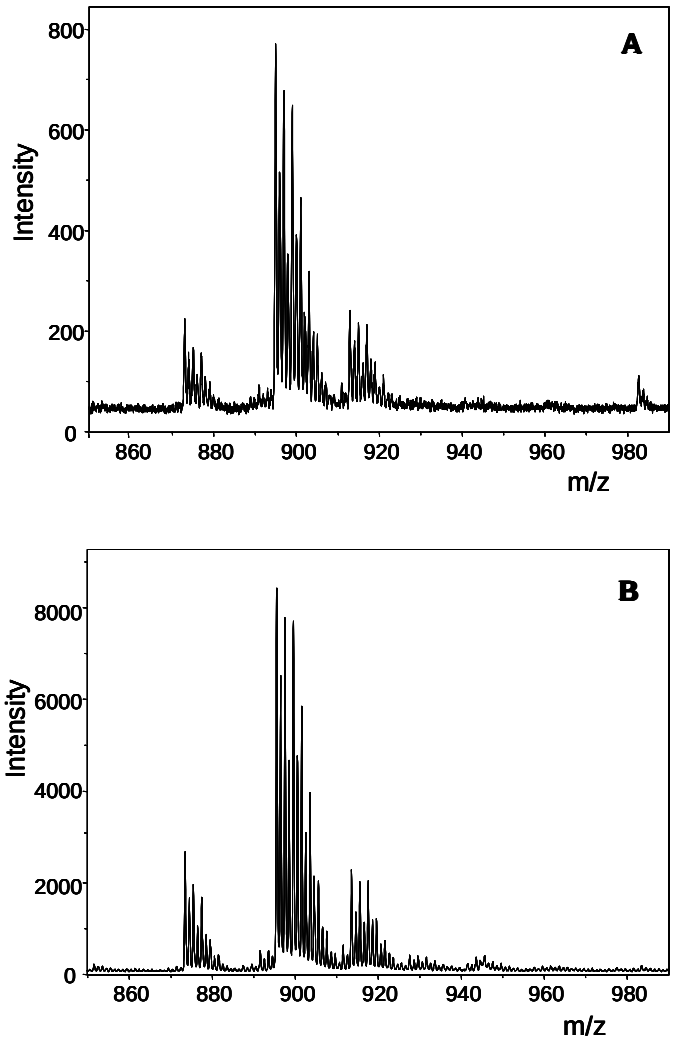
window (see Fig 10 for a comparison of two of the oils). There only seems to be a slightly better sensitivity for the oxidised species with ESI.

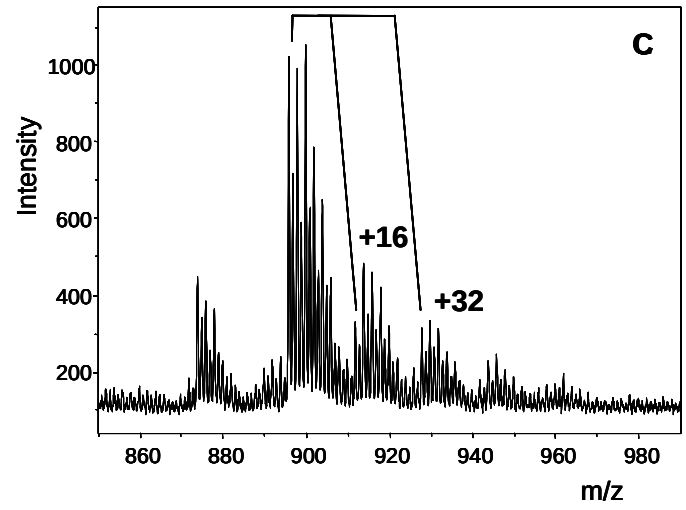
Table 10. Masses observed by ESI-FTICR-MS analysis of oil sample F150.

Material	True Mol. Wt.	Measured Mol. Wt.	Error (ppm)
TAGs[a]			
LnLnLn	890,7238	890,7307	7,79
LLnLn	892,7394	892,7445	5,70
OLnLn/LLLn	894,7551	894,7607	6,30
OLLn/LLL/SLnLn	896,7707	896,7755	5,34
OOLn/OLL/SLLn	898,7864	898,7914	5,60
OOL/SLL/SOLn	900,8020	900,8060	4,42
OOO/SOL/SSLn	902,8177	902,8217	4,47
SOO/SSL	904,8333	904,8364	3,41
All C18 DAG-TAG Dimers			
n=14[b]	1505,2148	1505,2406	17,15
n=13	1507,2304	1507,2588	18,82
n=12	1509,2461	1509,2720	17,17
n=11	1511,2617	1511,2815	13,08
n=10	1513,2774	1513,3027	16,73
n=9	1515,2930	1515,3132	13,31
n=8	1517,3087	1517,3320	15,36
n=7	1519,3243	1519,3435	12,61
All C18 TAG-TAG Dimers			
n=18	1763,4132	1763,4532	22,7094
n=17	1765,4288	1765,4657	20,8992
n=16	1767,4445	1767,4751	17,3392
n=15	1769,4601	1769,4893	16,4999
n=14	1771,4758	1771,5059	17,0174
n=13	1773,4914	1773,5214	16,9135
n=12	1775,5071	1775,5378	17,3167
n=11	1777,5227	1777,5547	18,0003
n=10	1779,5384	1779,5687	17,0527
n=9	1781,5540	1781,5883	19,2506
All C18 TAG-TAG Dimers with 1 oxygen			
n=15 + O	1785,4550	1785,509	30,17774
n=14 + O	1787,4707	1787,501	16,74489
n=13 + O	1789,4863	1789,51	13,12164
n=12 + O	1791,5020	1791,547	25,02425
n=11 + O	1793,5176	1793,557	21,73438
n=10 + O	1795,5333	1795,564	17,00386
All C18 TAG-TAG-TAG Trimers			
n=23	2644,1651	2644,272	40,4120

n=22	2646,1808	2646,276	35,8275
n=21	2648,1964	2648,3	38,9533
n=20	2650,2121	2650,282	26,4530
n=19	2652,2277	2652,329	38,0269
n=18	2654,2434	2654,337	35,1535

[a] P=palmitic acid, S=stearic acid, O=oleic acid, L=linoleic acid, Ln=linolenic acid
[b] n denotes the total number of double bonds within the material.

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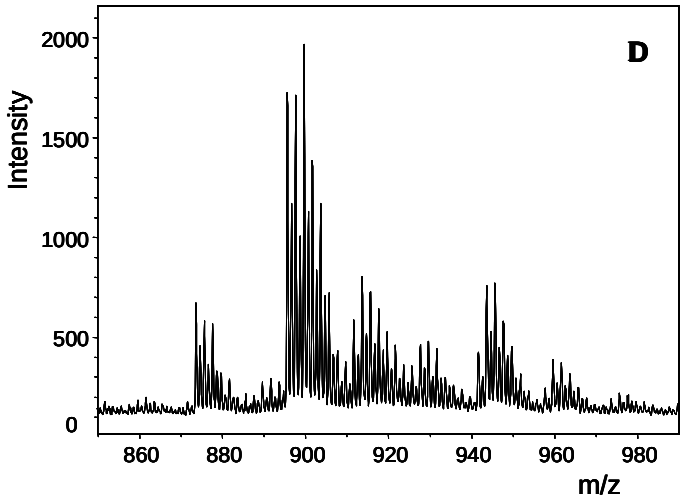
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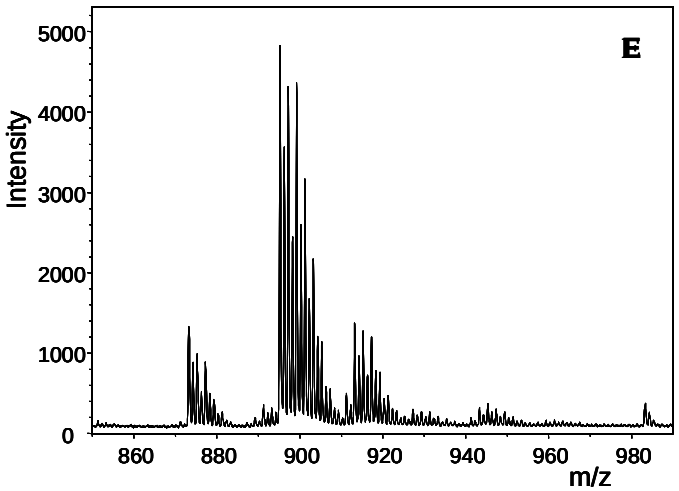
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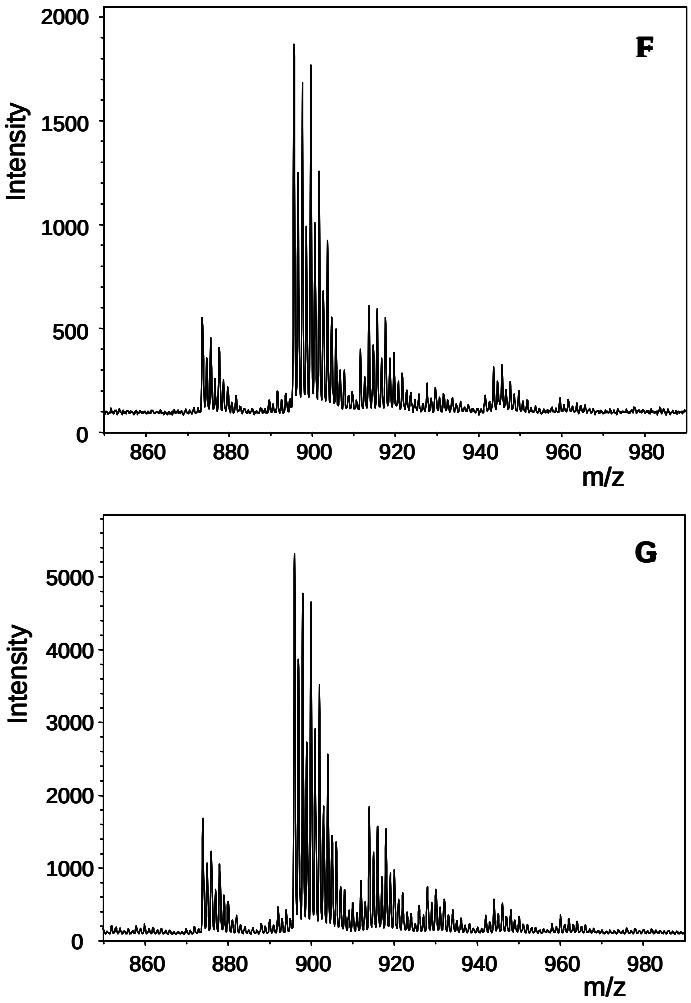


Figure 8. Mass window of MALDI-TOF-MS spectrum (Reflectron mode) of triacylglycerols and oxidation products of oil (a) F; (b) F150; (c) F300; (d) D; (e) D150; (f) D150Precipitate; and (g) W.

Table 11. Overview of ESI-FTICR-MS results of the processed oils.

Sample	Maximum number of incorporated oxygen atoms in TAGs	Dimers	Trimers	Maximum number of incorporated oxygen atoms in $(TAG)_2$s
F	1	DAG-TAG, $(TAG)_2$	$(TAG)_3$	1
F150	4	DAG-TAG, $(TAG)_2$	$(TAG)_3$, DAG-$(TAG)_2$, $(DAG)_2$-TAG	2

F300	5	$(DAG)_2$, DAG-TAG, $(TAG)_2$	$(TAG)_3$, DAG-$(TAG)_2$	2
D	5	$(TAG)_2$	$(TAG)_3$	3
D150	4	$(TAG)_2$	$(TAG)_3$	2
D150 Precipitate	7	$(TAG)_2$	$(TAG)_3$	> 7
W	4	(DAG-TAG), $(TAG)_2$	$(TAG)_3$, DAG-$(TAG)_2$, $(DAG)_2$-TAG	3

3.4. Discussion

From the above results it is clear that the initial TAGs, which are already slightly oxidised, do change upon processing. Of the oil processing methods investigated two treatments had a very significant effect on the chemical properties of the oils: heating the fresh oil to an end-temperature of 300 ºC and the heating a mixture of oil and drier (lead (II)oxide) to 150 ºC. Both of these oils were shown to contain constituents with a significantly increased molecular weight and number of incorporated oxygen atoms. The average molecular weights of the other oils investigated (heat treatment alone to 150 ºC (F150), lead and oil mixed at room temperature (D), and water washing (W)) were less affected by processing. According to the results reported here, it is also evident that hydrolysis is not an important process during the oil processing. The comparison between sample D150 and the precipitate D150Precipitate, which showed higher molecular weight material concentrated in the precipitating material, is important in terms of sampling for analysis and in the preparation of historically accurate paint samples. It indicates that the oil constituents are not uniformly distributed throughout the oil. Therefore if the oil is not well stirred prior to use or sampling, false conclusions could be reached regarding the degree of chemical alteration, i.e. chemical drying in the mixture. This phenomenon also implies that during use, artists may be experiencing a difference in the management and drying characteristics of their paint according to the age of their oil and whether they poured from the top of the container or used the oil remaining at the bottom of the container.

Although reasonable amounts of cross-linked material was demonstrated by SEC in almost all of the oils, it was very difficult to identify this material with the three mass spectrometric techniques, DTMS, MALDI-TOF-MS, and ESI-FTICR-MS. MALDI analysis in the linear mode was most effective. With this technique it was shown that oligomers as high as hexamers were formed in oil heated to 300 ºC (F300). This is in agreement with the SEC results. Surprisingly, upon mass spectrometric analysis the other, less cross-linked, oils showed only minor differences in their molecular weights despite their different SEC traces. Clustering of gaseous TAGs leading to dimers and trimers could partially cause this. The big advantage of the mass spectrometric techniques is that the incorporation of oxygen

into the TAGs can be monitored easily. The benefit of ESI-FTICR-MS compared to MALDI in this process is its capability of exact mass determination. This is indispensable in the case of multiply oxygenated species formed upon ongoing oxidation of TAGs containing both C16 and (unsaturated) C18 fatty acids. Surprisingly, the major products of oxidation observed contain only 1 oxygen atom (see Figures 8-10) whereas a hydroperoxide, a 2 oxygen atom containing functional group, is expected to be formed based on the autoxidation theory [52, 293, 297, 327]. Comparing the ion distribution of the non-oxidised TAGs with that of the mono oxidised species indicates that in this case oxidation is also accompanied by the loss of a double bond, as if water is added. This has been observed in mass spectra of autoxidised ethyl linolenate [123, 124] and of certain oils [325] as well, although it never has been explained. In almost all oil samples analysed, however, species are detected which do have a mass increment of 32, in accordance with the formation of hydroperoxides. When comparing oils F and F300 on one hand and oils D, D150 and the precipitate D150Precipitate on the other hand, it can be seen immediately that the oxidation seems to follow a different path for the lead containing oils. After the first oxygen is incorporated, there seems to be a preference for the simultaneous incorporation of two more oxygen atoms in the next step rather than one, as indicated by the higher intensity of triple oxygenated TAGs relative to double oxygenated species. Surprisingly, double oxygenated TAGs are present in lower amounts compared to the single and triple oxygenated species. Autoxidation as described in the literature, starts with the formation of hydroperoxides (ROOH), which would lead to a mass increase of 32 amu for the TAGs. A number of secondary oxidation products can then be formed: either a hydroperoxide attaches to previously unsubstituted fatty acyl chains or a second hydroperoxide adds to the fatty acyl chain already oxidised. In the latter case, an epidioxide is formed preferentially [97, 297] (See Scheme 1). This would lead to a total increase of 64 amu in the corresponding mass spectra. The thermal decomposition of the hydroperoxides, which can give rise to the radicals RO• and •OH, is slow and rather complex [232]. However, the ions of (transition) metals are effective as secondary catalysts of the autoxidation. Initially they act as an one-electron donor to give RO•. This radical can then pick up hydrogen from RH to give ROH and R•. The epidioxides are thought to have a higher stability towards breakdown by metal ions, relative to the hydroperoxides, which will lead to an oxygen distribution similar to that observed for the lead containing oils. The increase in oxidation observed for samples F150 and F300 is postulated to be caused by a synergy of both the higher amount of more reactive conjugated fatty acyl strands formed upon heating and the thermal breakdown of hydroperoxides. The fact that only monoxygenated species are seen in oil F suggests that the initially formed hydroperoxides are not stable under the conditions used.

DTMS analysis takes its place in between SEC and MALDI. Information can be obtained rapidly on the nature of the oil sample, based on polarity and molecular weight through the desorption profile, as well as its composition via the mass spectrum. The differences in the oils as can be observed with DTMS are mostly related to changes in the composition of the residual TAGs after processing.

For the oil samples investigated clear differences in the degree of oxygenation of the TAGs were observed with both ESI and MALDI analysis. In general the number of newly incorporated oxygen atoms correlates positively with the average molecular weight of the oil. Oil sample D, with lead drier but not heat-treated, is a good example of this phenomenon. All other oils with high oxygen content have also been heated. The heating has been shown to lead to oligomerisation. In both the MALDI- and ESI-experiments rather large amounts of oxidised species were detected. However, with DTMS only a slight indication was found for oxidation products, whereas with FTIR not even a slight trace of hydroxy groups or hydroperoxides was observed. The reason for these differences in sensitivity are not entirely clear, but may be due to higher ionisation efficiency of the more oxidised fractions in MALDI- and ESI-MS.

3.5. Conclusion

This study shows that the processing methods of freshly pressed linseed oil, as used by painters in the past, all have a clear effect on both the physical and chemical properties of the linseed oil. This supports the observations made during paint preparation with lead white pigment and oils where there were significant differences in paint rheology according to the oil treatment. Two major chemical processes were observed for the oils: oxidation and oligomerisation. This is accompanied by a relative decrease of the percentage of the triple unsaturated fatty acids (linolenic acid), the most reactive component of the TAGs. High temperatures applied, with or without the addition of a lead drier are shown to stimulate the observed changes. Litharge, when added to the oil as drier, clearly influenced the way autoxidation proceeds. Higher amounts of oxidised TAGs are found in these oils. The prepolymerisation by heating of the oil with a lead drier led to the formation of a precipitate on the bottom of the vial. This was enriched in oxidised polymeric material, compared to the upper part of the oil.

Both MALDI-TOF-MS and ESI-FTICR-MS are shown to be very useful for the analysis of oxygenated TAGs and their oligomers. Similar results for the composition of the TAGs and the degree of oxygenation were obtained with both techniques. FTIR, and also DTMS, are shown to be less useful for the characterisation of oxygenated TAG species. These techniques are more focussed on the bulk of the material whereas MALDI- and ESI seem to emphasise the oxidised TAGs.

Chapter 4

Direct temperature resolved mass spectrometry of oil paint constituents and aged oil paints

Direct temperature resolved mass spectrometry (DTMS), a direct mass spectrometric method that combines evaporation and thermal degradation of organic materials that vary in polarity as well as in molecular weight, is investigated and discussed as a fast and reliable fingerprinting technique for the characterisation of oil paints.

Dried oil, oil paints and a broad range of compounds like free (oxidised) fatty acids, metal carboxylates, triacylglycerols present in fresh, cured and aged paints were analysed. The desorbed compounds and pyrolysed polymeric material are ionised with 16 eV electron ionisation to prevent extensive fragmentation. The different classes of compounds that can be released by heating the paint sample are introduced into the ion source at different time intervals as a function of the differences in their physical and chemical properties. Specific fragment ions were observed for the metal carboxylates (sodium palmitate, lead distearate, and copper dipalmitate) and triacylglycerols investigated, apart from more general fragment ions also observed for free fatty acids. However, due to their appearance in different time windows additional information could be obtained on the nature of the constituents and the relative ratio of the different types of compounds present. Cross-linked material formed upon drying and/or heating of oil (paints) was identified as a broad envelope of specific small (fragment) ions formed at higher temperatures by pyrolysis. Ions indicative for alkylated benzenes, especially m/z 91 and 105 are in particular observed in the time/temperature analytical window of that part of the paint that cannot evaporate at low to medium temperatures. Curie-point pyrolysis (610 ºC) gas chromatography-mass spectrometry applied to a 25-year old lead white pigmented oil paint shows the formation of alkylated benzenes and other benzene derivatives upon pyrolysis at high temperature. Apart from these compounds a series of pyrolysis products of fatty acid soaps i.e. cycloalkanones, alkanals, alkanes and alkenes, and thermally released palmitic- and stearic fatty acids were identified. The distribution of ions of the summation

spectrum from these compounds resembles the DTMS ion pattern thus validating the latter method for oil paint fingerprinting.

4.1 Introduction

Traditional artists paints used in works of art consist of one or more pigments mixed with a binding medium. Drying oils like linseed, walnut and poppyseed have been widely used as binding medium since the fifteenth century. The fresh oils consist of relatively apolar mixtures of triacylglycerols with different fatty acid profiles. The fatty acids of linseed oil, which has been used most often in Northern Europe consists of 65-80% polyunsaturated fatty acid and only a small percentage of fully saturated fatty acids (FAs) (7-12%). The precise composition of the fresh oil is mostly determined by the geographical origin of the flax and the way in which the oil has been obtained from its seed [292]. These oils are highly susceptible to oxidation owing to the high content of polyunsaturated fatty acyl moieties. The oil forms a dry film during this autoxidation stage, a process that is relatively well-understood [293]. In this case drying is not the evaporation of a solvent but chemical drying which involves the cross-linking of triacylglycerols (TAGs) to high molecular weight substances. These "binding" substances are unstable end products due to the chemical environment in paintings. Further chemical changes in the oil paint film involve hydrolysis of the ester bonds, formation of new oxygen containing functional groups, oxidative cleavage of the fatty acid hydrocarbon chains, and metal-ion coordination of the fatty acid groups of the cross-linked material and non cross-linked fractions. It is likely that the different processes partly overlap in time. A first description of these processes and preliminary chemical data has been reported by Boon et al. (1996) [28] and Van den Berg et al. (1998) [328] (see Figure 1a-c for a simplified model of the different stages in the development of the oil paint).

A relatively good understanding of the process of curing exists because of detailed chemical studies on model compounds and alkyd paint [93, 123-125]. The transition of the initial "polyester" to other forms of polymeric systems in ageing traditional oil paint is much less understood. However, studies on both ionomeric systems and more recently alkyd ceramers indicate that interaction between metal ions and free carboxylic acid groups consist of both ionic and acidic interactions [275, 278].

Monitoring the progression of each of these processes in an oil paint sample by a single analytical chemical approach requires the determination of a broad range of compounds because of the presence of both an organic and inorganic fraction.

Gas chromatography(-mass spectrometry) (GC(/MS)), the traditional analytical method used for characterisation of oil paints in conservation science, is only suitable for the identification of low molecular weight compounds present in

oil paint samples like acylglycerols and free fatty acids [11, 13]. A big disadvantage of this method is that no information is obtained on both the inorganic fraction and the polymeric fraction directly and furthermore, the relatively long analysis time due to chemical work-up and the chromatographic separation. Fourier transform infrared, on the other hand, is suitable for the identification of the presence of specific functional groups present in both the low and high molecular weight material, including the interaction of (in)organic pigments with the oil paint material [270]. Although that analysis takes relatively little time, almost no structural information is obtained. It would be most desirable for a conservation scientist to have an analytical technique available, which gives detailed information on both the low molecular weight fraction as well as the cross-linked material in the same analytical run. The analytical method should maximise the information on most of the organic and inorganic constituents but require at the same time a minimum amount of paint sample for the analysis.

In this chapter the fingerprinting of oil paint samples by direct in-source pyrolysis mass spectrometry (PyMS) is investigated using a direct temperature resolved mass spectrometry (DTMS) approach. This method is used in our laboratory for the microchemical analysis of samples from paintings, mock paintings, reconstructions of traditional paints [9, 329, 330], and organic materials that can be found in samples taken from paintings such as beeswax, synthetic pigments and varnishes containing diterpenoid acids and triterpenoid alcohols [331-335]. Not only organic material can be investigated with this method but information is also obtained on certain inorganic materials such as mineral pigments and fillers in paintings. Metals that can be detected with this technique include sodium, potassium, iron, mercury, lead, copper, zinc, and cadmium. An extensive description of the technique and its applications is given by Boon [329]. A very important advantage of DTMS is that there is a narrow spatial correlation between the production space of neutral molecules, the ionisation beam volume and the extraction axis into the mass spectrometer thus minimizing the interaction of the compounds produced from the sample with the walls of the ionisation chamber. This is in strong contrast with a gas phase pyrolysis unit and a chromatographic separation system. Therefore, more polar or high molecular weight compounds can be analysed compared to GC systems. The selective decrease of specific compounds is very unlikely. Another advantage of DTMS is the simplicity of the sample pretreatment that only consists of grinding a micro amount of sample (about 20-50 microgram) in an organic solvent, application of the suspension to the analytical filament wire and subsequent drying prior to insertion of the probe into the ion source. The low sample requirement makes it possible to combine DTMS analysis with micropicking samples from specific layers in cross sections.

Since a resistively heated Pt/Rh filament is used for desorption and pyrolysis, the separation of different types of material can be achieved within the two minutes of the analytical run. As a result of the volatility and pyrolytic behaviour of the different components present in the sample, an in-source separation based on physical properties of the different sets of compounds is achieved giving this method its name of direct temperature resolved mass spectrometry (DTMS). The rapid increase to a high-end temperature makes it

possible to analyse paint samples, which consist of both inorganic and organic fractions. Whether a certain material will be detected depends on the volatility and ionisation potential of the desorbed compounds.

The thermal degradation (i.e. pyrolysis) under controlled conditions with this technique has proven to be a fast and reproducible method of investigating complex high molecular weight material like synthetic polymers, biopolymers, biomacromolecules and geomacromolecules [336, 337]. The analytical pyrolysis of these types of materials aims at the collection of information on structural elements in the macromolecular complexes by identifying the volatile material and the products of thermal degradation. For the analysis of oil paints two relevant classes of compounds have been investigated in the past using pyrolysis: oils and fats, and (modern) alkyd coatings [338, 339]. However, most of the studies were done using a pyrolysis device coupled to a GC or GC/MS system, in order to separate and characterise the pyrolysis products. A similar pyrolysis-GC/MS technique has been used in our lab, although often with the aid of a tetramethylammonium salt as an on-line (trans)methylation reagent [340]. In this way information could be obtained on the methylated (oxidised) fatty acids.

The suitability of DTMS for oil paint analysis was tested by analysis of standard reference samples related to the drying of oils, and a collection of oil paint samples of different age and composition. The main goal of the study is to identify diagnostic ions and ion patterns, in relation with desorption temperature, of the different types of compounds found in oil paint systems. The selected set of samples is relevant for a better understanding of drying/ageing of oil paint modelled in Fig. 1a-c. Samples representing hydrolysed and oxidised fatty acids are the free heptadecanoic-, octadecadienoic-, 12- hydroxy 9-octadecenoic-, and octanedioic fatty acids. A second class of hydrolysed compounds that can be present in an aged oil paint are the metal carboxylates. A sodium- and copper salt of hexadecanoic fatty acid and a lead salt of octadecanoic fatty acid are subjected to DTMS analysis. Subsequently DTMS data on three pure TAGs, i.e. tristearoyl-, trimyristoyl glycerol and 1-palmitoyl-2- oleoyl-3-linoleoyl-glycerol are investigated, and compared to spectra of fresh or prepolymerised linseed oil, which are mixtures of different TAGs. The last part of the research focuses on the aged paint itself and the different extractable fractions that can be obtained with hexane or methanol. A comparison is made between both the physical data in the form of total ion current traces and mass spectrometric data of the analysed reference materials and paints. Furthermore, the DTMS work on 25 year-old lead white pigmented oil paint is compared with the result of a Curie-point pyrolysis-GC/MS measurement in order to compare the mixture of identified compounds obtained by GCMS with the fingerprinting information from DTMS.

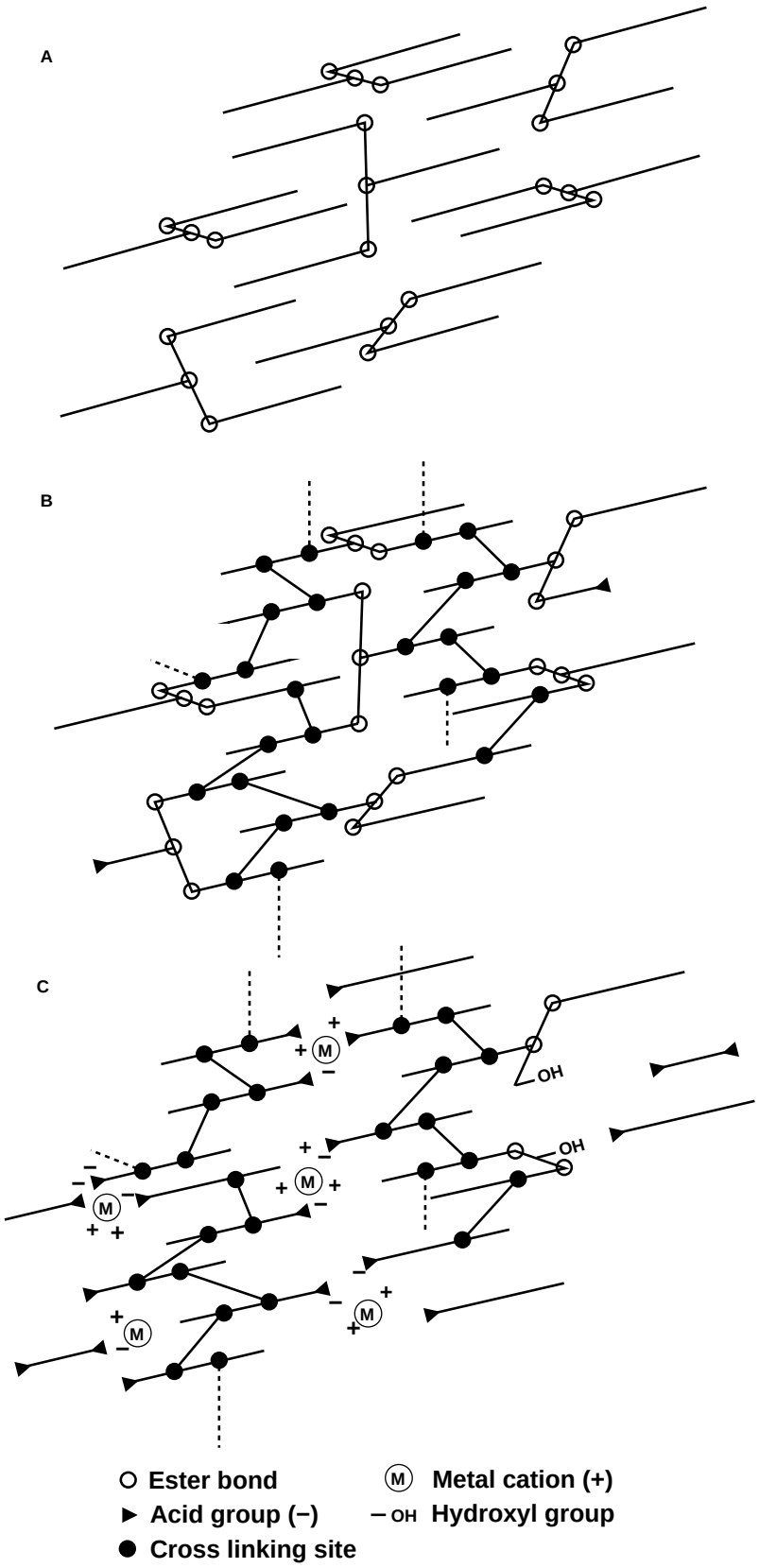


Figure 1. Schematic model depicting different stages in the development of oil paints. (a) fresh oil; (b) after curing, and (c) after maturation.

4.2 Experimental

4.2.1 Chemicals

Trimyristoyl-, tristearoyl-, and 1-palmitoyl-2- oleoyl-3-linoleoyl-glycerol, 9,12,15-octadecatrienoic acid, 12-hydroxy-9-octadecenoic acid, copper sulphate (all approximately 99%), nonanedioic acid (98%), sodium palmitate (98%), heptadecanoic acid and hexane (>99.5%) were obtained from Sigma-Aldrich Chemie Bv., Zwijndrecht, The Netherlands. Methanol was purchased from Merck, Amsterdam, The Netherlands.

4.2.2 Synthesis lead distearate

30 ml p-xylene (Acros, Geel, Belgium, >99%), together with 0.3 g lead oxide (Sigma-Aldrich, 99.999%) was de-aerated, 0.75 g stearic acid (Sigma-Aldrich, 99%) was added, the system was de-aerated again and maintained under a nitrogen flow. The mixture was heated with an oil bath to an end temperature of 138 °C at which the mixture was refluxed for 15 min. The hot solution was decanted over a glass filter in another round bottom flask to remove remaining unreacted lead oxide. This was also done under a stream of nitrogen. The obtained solution was gradually cooled so that crystals could precipitate. After cooling the solution to 0 °C, the crystals were collected on a glass filter, and washed with demineralised water and freshly distilled ethanol and acetone (both from Acros, Geel, Belgium), respectively.

After washing and drying of the crystals, the total amount of silver-white crystals was 1,97 g (yield: 95%). The melting point was determined and was found to be 117/8 °C. This agrees with melting points (110-125 °C) mentioned in the literature [341-343]. An infrared spectrum was recorded to see if residual free fatty acids were present. This appeared not to be the case and the spectrum was identical to a reference spectrum [205, 344].

4.2.3 Synthesis copper dipalmitate

Solutions of sodium palmitate and copper sulphate in demineralised water were mixed in a molar ratio of 0.6. The resulting suspension mixture was shaken

briefly for several minutes, and the resulting solution, containing excess copper- and sodium sulphate was decanted from the precipitated copper dipalmitate by centrifuge. Subsequently, the precipitate was washed with demineralised water three times.

4.2.4 Reconstructed paint systems

Raw purified linseed oil and stand oil (an oil of increased viscosity due to heat bodying) were obtained from Talens, Apeldoorn, The Netherlands. The oil was applied on glass plates with a thickness of approximately 0.5 mm and aged at room temperature under normal daylight conditions until samples were taken.

The 25-year old lead white pigmented paint was made by H.C. von Imhoff with cold-pressed linseed oil (Mühlfellner-Rupf, Zurich, Switserland) that was allowed to stand in flat dishes of 4 mm for 3 weeks. After the skin had been removed the oil was mixed with basic lead carbonate ($PbCO_3$ $Pb(OH)_2$, Schmincke, Germany), until a workable paint was obtained. The paint was applied on pre-sized and primed linden wood and aged under normal conditions. This particular paint is presently stored at the Canadian Conservation Institute (CCI), Ottawa, Canada.

The oil paint system made by A. Eibner in 1941 was made with Künstler Ölfarbe and Kobalt Blau Dunkel and initially stored under normal conditions. When the sample was taken it was found in a closed and dark cupboard. It is documented as No. 18b at the Doerner Institute, Munich, Germany.

The 17th century white impasto paint sample was taken from the painting "The Jewish Bride" by Rembrandt (SK-C-216), which is part of the collection of the Rijksmuseum, Amsterdam, The Netherlands.

4.2.5 Direct temperature mass spectrometry

Samples of typically 20 to 60 µg were taken from the paint films and homogenised in methanol using a mini-glas mortar. Aliquots of the obtained suspension or extracted material were applied onto the analytical filament and dried *in vacuo*. The analyses were performed on a Jeol SX-102 double focussing mass spectrometer (B/E) using a direct insertion probe equipped with a Pt/Rh (9/1) filament (100 micron diameter). The probe filament was temperature programmed at a rate of 0.5 A/min to an end temperature of about 800 °C. Compounds were ionised at 16 eV under electron ionisation conditions in an ionisation chamber kept at 180 °C, mass analysed over the range *m/z* 20-1000, with 1 second cycle time. Data were processed using a JEOL MP-7000 data system.

4.2.6 *Curie-point pyrolysis gas chromatography – mass spectrometry*

Typically 20 to 60 µg of paint sample was taken and homogenised in methanol using a mini-glas mortar. About 15 µl of the suspension was applied onto a rotating 610 °C Curie-point wire and subsequently the sample was dried in vacuo. The ferromagnetic wire was inserted in a glass liner, flushed with argon to remove air and subsequently placed into the pyrolysis unit. Curie-point pyrolysis was performed with a FOM 5-LX pyrolysis unit [345]. The ferromagnetic wire was inductively heated in a 1 MHz Rf field for 6 s to its Curie-point temperature (610 °C). Pyrolysis fragments were flushed (splitless) into a SGE BPX5 column (25 m, 0.32 mm i.d., 0.25 µm film thickness) mounted in a Carlo-Erba gas chromatograph (series 8565 HRGC MEGA 2) which was coupled directly to the ion source of a JEOL DX-303 double focussing (E/B) mass spectrometer via a home built interface which was kept at 280 °C. Helium was used as carrier gas at a flow rate of approximately 2 ml/min as regulated with a CP-CF 818 pressure/flow control box (Fisons Instruments). The initial temperature of the gas chromatograph was 50 °C, which was maintained for 2 minutes. The oven temperature was programmed with a ramp of 6 °C to an end temperature of 320 °C (50(2)-6-320). Ions were generated by electron ionisation (70 eV) in the ionisation chamber (180 °C), accelerated to 3 keV, mass separated and postaccelerated to 10 keV before detection. The mass spectrometer was scanned from m/z 40-700 with a cycle time of 1 s. A Jeol MP-7000 data system was used for data acquisition and processing.

4.3 *Results*

Well-defined reference materials were analysed first to determine the temperature domains in which the different chemical compound classes appear, what the chemical effect is of the heating process on the standard materials and which specific (fragment) ions can be detected after 16 eV electron ionisation of the volatile materials and pyrolysis products.

4.3.1 *Well defined reference materials*

4.3.1.1 *Free Fatty Acids*

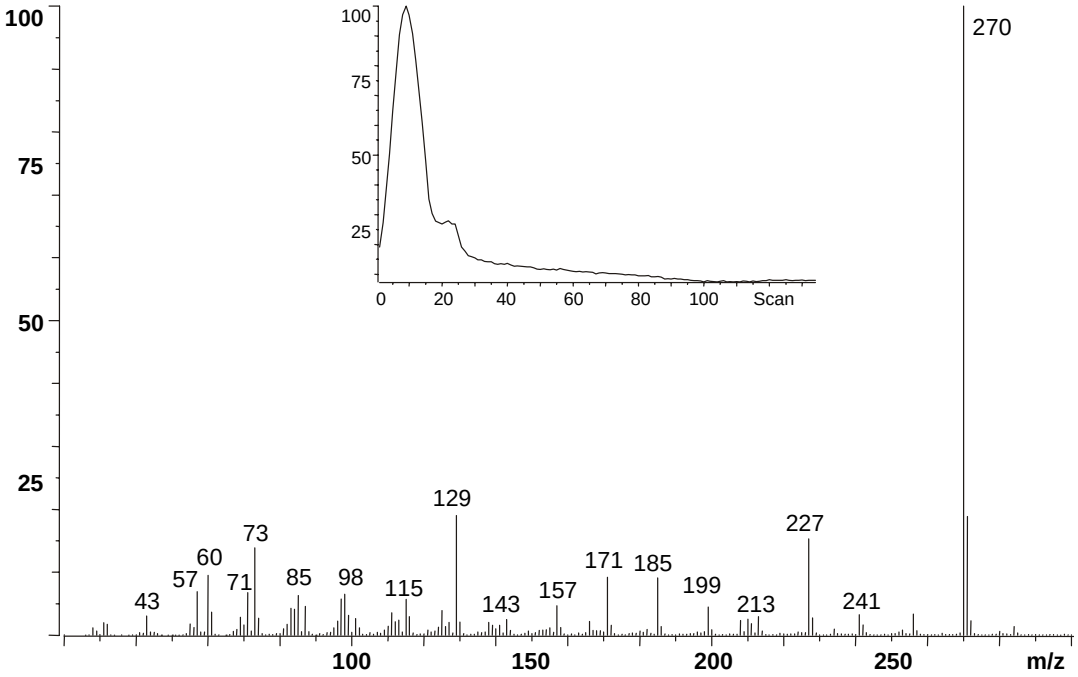


Figure 2. DTMS summation spectrum of heptadecanoic acid (scans 3-15). Insert: TIC.

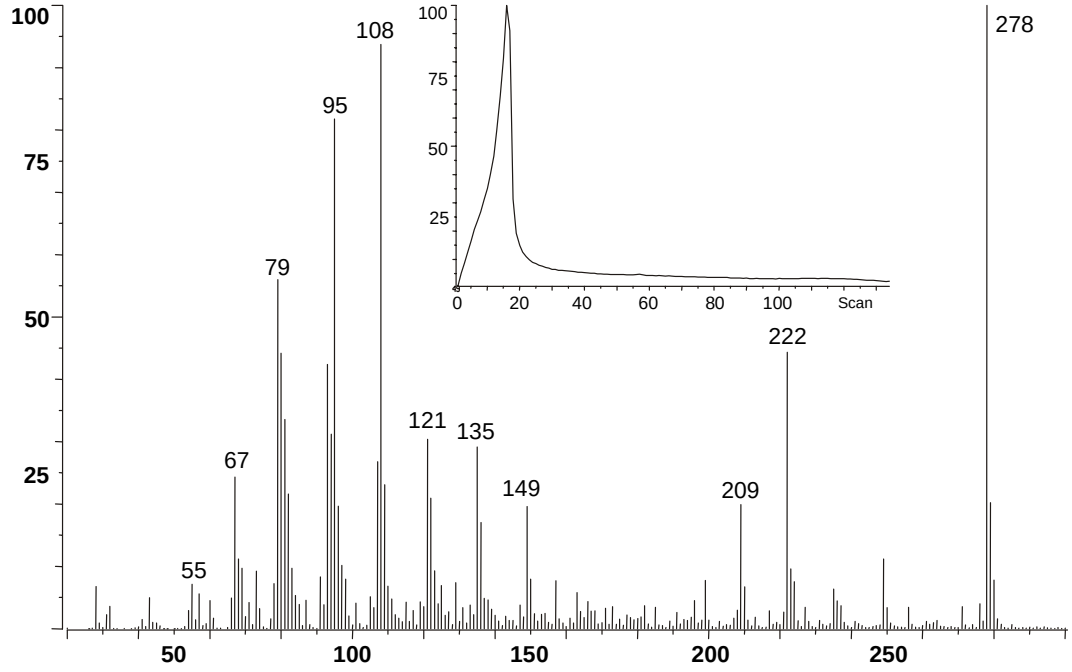


Figure 3. DTMS summation spectrum of 9,12,15-octadecatrienoic acid (linolenic acid) (scans 11-17). Insert: TIC.

Low molecular weight compounds representative for hydrolysed fatty acyl moieties formed within oil paint were analysed. The results for heptadecanoic acid (C17 fatty acid) are depicted in Figure 2. Most of the desorbed ions are detected from scan 1 to 17 in the total ion current (TIC, see insert Figure 2). This is indicative for the relative volatile nature of this compound. The molecular ion at m/z 270, together with m/z 227 (M-43), is clearly visible in the mass spectrum obtained after summation of scans 3-15 (Fig 2). Fragment ions formed upon cleavage of the carbon backbone are observed at m/z 185, 171, 157, 143, 129 and 73 ($[HOC(O)C_nH_{2n}]^+$). These are not formed by simple cleavage between the two adjacent carbon atoms of the backbone, but a more complex mechanism involving distonic ions has been suggested [346]. At low mass, a series of alkyl ions is visible at m/z 43, 57, 71, 85, ….. (C_nH_{2n+1}), in combination with an unsaturated ion series at m/z 41, 55, 69, 83,…(C_nH_{2n-1}).These types of ions carry little structural information and are normally abundantly present in the spectra of fatty acids but the low electron ionisation energy employed in this study reduces the intensity of these ions. M/z 60 is formed by a McLafferty (H) rearrangement. All these fragments are also observed in 70 eV mass spectra of free saturated long chain fatty acids although with different relative ratios [322] due to the differences in the internal energy of the ions.

Table 1. Characteristic fragment ions observed in the mass spectra of typical fatty acids analysed with 16 eV direct temperature resolved mass spectrometry

m/z	Fragment(s) (lost)	Compounds
$[M-17]^+$	-OH	Hydroxy substituted fatty acids, Diacids
$[M-18]^{+\cdot}$	$-H_2O$	Hydroxy substituted fatty acids
$[M-29]^+$	$-CH_2CH_3$	Normal chain fatty acids
$[M-35]^+$	$-H_2O+H$	Diacids
$[M-36]^{+\cdot}$	$-2H_2O$	Diacids
$[M-43]^+$	$-(CH_2)_2CH_3$	Normal chain fatty acids
$[M-56]^{+\cdot}$	$-C_4H_8$	Normal chain unsaturated fatty acids
$[M-59]^+$	$-CH_2-COOH$	Diacids, Normal chain saturated fatty acids
$[M-77]^+$	$-CH_2-COOH+H_2O$	Diacids
$[M-78]^{+\cdot}$	$-CH_2-COOH+H_2O+H$	Diacids
$[84 + nx14]^{+\cdot}$	$C_6H_{12}O + (CH2)_n$	Diacids, Ricinoleic acid, Normal chain fatty acids
$[59 + nx14]^+$	$HOOC-(CH2)_n$	Normal chain saturated fatty acids
$[29 + nx14]^+$	$C_2H_5-(CH2)_n$	Normal chain saturated fatty acids
$[27 + nx14]^+$	$C_2H_3-(CH2)_n$	Unsaturated fatty acids
60	$C_2H_40_2$	All fatty acids

Unsaturated long chain fatty acids show a different fragmentation behaviour as is shown for linolenic acid (C18:3) in Figure 3. Here the summed mass spectrum of scans 11-17 is depicted. Again the molecular ion is clearly visible (m/z 278). Characteristic for this compound is the fragment ion M-56 at m/z 222 with relatively high intensity, which is thought to be formed upon the elimination of C_4H_8. Furthermore, relatively intense fragment ions at m/z 67, 79, 80, 81, 93, 95, and 108 are detected, which are assigned to the series $C_nH_{2n-5/4/3}$ and are hardly seen in spectra of saturated fatty acids. These peaks can serve as a marker for the presence of aliphatic unsaturated molecules. The ions found are similar to the ones produced with 70 eV electron ionisation but again in different relative intensities [322, 347].

In order to test the influence of underivatised polar groups on the behaviour upon DTMS analysis of fatty acids, a hydroxylated unsaturated C18 fatty acid (ricinoleic acid, 12-hydroxy, 9-octadecenoic acid) was also analysed. This compound has a lower volatility than the previous fatty acids, resulting in a temperature profile, which is slightly shifted towards higher temperature in the TIC of Figure 4. The molecular ion, m/z 298, is only present in low abundance in the summed mass spectrum (scan number 15 to 27). Loss of water is observed as indicated by the presence of an ion at m/z 280 in the mass spectrum. Characteristic for this specific compound are the mass fragments at m/z 184 and 213 which are formed by -cleavage on either side of the hydroxy group on the 12-position. Additional loss of water is expected to occur, giving rise to ions at m/z 166 and 195, respectively. Even-mass ions are found at m/z 148, 124, 122, 98, 96, 84 and 82. Furthermore, in the low-mass region numerous other non-specific masses are visible like m/z 43, 55, and 69. A comparison between the mass spectrum of octadecadienoic acid (C18:2) and ricinoleic acid shows that both display a peak at m/z 280, but that most other fragment ions are qualitatively different.

A second and important group of polar fatty acids analysed is the diacids. These are relatively stable end-products formed upon autoxidation of unsaturated fatty acids and are encountered in relatively high amounts in aged oil paint systems. Azelaic acid, the C9 diacid, is detected from scans 10 to 21. The most characteristic and intense fragment ion in the summed mass spectrum (Fig. 5) is m/z 152. This ion is formed upon loss of two methanol moieties [348]. No molecular ion (m/z 188) is observed, whereas general fragment ions, including a McLafferty rearrangement (m/z 60), are seen which are also observed in the mass spectra obtained for other diacids investigated (results not shown). These ions, which are also found in 70 eV mass spectra [348], are listed in Table 1 and their nature is given. It should be noted that part of the fragment ions belonging to the series m/z 84 + (n x 14) were also observed in the mass spectrum of ricinoleic acid up to m/z 124 but with a higher relative abundance. The use of these ions as a marker for diacids therefore is not recommended for a complex sample like young oil paint where a number of (unsaturated) oxidised fatty acids is expected.

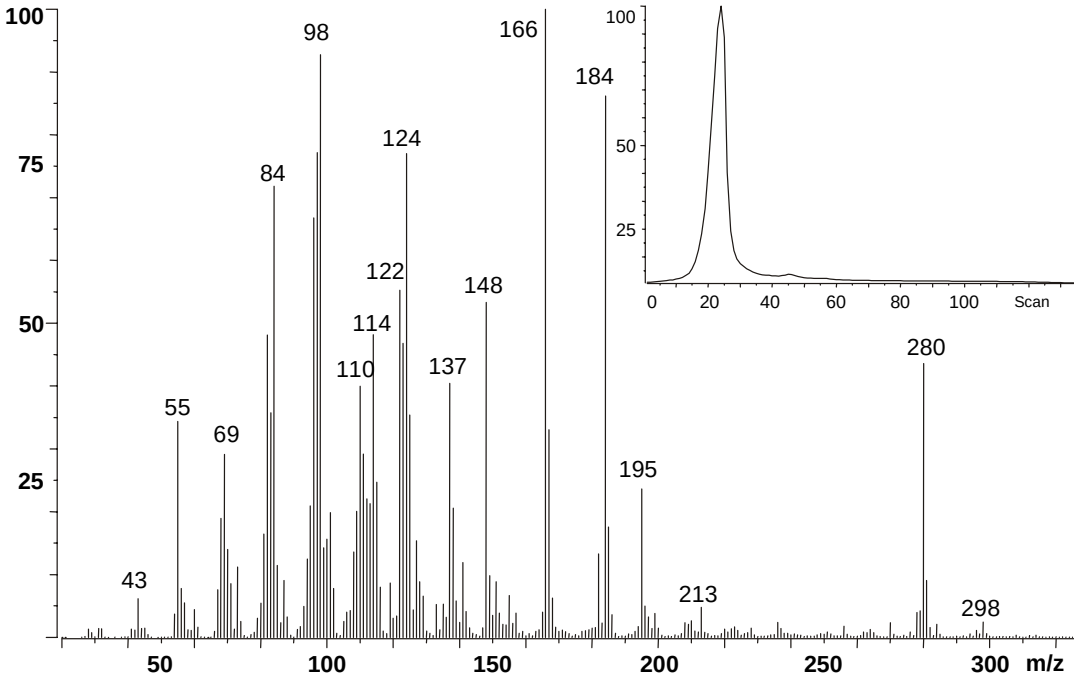


Figure 4. DTMS summation spectrum of 12-hydroxy-9-octadecenoic acid (ricinoleic acid) (scan 15-27). Insert: TIC.

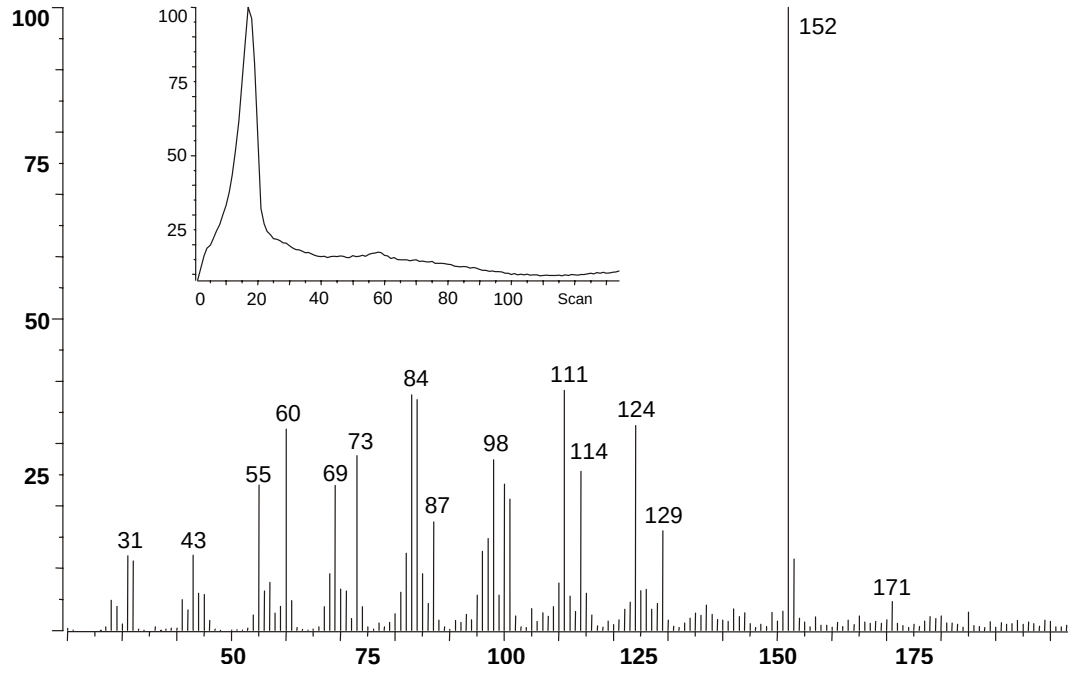


Figure 5. DTMS summation spectrum of nonanedioic acid (azelaic acid) (scan 10-21). Insert: TIC.

4.3.1.2 Metal Carboxylates

Three different fatty acid salts were investigated by DTMS because in oil paints free fatty acids as well as their metal salts are expected to be present. DTMS analysis of sodium palmitate ($C_{15}H_{31}(CO)ONa$, an example of an ionic carboxylate gave two peaks in the TIC at scans 1-14 (A) and 55-70 (B), respectively. The summed mass spectrum of the first peak (Fig. 6a) shows ions indicative for free palmitic acid, with the combination of m/z 256, and 213 ($[M-43]^+$) being typical. The series of fragment ions as reported for heptadecanoic acid can be seen as well (see also Fig. 2 and Table 1). The ions observed in Figure 6b originating from the second peak in the TIC are identified as sodiated sodium palmitate (m/z 301) and as clusters with an additional sodium palmitate moiety at masses 579 and 857 (mass increments of 278 amu). With DTMS, a comparable clustering of sodium salts in DTMS data has been observed for NaCl containing samples [349]. DTMS analysis with the electron ionisation turned off resulted in a mass spectrum with identical masses, indicating that thermo-ionisation is occurring. The high scan number i.e. high temperature at which this compound is desorbed from the pyrolysis wire confirms the reduced volatility of the sodium salt compared to the free fatty acid. Besides the intact sodiated salt also ions are detected at the molecular mass of the free fatty acid (m/z 256), albeit with a much lower intensity compared to the ones in scan range 1-14. The formation of these ion indicates that thermally induced rearrangements are taking place. In the mass region below m/z 256 it can be seen that the same fragment ions are formed as observed for the free fatty acids. It is clear from the above results that two types of fatty acids can be identified in the sodium palmitate sample on the basis of the time of appearance in the TIC: the free fatty acid and its sodium salt, respectively.

DTMS on the lead salt of stearic acid ($(C_{17}H_{35}(CO)O)_2Pb$) resulted in only one peak which ranges from scan 51 to 66 in the TIC (see insert Figure 7), and is clearly different from the TIC profile observed for the free fatty acids. The most prominent peak in the summed mass spectrum (Figure 7) is m/z 267, which is the acylium ion of stearic acid formed upon -cleavage. No molecular ion (m/z 774) is visible, but two fragment ions are observed at m/z 507 and 491, respectively that can be ascribed to the loss of the acylium ion or fatty acid carboxyl radical. Due to the presence of the lead moiety these last two masses can be observed as isotopic clusters because of the three isotopes of lead (m/z 206:207:208 ~ 1:1:2). Comparison with the theoretical isotope distribution of the ion at m/z 507 points to discrepancies. M/z 506 and 505 are present at lower relative abundance in the set of peaks, which suggests some uptake of hydrogen has taken place and therefore positive ions are formed instead of the expected radicals. This also explains the higher relative intensity of m/z 508 when compared to the theoretical distribution. Peculiar features in the mass spectrum of scans 51-66 are peaks at m/z 282 and 284. These peaks cannot be explained by simple homolytic cleavages.

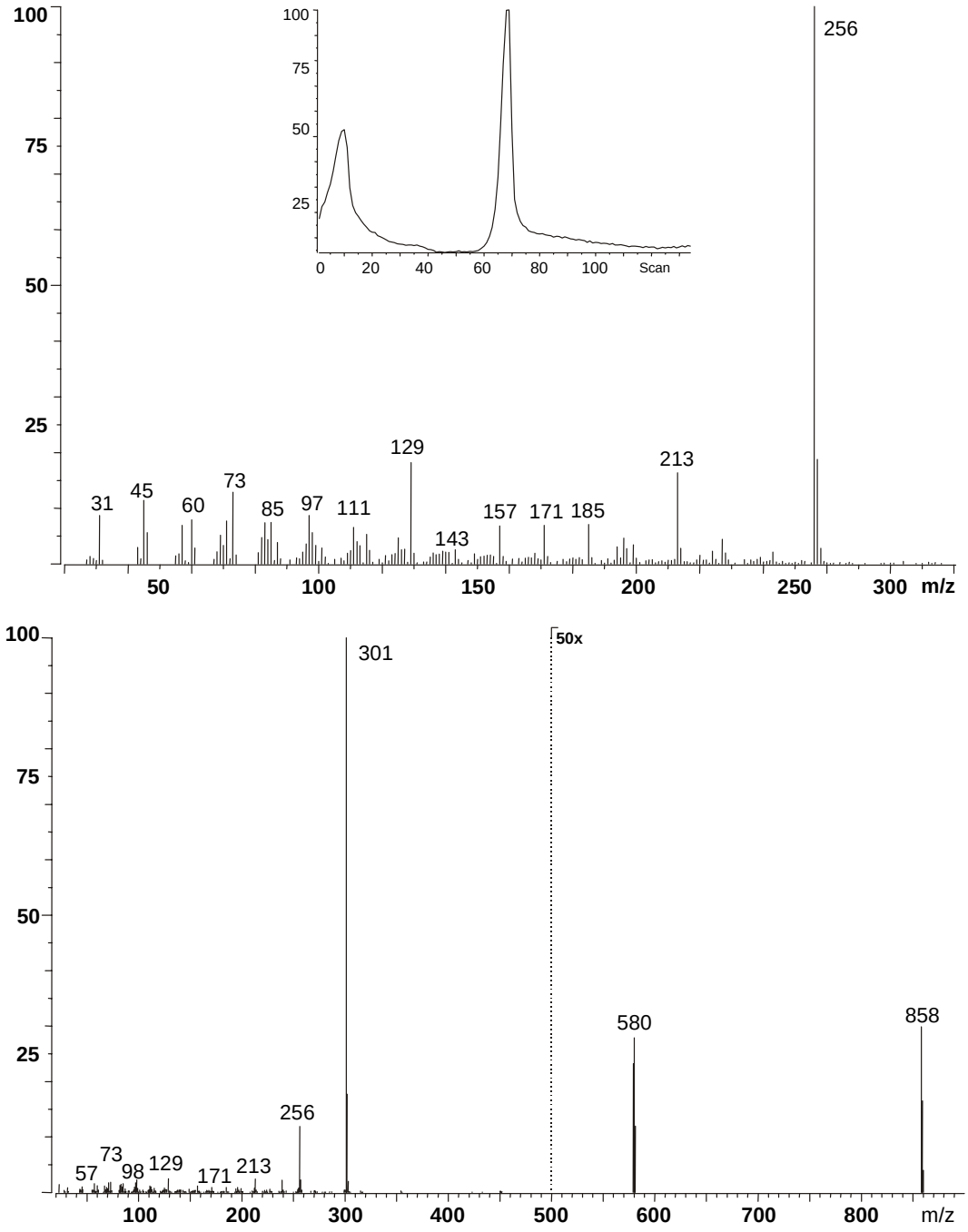


Figure 6. DTMS summation spectrum of sodium hexadecanoate (a) scan 1-14. Insert: TIC, and (b) scan 55-70.

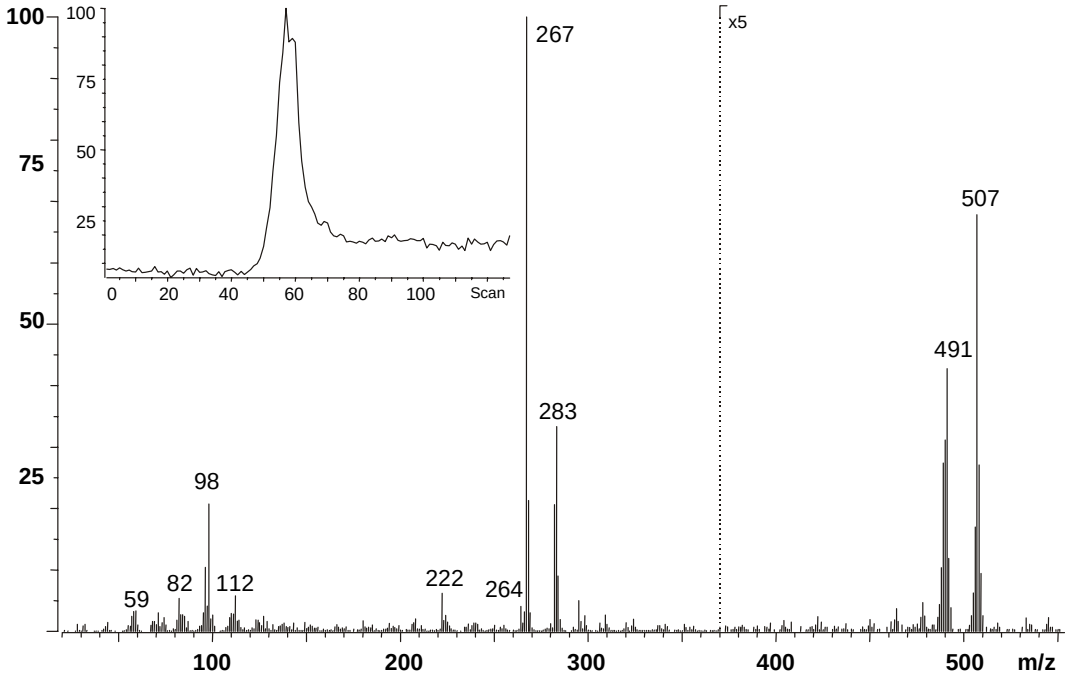


Figure 7. DTMS summation spectrum of lead dioctadecanoate (scan 51-66). Insert: TIC.

We postulate that hydrogen stripping takes place to a certain extent on the platinum wire upon pyrolysis of the lead dicarboxylate. This process will lead to the formation of unsaturated fatty acyl moieties. Part of these compounds are thought to take up hydrogen leading to positive fragment ions, e.g. m/z 282 ($[C_{17}H_{33}(CO)OH]^+$) and m/z 283 ($[C_{17}H_{33}(CO)OH_2]^+$). This hydrogen uptake of the deesterified carboxylic acid group also has been observed in DTMS measurements of intact palmitic acid containing wax esters, where formation of an ion at m/z 257 ($[C_{15}H_{31}(CO)OH_2]^+$) was observed. In the scan range 51-66 almost no signal is visible at m/z 206, 207 and 208, the isotopes of elementary lead, respectively. A small peak is visible at m/z 98. This mass was also encountered in the DTMS spectra of linolenic-, ricinoleic- and the diacids with a carbon number of 7 and higher. Furthermore, the peaks that are observed at m/z 222 (M-60) and 264 (M-18) can also be found in the 70 eV EI mass spectrum of octadecenoic acids and confirm the formation of unsaturated fatty acyl moieties. A trace of elementary lead and its isotopic pattern is clearly detected at higher scan numbers (scan 70 >; mass spectrum not shown). The lead is first reduced by the residual char on the hot platinum surface before evaporation and ionisation. In the time course of the analysis the presence of lead is detected in two temperature domains. This indicates that there is a difference in the binding efficiency of the lead within the sample. Lead in a mineral form, such as lead oxide or basic lead carbonate (lead white, a common pigment), is desorbed and detected at a high temperature at the end of the analytical run (results not shown here), whereas in the case of a lead carboxylate such as lead stearate or octoate (result not shown) the compound evaporates much earlier.

The third metal carboxylate investigated is the freshly prepared binuclear copper salt of palmitic acid ($(C_{15}H_{31}(CO)OCu)_2$). The TIC depicted in Figure 8 clearly differs from the one obtained for lead distearate and sodium palmitate. A complex profile with two distinct peaks is visible in the TIC, ranging from scan 50-60 (A) and 74-80 (C), respectively.

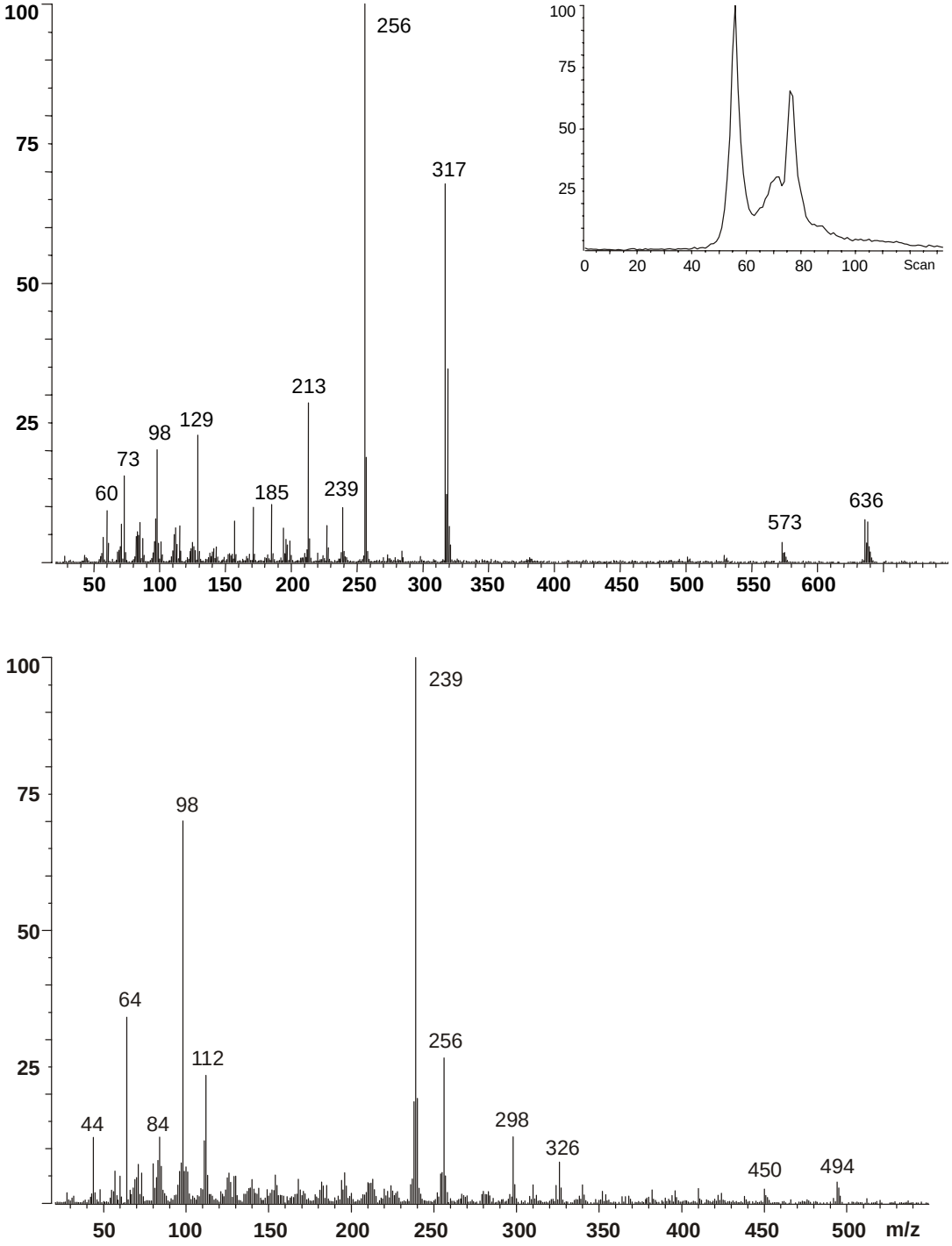


Figure 8. DTMS summation spectrum of copper dihexadecanoate (a) scan 50-60 Insert: TIC, and (b) scan 74-80.

The mass spectrum of peak A (Figure 8a) is almost identical to the mass spectrum obtained for a free palmitic fatty acid but three clusters are seen in addition at m/z 317, 573 and 636, which can be ascribed to copper palmitates. M/z 636 can be identified as the intact copper palmitate, consisting of two fatty acyl moieties and two copper ions ($(C_{15}H_{31}(CO)OCu)_2$). The isotopic distribution is in agreement with the theoretical isotopic distribution for a compound with two copper ions (m/z 636:637:638:639:640 ~ 100:35,8:95,6:32,5:25,6). At m/z 573, 63 mass units lower, a cluster with a different isotopic distribution is seen, which indicates the loss of one copper from the intact molecular ion. The isotopic distribution of the cluster at m/z 317 suggests that this fragment ion consists of a copper and a fatty acyl moiety. However, the mass is one unit lower than is expected for a "normal" copper carboxylate. Most likely, Cu insertion has taken place on the fatty acyl chain, accompanied with the loss of hydrogen and resulting in a cyclic structure. The formation of $[C_{15}H_{31}(CO)OH_2]^+$, as has been discussed previously, is seen as well. Another feature of the mass spectrum is the increased intensity of m/z 98 ($[C_6H_9O]^+$), compared to the spectrum of free long chain fatty acids. In the mass spectrum of peak C emerging at higher temperatures (and also peak B (scan 63-73), not shown), a high intensity acylium ion is present at m/z 239 (See Figure 8b). Furthermore, ions have been observed at m/z 84, 98, and 112, with m/z 98 with a high intensity (70%), which are thought to have a cyclic structure and molecular formula $C_5H_7 + nCH_2$ (n=0-2). In the higher mass region low intensity peaks at m/z 450 and 494 are observed. These ions can be ascribed to the heat-induced formation of long-chain ketones ($C_{15}H_{31}(CO)C_{15}H_{31}$) and anhydrides ($C_{15}H_{31}(CO)O(CO)C_{15}H_{31}$), respectively, by ketonic decarboxylation (a type of head-to-head condensation) [350]. The origin of ions at m/z 298 and 326 is presently unknown. In the mass spectrum also residues of the sulphate, which has been used for the synthesis, are visible at m/z 64 and m/z 80. This is thought to be SO_2 and SO_3, respectively, which evolves upon pyrolysis. Copper itself (m/z 63: 65 ~ 2:1) is desorbing from scan 115 onwards (not shown) pointing to high temperature reduction processes.

4.3.2 Acylglycerols

The third type of material present in (fresh) oil paints are acylglycerols. These are the most important sources of fatty acids in fresh oil paints, the amount being reduced in the course of time due to hydrolysis and oxidation. Two different saturated triacylglycerols were investigated, although these are not expected to be present in the drying oils used for traditional paints. Tristearoyl glycerol, with a molecular weight of m/z 890, is desorbed in the scan window 38-48. It is immediately clear from the TIC (insert Fig. 9) that this type of material is detected over a relatively large scan range. This is explained by condensation on the colder walls of the ion source and the "stickiness" of the material. Therefore it remains in the mass spectrometric system for a relative long period. Increasing the temperature of the pyrolysis filament will heat up the ion source and condensed material will be redesorbed. Inspection of the summed mass spectrum (scans 41-

46, Fig. 9) gave a small but distinctive molecular ion. An interesting feature of the mass spectrum is the presence of fragment ions of low intensity, corresponding to the loss of water. These are formed by enolisation followed by 1,4-elimination of water as was shown by Aasen et al. [324]. Two classes of ions are more intens: 1. ions containing two complete fatty acyl moieties and 2. ions containing only one intact fatty acyl moiety. The fragment ions formed by loss of an acylium group and an acylium group plus additional hydrogen are examples of class 1 ions. These fragments are seen at m/z 607 and 606, respectively. In the case of the saturated triacylglycerols, the $[M\text{-}RCOO]^+$ is more intense than the $[M\text{-}RCOOH]^+$ peak. A second class 1 ion is formed upon a McLafferty-rearrangement leading to a fragment ion of m/z 666. The loss of water from this fragment ion is observed as well (m/z 648). Furthermore an important diagnostic ion is observed with low intensity at m/z 593 and is formed upon cleavage of the glycerol backbone between positions 1,2 and 2,3. In the case of this particular triacylglycerol, all acyl chains are identical so no distinction can be made between the different positions. The simplest class 2 ion is the acylium ion m/z 267. A fragment ion at one amu lower is also observed. The second important fragment ion is seen at m/z 341 ($[RCO+74]^+$). This ion corresponds to an ion containing the glycerol backbone, minus 1 hydroxy group. The fragment ion at m/z 382 ($[RCO+115]^+$) can be ascribed to a glycerol backbone with an additional ethene group, formed by a McLafferty rearrangement upon the loss of an alkyl chain. Almost similar to this structure is the fragment ion at m/z 395 ($[RCO+128]^+$), which contains the first three carbons from one of the other alkyl chains as a consequence of a fragmentation at the allylic position. Close inspection of the less abundant ions at higher masses reveals that peaks are recurring at intervals of 14 m/z units. These ions are thought to be formed by a simple homolytic cleavage of the alkyl chain of fragment ion $[M\text{-}RCOOH]^+$. One of these ions, m/z 451, is more intense. This is rationalised by the possible formation of a six-membered ring structure [324, 351]. Comparison of the 70 eV EI spectrum of tristearoyl glycerol [307] with Figure 8 makes clear that identical fragment ions are observed but that relative ion intensities are higher for m/z 267, 341, and the ions at higher masses (> m/z 600). Formation of a fragment ion at m/z 98 and 112 was also observed in this spectrum.

For trimyristoyl glycerol the same type of fragment ions were observed (see Figure 10) but at 112 or 56 amu lower relative to the fragment ions found for tristearin, corresponding to the number of fatty acyl moieties present in the fragment ions.

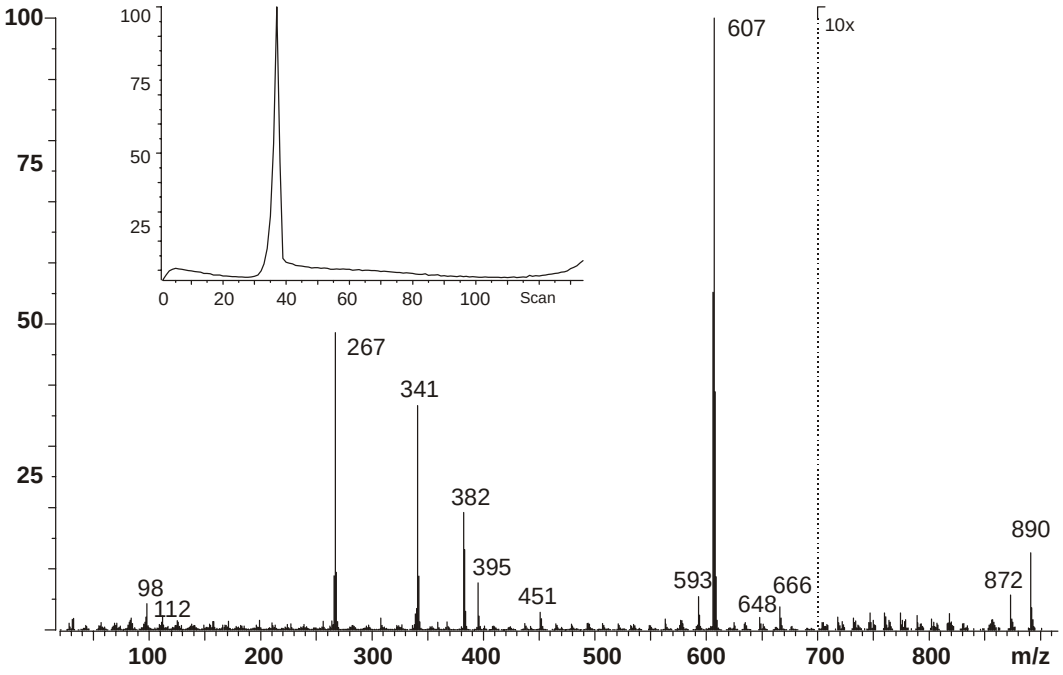


Figure 9. DTMS summation spectrum of tristearoyl glycerol. (scan 41-46). Insert: TIC.

The mass spectrum becomes more complicated when triacylglycerols with different (unsaturated) fatty acyl moieties are investigated. These types of molecules are the major constituents of fresh oil paint. The TIC (not shown) is almost identical to that of the saturated TAGs, the only difference being the onset of desorption. In Figure 11, the summed mass spectrum is depicted of 1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol (scans 32-38). The molecular mass is visible at m/z 856. Water loss is observed giving rise to a fragment ion at m/z 838. Several intense ions contain two fatty acyl moieties, e.g. the ions at m/z 601 and 600, which are formed by the loss of the palmitoyl unit. The remaining fragments contain two fatty acyl groups with a total number of 3 unsaturations and therefore the intensity of the $[M\text{-}RCOOH]^{+\cdot}$ fragments is higher relative to those of $[M\text{-}RCOO]^{+}$ [37]. For the class 1 ions with a palmitoyl moiety still esterified to the glycerol, these $[M\text{-}RCOOH]^{+\cdot}$ fragments are also observed at m/z 576 and 574 respectively. The latter fragment has a higher intensity relative to the $[M\text{-}RCOO]^{+}$ ion because of the two vs. one double bond present.

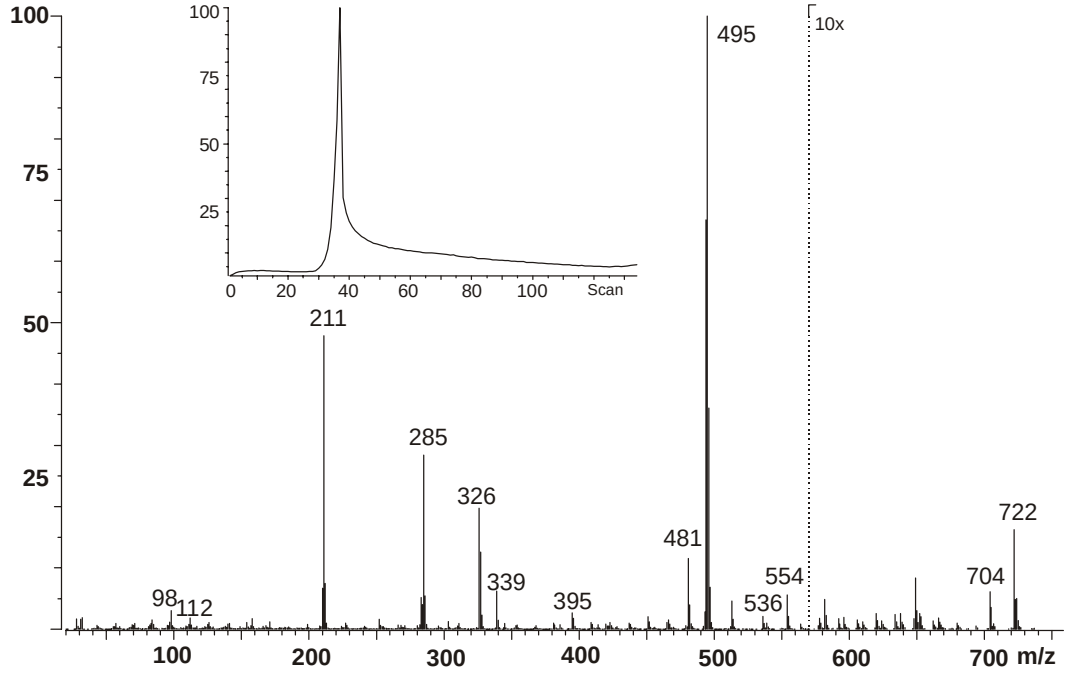


Figure 10. DTMS summation spectrum of trimyristoyl glycerol. (scan 41-46). Insert: TIC.

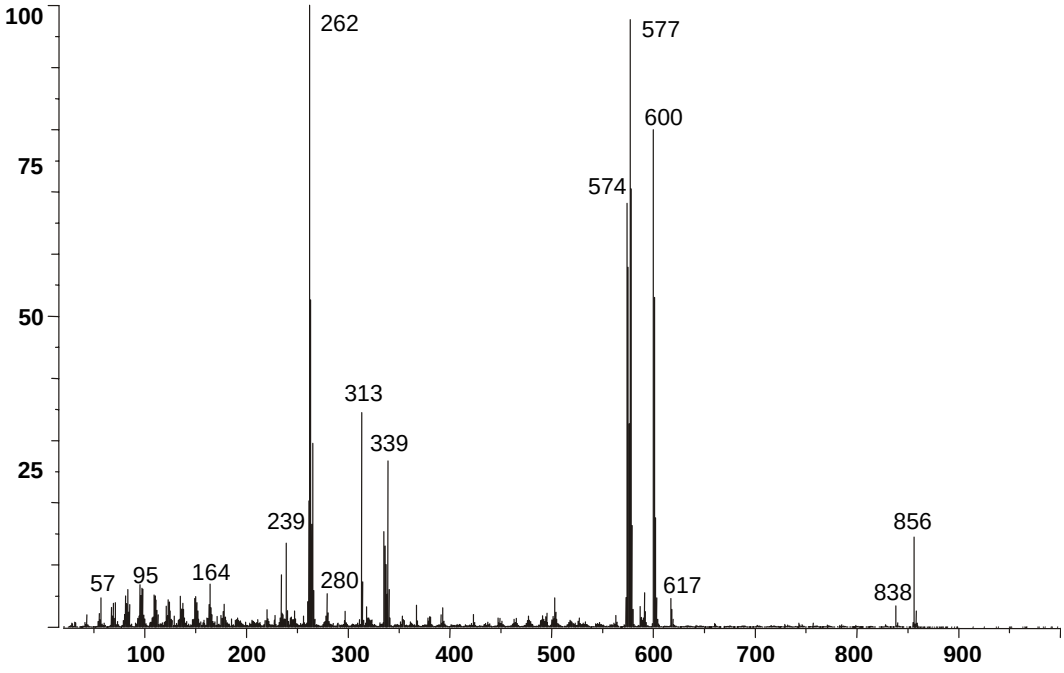


Figure 11. DTMS summation spectrum of 1-palmitoyl-2- oleoyl-3-linoleoyl-glycerol. (scan 32-38). Insert: TIC.

The position of the middle fatty acid group is confirmed by fragment ions formed upon cleavage of the glycerol backbone. Cleavage of the bond on the 1,2 and 2,3 position gives rise to low intensity ions at m/z 587 and 563, whereas no

distinct ions are observed at m/z 561. This confirms the location of the oleyl moiety.

The acylium ion of the C18:2 fatty acids is of high intensity compared to those of C16 and C18:1 fatty acids. Furthermore, the RCO^+ ions of the unsaturated acylium ions are accompanied by prominent $[RCO\text{-}H]^{+\cdot}$ ions. The loss of the additional hydrogen might give rise to the formation of a ketene-type species rather than an acylium ion, which is thought to result in an energetically more favourable structure. Experiments performed by Lauer et. al. [307] showed that the intensity of the $[RCO\text{-}H]^{+\cdot}$ fragment ions increased relative to the RCO^+ ions when 15 eV EI was used for ionisation instead of 70 eV EI. The $[RCO+74]^+$ ions are observed at m/z 313 and 336-339 for the C16 and unsaturated C18 fatty acids, respectively. The $[RCO+115]^{+\cdot}$ and $[RCO+128]^+$ ions are of very low intensity, but still observable, i.e. m/z 354, 378-380, and m/z 367, 391-393. The same holds for the series of $[RCO+128+14n]^+$. Whereas for tristearin only one fatty acyl moiety was present and therefore relatively intense $[RCO+128+14n]^+$ ions were observed, in this case three different $[RCO+128+14n]^+$ ions are formed accompanied with the additional loss of hydrogen, giving rise to a distribution of ions that is more spread out in this region.

The presence of a second peak that is two mass units higher relative to the molecular ion suggests that this sample also contains traces of 1-palmitoyl-2-stearoyl-3-linoleoyl glycerol and/or 1-palmitoyl-2,3-dioleoyl glycerol, i.e. a triacylglycerol with a total number of 2 double bonds. Closer inspection of the mass spectrum suggests the latter since the ions at m/z 589 can only be formed from this compound. It is clear from this last observation that analysis of oil solely on the basis of DTMS will be complicated by the large number of isomeric triacylglycerols that are present.

4.3.3 Complex reference materials

4.3.3.1 Linseed Oil

Drying oils used for painting are a mixture of (isomeric) triacylglycerols, with fatty acyl moieties with different degrees of unsaturation and number of carbon atoms (see Table 2). This is clearly visible in the mass spectrum shown in Figure 12 that was obtained for a raw purified linseed oil. The bulk of the material was detected within scan numbers 40-54, but molecular information is detectable afterwards due to condensation/redesorption. The mass spectrum of scans 49-53 shows two clusters of molecular ions at m/z 850-860 and 852-886, corresponding to triacylglycerols with one C16, two C18 fatty acids and 6 to 1 double bonds and triacylglycerols with three C18 fatty acids and 9 to 2 double bonds, respectively. No distinct signals are seen for triacylglycerols with two or three saturated C16 fatty acids or three saturated C18 fatty acids. The ratios of the molecular ions are not in agreement with the absolute amounts of these species reported in literature

(Table 2). This is partially caused by the difference in fragmentation probability and ionisation efficiency. Furthermore, linseed oil is not a pure product and regional variations are expected. Fragment ions formed upon loss of an acylium ion from the triacylglycerols are observed at mass range m/z 619-611 with the lowest m/z representing a diacylglycerol containing two linolenic fatty acids (m/z 611). Complete loss of a fatty acyl chain is seen in the m/z range 605-594, with once more the last m/z representing a diacylglycerol containing two linolenic fatty acyl chains. Diacylglycerol fragment ions consisting of one C16 fatty acyl unit are detected from m/z 579 to 572, with the last ions consisting of a C16- and a linolenic fatty acyl moiety. Acylium and $[RCO+74]^+$ ions are seen for all fatty acids with those of the unsaturated fatty acids of highest intensity. The ratio of these, however, does not reflect the ratio of fatty acids present. The fragment ions $[RCO+115]^+$ and $[RCO+128]^+$ ions are hardly visible in the mass spectrum. Fragment ions arising from fragmentation of unsaturated fatty acids are observed in the low mass region with m/z 108 being the most intense one (50%). These are identical to those observed in the DTMS spectrum of linolenic acid previously described in Figure 2 and in the literature [322].

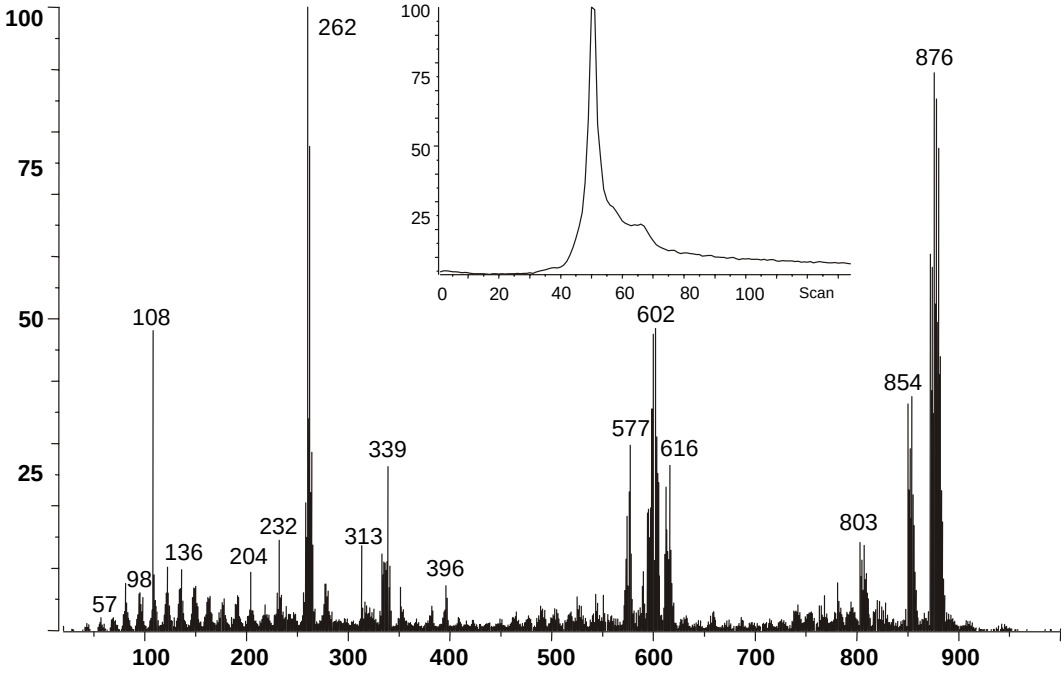


Figure 12. DTMS summation spectrum of fresh linseed oil (Talens). (scan 49-53). Insert: TIC.

Table 2. Relative composition (%) of triglycerides from linseed oil [36]

TAG[a]	RP-HPLC	DCI-MS
PLnLn	4.2	4.3
PLLn	3.1	3.2
PLL	0.6	0.7
POL	0.8	1.0
LnLnLn	50.7	49.3
LLnLn	19.2	18.9
LLLn	3.6	13[b]
OlnLn	9.2	
LLL	2.4	5.6
OLLn	3.2	
OLL	1.4	2.9
SLLn	0.8	
OOL	0.5	0.8
OOO	0.3	0.3

[a] P=palmitic; S=stearic; O=oleic; L=linoleic; Ln=linolenic acid
[b] No discrimination could be made between TAGs with the same molecular weight

4.3.3.2 Prepolymerised oil and oil paint systems

So far, the emphasis of the DTMS analyses lied on relatively simple model compounds. Oil paint however, once it starts to dry, is much more complex due to the formation of cross-linked material, oxidised compounds, hydrolysis products or combinations thereof. Furthermore, heating of the oil prior to paint manufacturing will lead to alterations of the TAGs, including cross-linking processes. In the next paragraphs, DTMS analyses of both prepolymerised oil, as well as several oil paint systems of different age, composition and history will be described. DTMS is also performed on the extractable material of an aged linseed oil film when immersed in the organic solvent hexane or methanol. These last experiments are thought to provide a better insight into the relatively volatile fraction of oil paints. Additional data is obtained on the evolution of solvent extractable components of oil paint films at the same time, a subject that already has been described extensively by K. Sutherland [8].

4.3.3.3 Prepolymerised linseed oil

The DTMS TIC of commercially available non-aged heat bodied oil (stand oil) in Figure 13a consists of two peaks. The peak at low scan numbers (45-55; A) maximises in the same scan range as the peak observed, when analysing fresh linseed oil. Inspection of the corresponding mass spectrum shows (see Figure 13a) a slight decrease in the relative abundance of ions with triply unsaturated fatty acyl moieties. This is expected since the polyunsaturated fatty acids react away during

heat bodying by Diels-Alder cyclisation reactions, which result in cyclic fatty acids and oligomeric material [113, 178]. However, it should be recalled that oils of different natural sources are analysed and that the relative amount of the unsaturated fatty acids could have been lower prior to the heat treatment. In the low mass region the peak of high intensity at m/z 108, also present in mass spectrum of fresh linseed oil and ascribed to C18:3 fatty acyl moieties, has now decreased strongly indicating that these moieties have been altered upon treatment. The second peak in the TIC (B), which is of higher intensity and ranges from scans 65 to 72, suggests that less volatile compounds have been formed upon heating of the oil. The formation of high molecular weight material due to cross-linking by Diels-Alder reactions can account for this. The summed mass spectrum in Figure 13b shows typical m/z values in the high mass region also seen in the spectra of the reference triacylglycerols and the material desorbing at scan 45-55, but with a relatively low intensity. These are arising from material retained in the ion source. The acylium ions of the C18:1 moiety (m/z 264) show up with a higher intensity relative to the multiply unsaturated compounds in the fresh oil, again implying that upon formation of the oligomeric material the multiply unsaturated fatty acyl moieties have a higher reactivity and react away leading to a relative enrichment of the monounsaturated species. As a consequence of the high temperature at which this non-volatile material is desorbed from the pyrolysis wire typical pyrolysis breakdown products of fatty acyl groups are formed like short chain alkanes and alkenes, which give rise to a variety of peaks in the low mass region [352]. At the same time peaks emerge that correspond to the molecular masses of the fatty acids originally present in the TAGs (m/z 256, 284, 280, 278). Up to now these were indicative of free C16 and C18 fatty acids or metal carboxylates thereof. This is however not the case in this sample: metals are not present in high amounts in this sample since pigments or driers were not added and the fresh oil itself only contains trace amounts. Therefore, the fact that fatty acids only appear at relative high temperature indicates that the fatty acids are released upon thermally induced rearrangement of ester bound fatty acids that are part of higher molecular weight networks. Free fatty acids would have evaporated at a much lower temperature.

4.3.3.4 Aged linseed oil

Although the mixture of compounds analysed in these last two measurements is relatively simple, already a rather complex mass spectrum is generated. In the case of a cured or aged oil paint, a complex mixture of oxidised acylglycerols is expected due to incorporation of different numbers and types of functional groups containing oxygen [293], specific breakdown reactions occurring upon autoxidation [135, 141] and hydrolytic processes [29]. In Figure 14a,b the result of a DTMS analysis of a 4-year old aged linseed oil paint is depicted. Most of the material is desorbed and / or pyrolysed at higher scan numbers relative to the fresh oil (see for example Figure 11) as can be seen in the insert of Figure 14a.

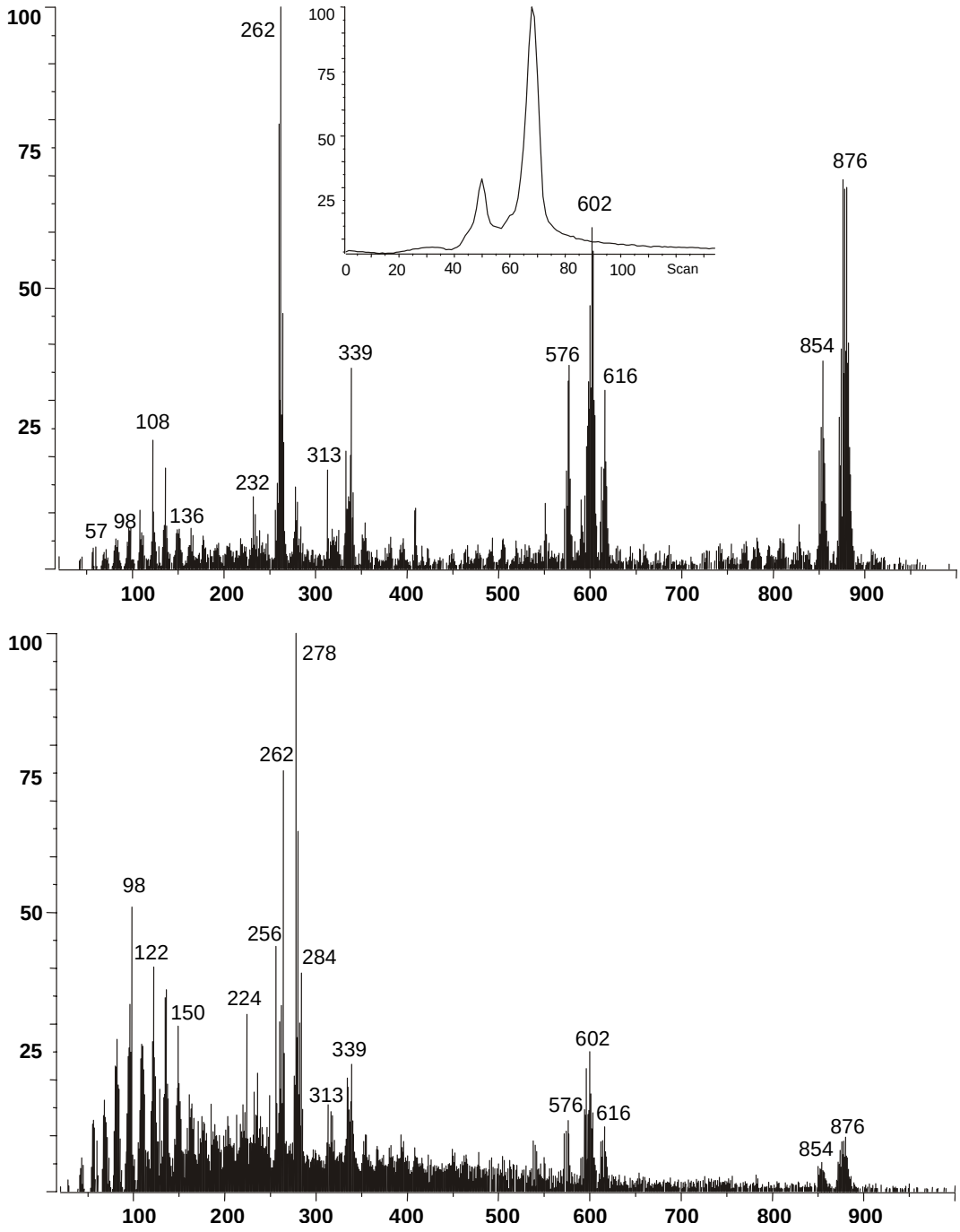


Figure 13. DTMS summation spectrum of fresh stand oil (Talens) (a) scan 45-55 Insert: TIC, and (b) scan 65-72.

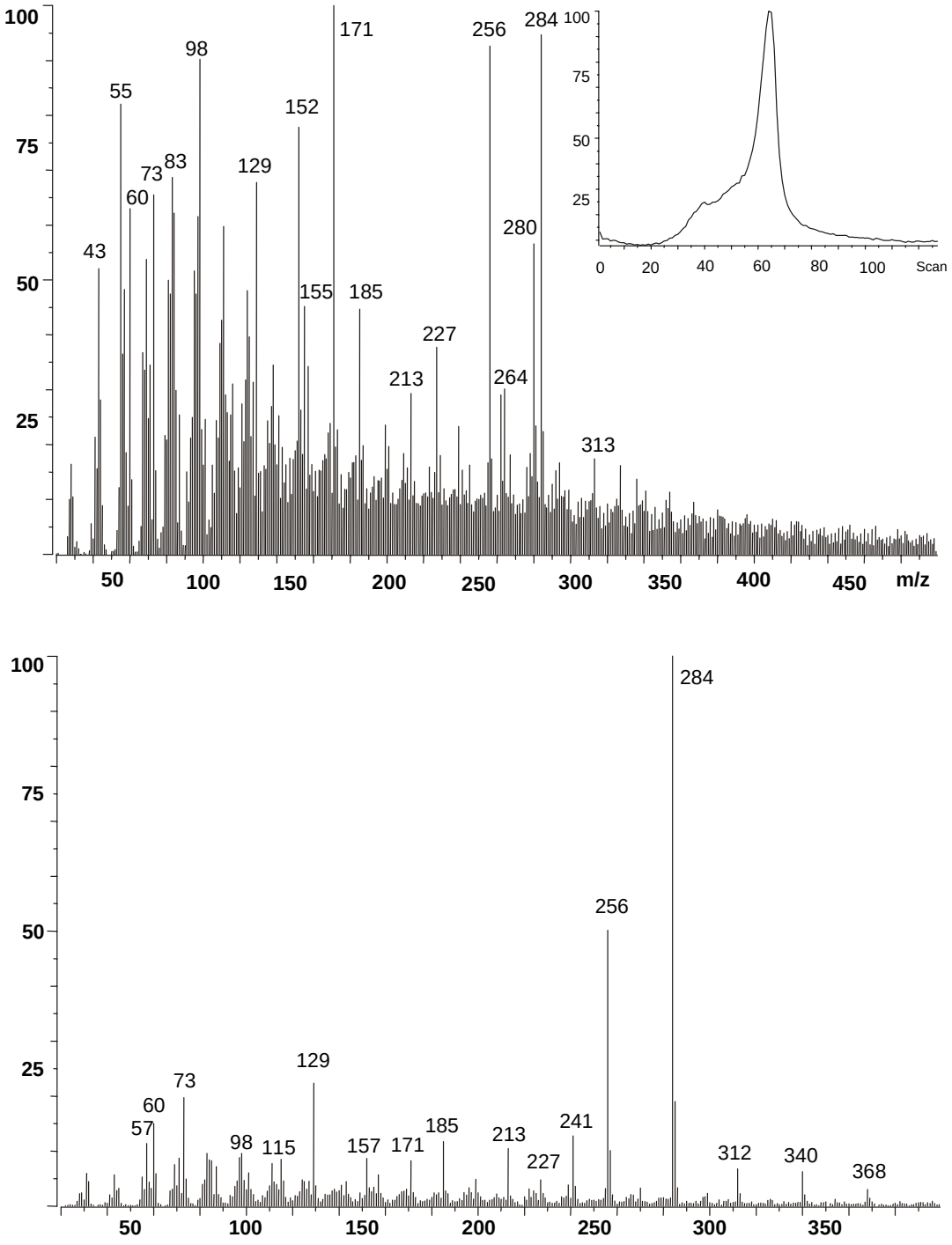


Figure 14. DTMS summation spectrum of 4-year-old dried linseed oil (Talens) (a) scan 56-64 Insert: TIC, and (b) scan 1-15.

This is indicative for the presence of more polar and high molecular weight material. Peaks representative for polar compounds are seen at m/z 152 and m/z 155 in the summed mass spectrum of scans 56-64. These masses can be ascribed to fragment ions of oxidation products: a C9 diacid (see Figure 4) and a 9,10-

epoxyoctadecanoic acid, respectively [353]. Furthermore, a high intensity peak at m/z 171 is observed which is seen in a number of spectra already encountered, but always with a relatively low intensity. This fragment ion is thought to be indicative for FAs that are substituted with oxygen on the 9-position. The large number of low molecular weight masses in combination with the absence of diacyl- or triacylglycerols (fragment) ions suggests that most of the TAGs originally present have reacted away and that pyrolysis of cross-linked material has taken place.

The molecular ions of palmitic and stearic acid at m/z 256 and 284 were observed within two scan regions. Besides the detection at scans 56-64, inspection of the early region of the TIC in Figure 14a shows that these peaks are also present at low temperature. This indicates that free fatty acids are present. This is obvious from Figure 14b, which depicts the summed mass spectrum of scans 1-15. The ratio of the molecular ions of palmitic (m/z 256) and stearic (m/z 284) acids (P/S ratio = 0.5) in this figure is not in agreement with the range of 1.1 to 2.1 as has been determined for different linseed oil paints [12]. This may be explained by unequal evaporation during drying of the sample and /or insertion of the analytical probe into the hot ion source. Upon heating the relative amount of C18 fatty acids increases due to enhanced evaporation of the more volatile C16 fatty acids with a factor of approximately 4, as has been suggested by Schilling et al. [15]. The P/S ratio of 1 observed in Figure 14a seems to match better. These fatty acids, at the time of analysis, are derived from TAGs and their oligomeric material and therefore no discrimination will occur upon drying. Besides these two saturated fatty acids also low intensity molecular ions of C20, C22 and C24 fatty acids are observed at m/z 312, 340, and 368, respectively. These are known to be present in fresh oils in trace amounts [292] and most probably are better retained within the paint due to their reduced volatility. No (fragment) ions are observed that point to the presence of unsaturated fatty acids. These must have reacted away by autoxidation forming oxidised compounds and/or are incorporated into cross-linked material.

4.3.3.5 Extractables

The extractive power of certain solvents applied to a paint sample can also be monitored using DTMS. This is demonstrated for a hexane extract of the same 4-year old aged linseed oil paint obtained by immersion of the paint. It is expected that free (oxidised) fatty acids, mono-, di- and triacylglycerols will be extracted apart from extractable low molecular weight oligomeric material. The TIC (insert Figure 15a) shows that most of the extracted material evaporates in the early stage of the analysis (scans 1-20), indicative for relatively volatile material. Other compounds are desorbed within scan range 32-48. The summed mass spectra of both peaks are depicted in Fig. 15a and b. First, free saturated fatty acids evaporate from the filament as is clear from m/z values 60, 73, 129, 171, 185 and 213 (Figure 15a). The corresponding molecular ions are observed at 256, 284, 312, 340, and 368, corresponding to C16-C24 fatty acids. The P/S ratio of 0,87 for this

analysis, on the basis of the relative abundance of the molecular ions, is rather low and not in accordance with the ratio of 1.17 as determined by GC analysis in our lab. In the high mass region no peaks are detected that can be ascribed to intact triacylglycerols, or their breakdown products.

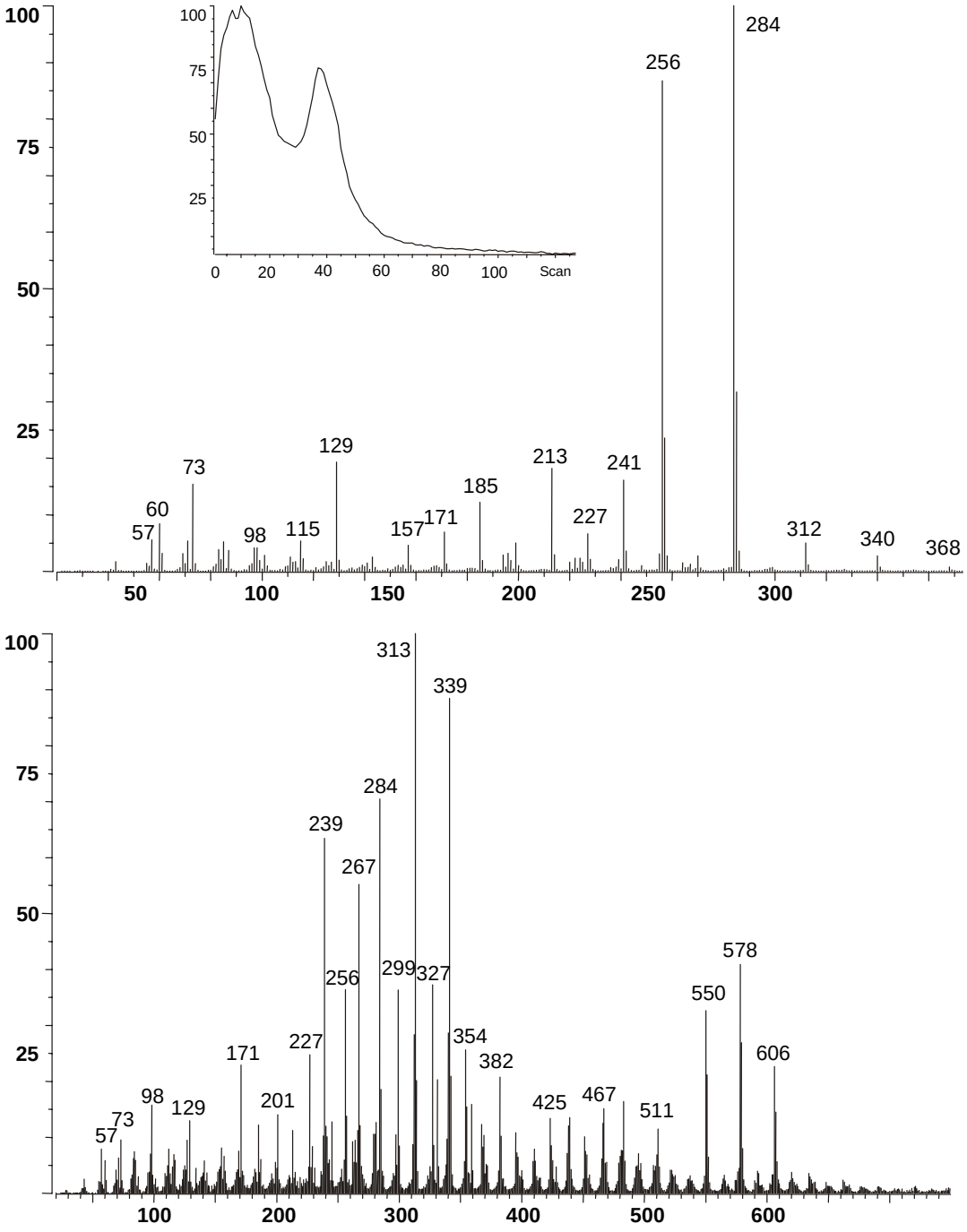


Figure 15. DTMS summation spectrum of a hexane extract of 4-year-old dried linseed oil (Talens) (a) scan 1-20 Insert: TIC, and (b) scan 32-48.

The mass spectrum of the second peak in the TIC (Fig. 15b), however, shows fragment ions typical for saturated acylglycerols as expected. No distinct peaks were observed above mass 700, which indicates that most of the original triacylglycerols have reacted away. Fragment ions of the diacylglycerols of saturated fatty acids are seen at m/z 550, 578 and 606. These masses can be explained by the loss of water from the molecular ions. Ions at m/z 256 and 284 show up in a ratio of 0.5, whereas fragment ions indicative of esterified palmitic and stearic fatty acids, m/z 239 and 267 $[RCO]^+$, and m/z 313 and 341 $[RCO+74]^+$, respectively, have a ratio of 1.15. This can be explained by the fact that the free fatty acids are derived from the tail of the first peak in the TIC, giving rise to a relative increase of the less volatile C18 fatty acids. No peaks indicative of unsaturated diacylglycerols are observed, mainly because these will have reacted away, giving rise to polar species that are less prone to extraction with hexane or that have cross-linked and / or are incorporated into the oil network(s). These compounds may fragment relatively easily as is suggested by the series of clusters of relatively high intensity ions with a spacing of 14 amu that is seen throughout the entire spectrum. This phenomenon can be ascribed to fragmentation of the saturated carbon backbone of the fatty acid chains of the (cross-linked) acylglycerols. There are however low intensity ions seen at m/z 262 and 264, which would be indicative for unsaturated fatty acids. These are supposed to be formed partially by retro Diels-Alder like fragmentation processes.

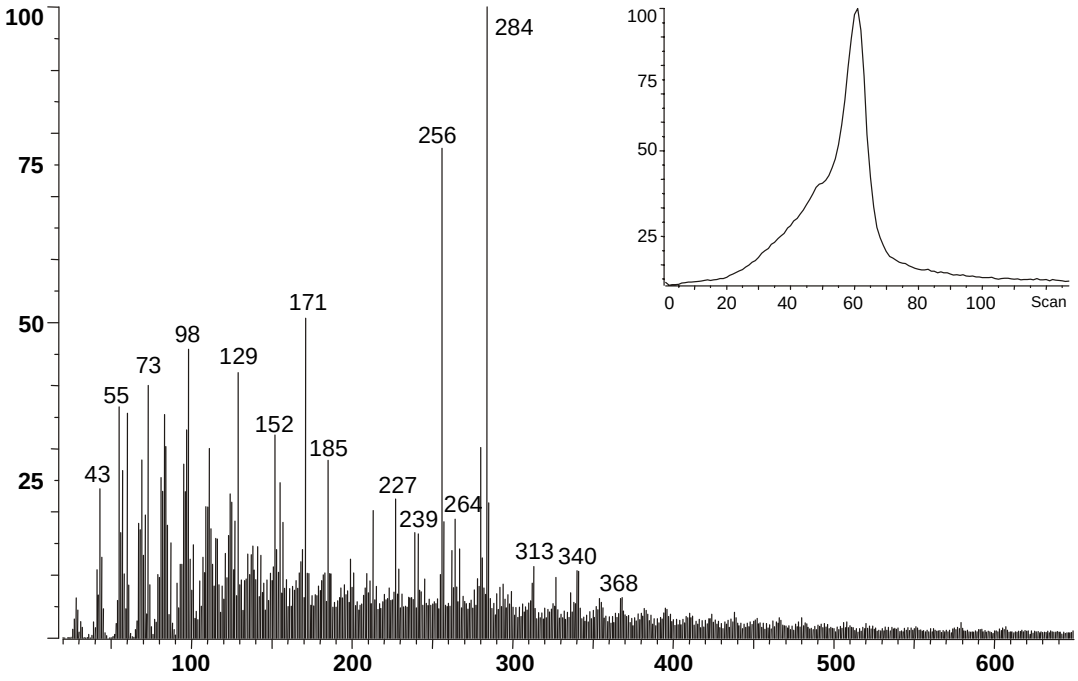


Figure 16. DTMS summation spectrum of a methanol extract of 4-year-old dried linseed oil (Talens) scan 60-68 Insert: TIC.

New ions are seen in the mass region of compounds with a single fatty acid strand. M/z 299 and 327 are thought to be formed upon cleavage of the glycerol backbone of the diacylglycerol between the 1,2 or 2,3 positions. The nature of the ions at m/z 331 and 359 is unknown. In the low mass region, a number of fragment

ions is visible that were encountered before: m/z 155, which is ascribed to the presence of oxidised fatty acids containing a 9,10 epoxy group, and the series of ions representative for carboxylic fatty acid groups (see above).

Extraction with the polar solvent methanol of the same relatively fresh and unpigmented linseed oil paint leads to a different result as can be seen in Figure 16. Now large amounts of material have been extracted and most of the film has dissolved. This is also reflected in the TIC, which looks very similar to the TIC obtained for the whole paint sample (Figure 14a). In the summed mass spectrum of scans 55-64, depicted in Figure 16, the same m/z values are seen when compared to Figure 14a, with a relative increase of the peaks at m/z 256 and 284. This is due to lower amounts of cross-linked material in the extract.

4.3.3.6 Lead white pigmented oil paint

The DTMS TIC (see insert Fig. 17a) from a 4-year old lead white pigmented paint (paint to pigment ratio 2,3:1) is clearly different compared to the unpigmented paint. The TIC indicates a relative shift towards material desorbing at lower temperatures (compare Figure 16 and 17a). This is caused partially by desorption of pigment related material. Carbon dioxide (m/z 44) is given off due to thermal breakdown of the basic lead carbonate and to a lesser extent by the decarboxylation of the ester bonds. The evolution of carbon dioxide (m/z 44) takes place within scan 45 to 60 as can be seen in the mass chromatogram depicted in Figure 18. Mass chromatograms of typical (fragment) ions previously identified for the reference materials show evaporation of acylglycerols (m/z 550 and 267) in combination with oxidised fatty acids (m/z 152) at scans 40-70, fatty acids at scan 1-15 and 45-65 (m/z 284).

In Figure 17a the mass spectrum of the summation of scans 40-60 is shown. Besides m/z 44 from CO_2, (fragment) ions of (oxidised) fatty acids are observed (m/z 98, 152, 155, 171, 185, 213, 241, 256, and 284)). Closer inspection of the low mass region of Figure 17b (summation mass spectrum of scans 65-70) shows that two new peaks have appeared in between the series of small (unsaturated) aliphatic fragment ions which shows up as an unresolved envelope in the mass spectrum. The peaks, with m/z values 91 and 105, can be attributed to alkylated benzenes that are formed from the cross-linked oil paint material upon exposure to high pyrolysis temperature. This has been shown for sodium salts of unsaturated and oxidised fatty acids [354]. The n-alkylbenzenes formed were not observed in experiments performed at lower temperature. It is inferred that the elimination of free fatty acids from the paint system by thermal processes is accompanied by the loss of hydrogen from the residual oil network leaves a more aromatic residue. There may be some resemblance to the thermal dissociation behaviour of polyvinyl chloride (PVC) in which pyrolytic removal of the chlorides by release of HCl left a very aromatic network structure that is subsequently characterised by small

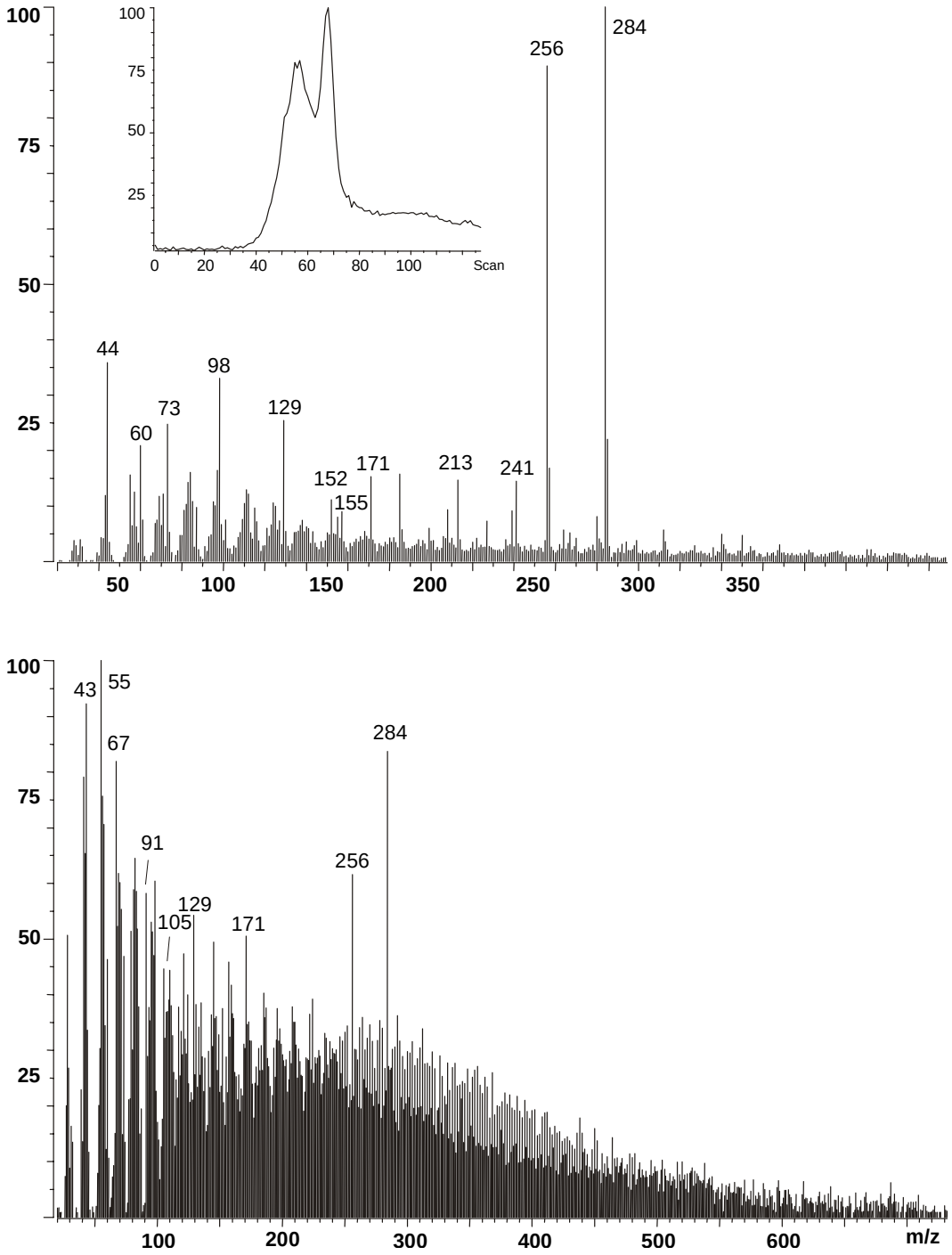


Figure 17. DTMS summation spectrum of 4-year-old dried lead white pigmented linseed oil (Talens) (a) scan 40-60 Insert: TIC, and (b) scan 65-70.

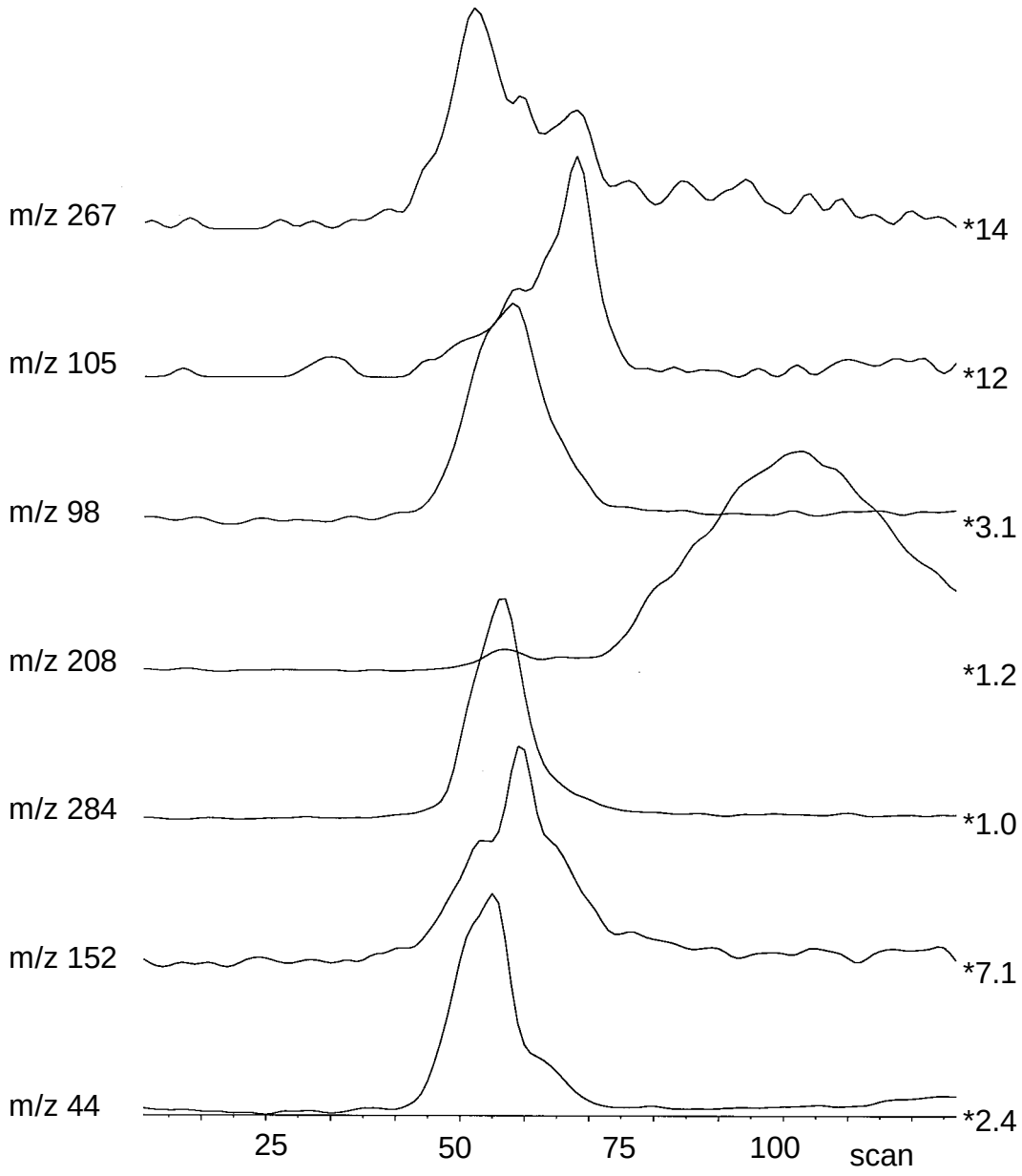


Figure 18. Mass chromatograms of 4-year-old dried lead white pigmented linseed oil (Talens) obtained with DTMS (see also Figure 17).

alkylbenzenes in the pyrolysate [355]. Relatively low amounts of free fatty acids are detected in the beginning of the TIC. This implies that hydrolysis has not proceeded extensively and that a large percentage of the fatty acids are still esterified and / or that free fatty acids have been "trapped" by formation of lead carboxylates. Inspection of the scan region between 55-65 for the presence of lead

salts shows the presence of acylium ions of both palmitic- and stearic acid, but no distinct signals are observed for those fragment ions encountered in the DTMS analysis of lead distearate (m/z 507 and 491). The high number of carboxylic acid groups that can be formed within the paint networks most likely leads to the formation of lead co-ordinated oil paint, which will also partially trap the hydrolysed palmitic- and stearic acids as lead carboxylates. This material cannot evaporate directly and is most likely pyrolysed. Not only the saturated fatty acids will react with the lead present but also a variety of smaller breakdown products which contain carboxylic acid groups. A whole range of ions will be formed upon pyrolysis and specific ions will be lost in the unresolved envelope of peaks. The fact that lead has three isotopes will make it even more problematic to detect distinct peaks of the low molecular weight lead carboxylates. Lead, traced by m/z 208 and appearing in a high temperature window (scan number 85-120; Fig. 18), evaporates as elementary lead after reduction from its metal ion state by the residual char on the hot platinum surface.

Solvent extraction of this film with hexane gave similar qualitative results compared to the unpigmented paint. Extraction with methanol led to a mixture, which contained relatively higher amounts of oxidised fatty acids as is visualised by a higher relative abundance of m/z 152 and 155 (results not shown). This can be ascribed to the catalytic effect of lead on the autoxidation process.

4.3.3.7 25-year old lead white pigmented oil paint

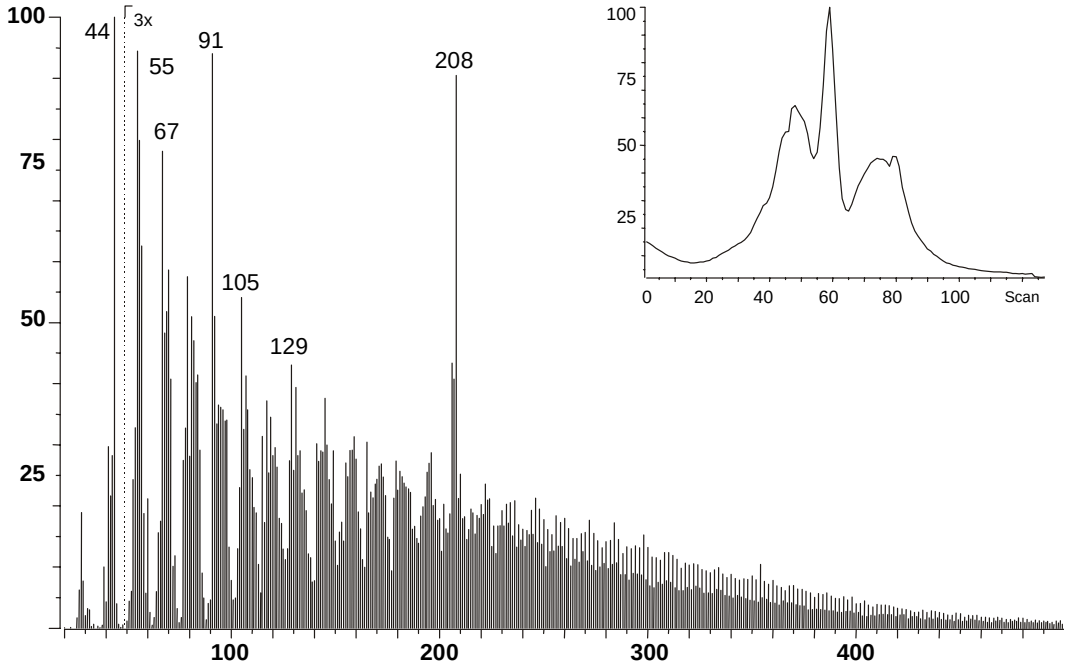


Figure 19. DTMS summation spectrum of 25-year-old dried lead white pigmented linseed oil (CCI) scan 55-62 Insert: TIC.

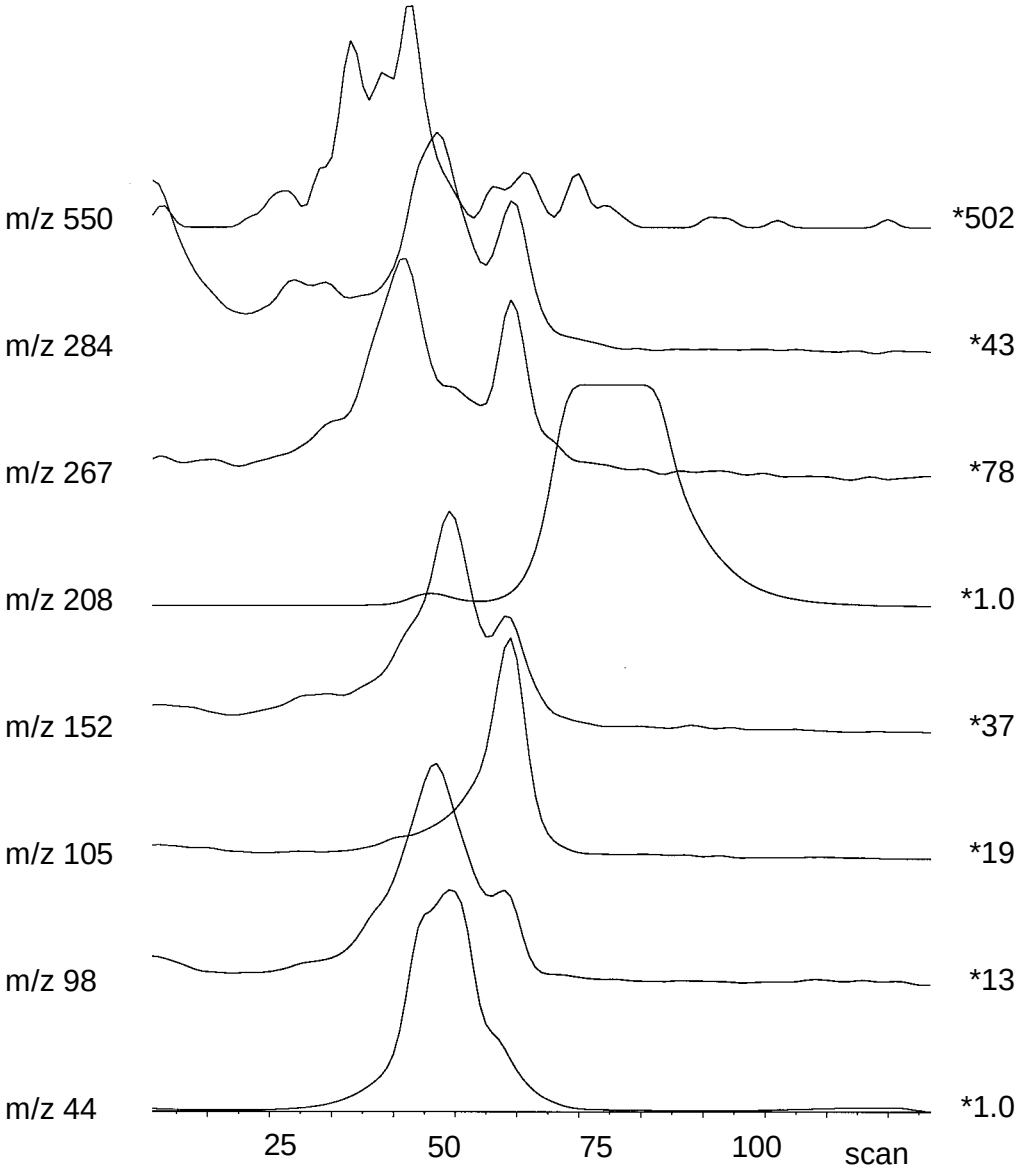


Figure 20. Mass chromatograms of 25-year old dried lead white pigmented linseed oil (CCI) obtained with DTMS (see also Figure 19).

The TIC of a 25-year old lead white ($PbCO_3$ $Pb(OH)_2$) pigmented linseed oil paint reconstruction depicted in Figure 19 shows a desorption/pyrolysis profile that is very similar to the profile of the 4-year old lead white pigmented paint, although more complex (insert Figure 19). Besides relatively higher amounts of volatile material desorbed in the beginning of the analysis, another peak has appeared at higher temperature. Inspection of the different regions by mass chromatograms (Figure 20) shows evaporation of free fatty acids mainly from scan 0 to 25 (m/z 284), oxidised fatty acids (m/z 152) and trace amounts of acylglycerols (m/z

550+267) within scans 30-45, and the evolution of carbon dioxide (m/z 44) from scan 35 to 62 largely due to thermal breakdown of the basic lead carbonate and to a lesser extent the decarboxylation of the cross-linked network. The lead itself (m/z 206-207-208) evaporates at higher temperature (scan number 60-100) and the intensity of the peak suggests that a rather high amount of lead hydroxy carbonate is present within the film. If this result is compared with the previous analysis it is clear that there can be some variation in the appearance time of a specific constituent in the TIC profile. This will depend on differences in the shape of the pyrolysis wire, the position and thickness of the sample deposited on the wire, and therefore the temperature profile within the sample. Besides, matrix effects are also not to be excluded. No distinct signals were found in the high mass region that could be ascribed to the presence of lead carboxylates of the two most dominant fatty acids, palmitic and stearic acid. However, a distinct increase in peak intensity is observed for m/z 239 and 267 in the scan range 55-65. Although it is difficult to positively identify lead salts as has been explained in the previous experiment, it is thought that the appearance temperature of the acylium ions is a good indicator of the nature of the lead soap desorbed. Ions indicative for the cross-linked oil fraction also are detected from scan 55 to 62. In this case no distinct signals are seen for the fatty acids or cross-linked material thereof in the summed mass spectrum (Fig. 17), but only an unresolved envelope of low molecular weight material with distinct ions at m/z 44, 91 and 105 due to pyrolytic breakdown of the cross-linked material. No distinct, identifiable peaks can be seen anymore in the mass spectrum above mass 210. Furthermore, it should be noted that lead already starts desorbing in this temperature range.

4.3.3.8 58-year old cobalt pigmented linseed oil paint

The analysis of another reconstructed linseed oil paint, made with cobalt blue ($CoO\ Al_2O_3$) as pigment and dating from 1941, gave an almost identical result. The TIC trace in Figure 21 resembles that of the 25-year old lead white paint except that the peak ascribed to lead is missing. The signals arising from palmitic- and stearic acid are more pronounced in the low temperature region of scan 20-39 whereas for the lead pigmented oil paint they were mainly desorbed at lower temperature in the beginning of the measurement. This phenomenon was observed for a whole range of 50 to 60-year old oil paints, which indicates that the fatty acids present are not as mobile as those in relatively young paints. This may be due physical entrapment within the cross-linked networks. The fragment ions previously ascribed to acylglycerols are hardly visible here. This suggests that either hydrolytic processes have proceeded to a larger extent in this cobalt pigmented paint or that cross-linking led to immobile acylglycerols. Whereas lead could be detected in the lead white pigmented oil paint no peaks are visible that can be ascribed to the inorganic pigment here. The mass spectrum of the cross-linked part (Fig. 21, scans 58-64) looks very similar to Figure 19 except for the lower abundance of m/z 44 and 206/207/208 due to the absence of lead carbonate in this sample. The fact that m/z 44 is still present is a good indication for the

pyrolytic decarboxylation of acid groups and carboxylates present in the cross-linked material.

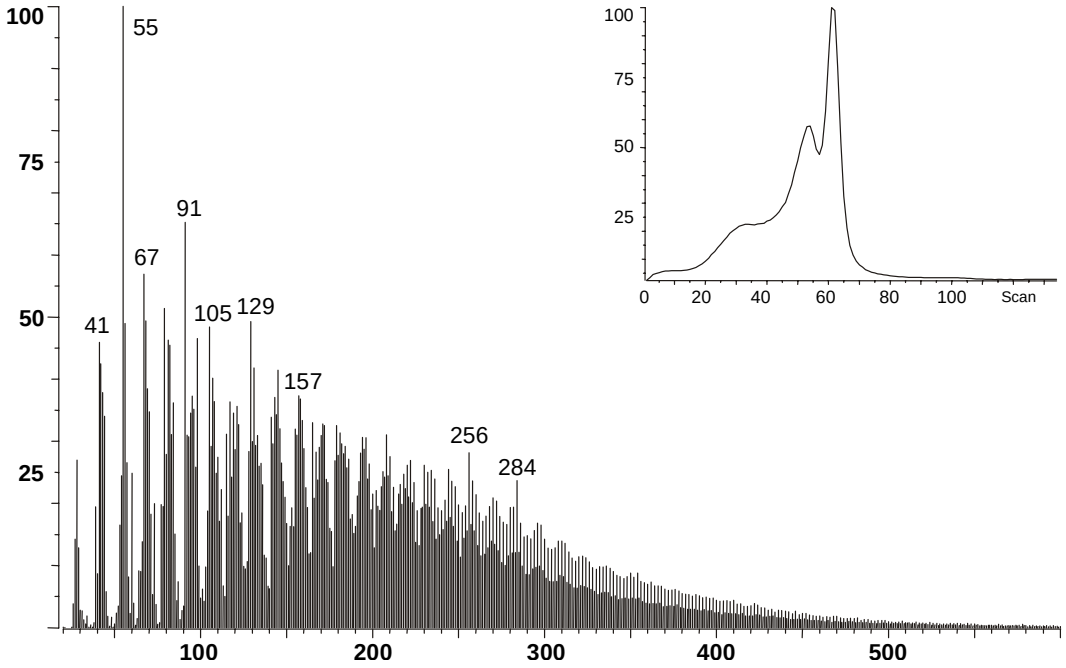


Figure 21. DTMS summation spectrum of 58–year old dried cobalt blue pigmented linseed oil (CCI) scan 58-64 Insert: TIC.

4.3.3.9 17th century white impasto paint

DTMS analysis of this unknown sample leads to a very similar desorption profile (Figure 22a) compared to the 25-year old lead white pigmented linseed oil paint. Figure 22b depicts a number of mass chromatograms of typical (fragment) ions. It is clearly seen that most of the saturated fatty acids are released within scans 25-46, identical to the situation observed for the 58-year old oil paint. The P/S ratio, based on the intensity of m/z 256 and 284, is close to 2. Although this number is within the range of linseed oil paints it cannot be said with absolute certainty that the paint has been made with linseed oil. Evolution of relatively large amounts of carbon dioxide is occurring within scans 50-59, indicative for the presence of carbonates. An unresolved envelope ascribed to small pyrolysis breakdown products is seen from scan 60 to 69, with low intensity ions 206, 207, and 208, indicative for lead on top of it (results not shown). A high intensity lead isotope cluster is observed at higher temperatures (scans 72-115). It can be concluded from these results that the impasto paint consists of a lead white pigmented oil paint. The age of the sample cannot be derived from the profile nor the type of materials detected as is obvious after comparison of both aged lead white pigmented linseed oil paints analysed.

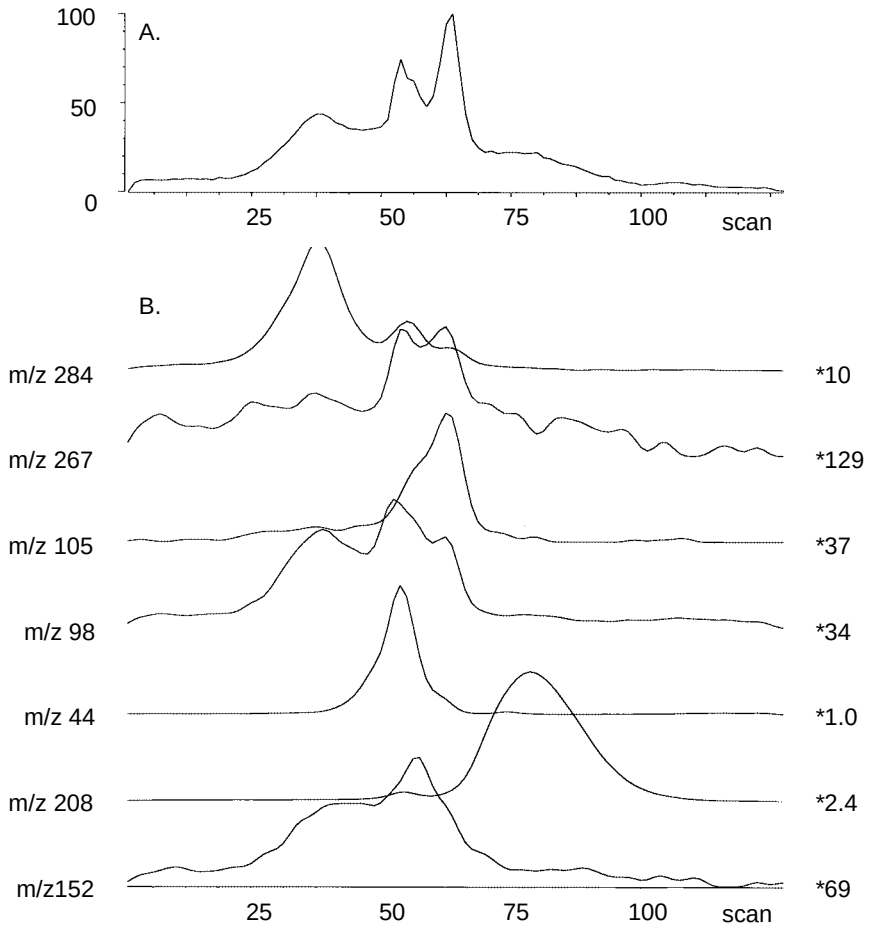


Figure 22. (a) DTMS TIC, and (b) mass chromatograms of a white impasto paint sample of "The Jewish Bride" by Rembrandt (SK-C-216). The probe filament was temperature programmed at a rate of 1 A/min. Compounds were ionised at 12 eV under electron ionisation conditions.

4.3.4 Comparison with Pyrolysis-GC/MS

The 25-year old lead white pigmented paint was also analysed with 610 ºC Curie-point pyrolysis GC/MS to separate and identify the products formed upon application of a high temperature to the paint system. Although the effect of the pyrolysis upon the oil paint will not be exactly the same when compared to DTMS measurements the type of products formed and their mass spectra will give a better understanding of the DTMS results and a more reliable interpretation of the analytical data. The main differences affecting the type of products formed are the heating time, pressure and temperature, hence the way and the amount of energy that is deposited into the sample.

Table 3. Identified compounds in the Curie-Point pyrolysis (610 ºC) – GC/MS measurement of 25-year old lead white pigmented oil paint. The numbers correspond to Figure 23.

No.	Rt. (min)	Compound[a]	MW
1.	3.74	methylbenzene	92
2.	4.00	octene	112
3.	4.15	octane	114
4.	4.28	cyclopentanone	84
5.	4.34	hexanal	100
6.	4.37	butanoic acid	88
7.	5.62	dimethylbenzene	106
8.	5.86	dimethylbenzene	106
9.	6.16	nonene	126
10.	6.32	nonane	128
11.	6.42	styrene	104
12.	6.61	cyclohexanone	98
13.	6.66	pentanoic acid	102
14.	8.41	benzaldehyde	106
15.	8.78	decene	140
16.	8.89	phenol	94
17.	9.15	hexanoic acid	116
18.	9.71	cycloheptanone	112
19.	11.51	2-decanone	156
20.	11.60	heptanoic acid	130
21.	11.89	nonanal	142
22.	12.52	cyclohexanone	126
23.	13.86	dodecene	168
24.	14.07	octanoic acid	144
25.	14.27	benzoic acid	122
26.	15.27	3,4,5,6-tetrahydropentalen-1(*2H*)-one[b]	122
27.	16.19	nonanoic acid	158
28.	16.27	tridecene	182
29.	16.43	tridecane	184
30.	18.33	decanoic acid	172
31.	18.55	tetradecene	196
32.	18.69	tetradecane	198
33.	19.07	3,4-dihydro-*1H*-isochromen-1-one[b]	148
34.	20.35	undecanoic acid	186
35.	20.70	pentadecene	210
36.	20.83	pentadecane	212
37.	22.40	dodecanoic acid	200
38.	22.75	hexadecene	226
39.	22.86	hexadecane	228

40.	23.07	diethylphtalate	222
41.	24.68	heptadecene	240
42.	24.79	heptadecane	242
43.	26.19	tetradecanoic acid	228
44.	27.02	N-butylbenzenesulfonamide	213
45.	27.13	hexadecanal	240
46.	27.87	pentadecanoic acid	242
47.	28.56	2-heptadecanone	254
48.	28.92	hexadecanoic acid, methyl ester	270
49.	29.65	dibutylphtalate	278
50.	29.88	hexadecanoic acid	256
51.	30.57	octadecanal	268
52.	31.84	2-nonadecanone	282
53.	32.16	octadecanoic acid, methyl ester	298
54.	32.59	6-tridecyltetrahydro-*2H*-pyran-2-one[b]	282
55.	33.03	octadecanoic acid	284
56.	35.21	5-tetradecyldihydrofuran-2(*3H*)-one[b]	282
57.	35.74	benzyl butyl phtalate	312
58.	36.52	triphenyl phosphate	326
59.	36.65	2-ethylhexyl diphenyl phosphate	362
60.	37.98	di-2-ethylhexyl phtalate	390

[a] Identification based on 70 eV electron ionisation mass spectra.
[b] Name obtained with ACD/IUPAC Name Free v4.5 using the ACD/I-Lab service

Figure 23 depicts the chromatogram of the Py-GC/MS measurement. The two main components that were chromatographable are palmitic- and stearic acid (Compounds 50 and 55; see Table 3 for the identification). A series cyclic ketones (C5-C8), alkanes/alkanes (C9-C17), fatty acids (C4-C15), and alkanals (C6-C18) is observed as well. The cyclic ketones are thought to be derived from diacids, comparable to the formation of lactones by heating of hydroxy fatty acids [133]. Shorter fatty acids, alkanes and alkenes are typical radical cleavage products of (oxidised) (un)saturated fatty acids. Alkanals are most likely produced by pyrolysis of oxidised fatty acid [133, 354]. These compounds give a whole range of low aliphatic fragment ions (<m/z150) upon 70 eV electron ionisation. Pyrolysis-GC/MS analysis of the same paint sample with the addition of tetramethyl ammonium hydroxide (TMAH) to achieve on-line (trans)methylation of the acid and hydroxyl groups shows a intact range of fatty acids and diacids while thermal breakdown products are almost absent [345]. This clearly confirms that the compounds detected in Figure 23 are formed by pyrolysis especially when the fatty acids are present as metal carboxylates. The typical fragment ions of these

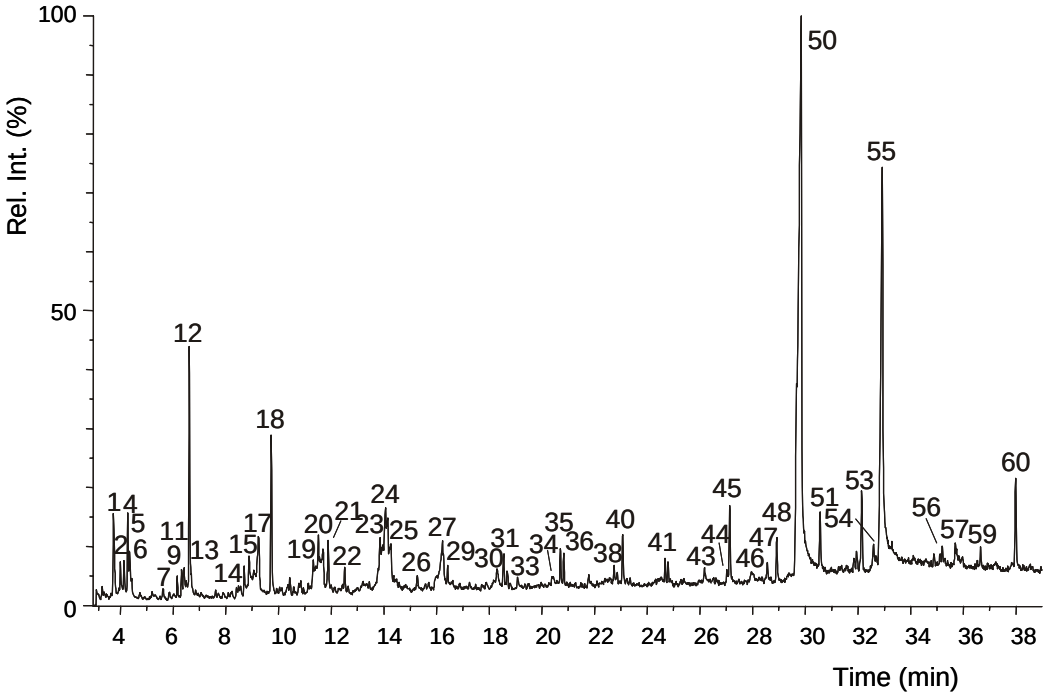


Figure 23. Gas chromatogram TIC of 25-year-old dried lead white pigmented linseed oil (CCI) obtained with Curie-point pyrolysis (610 ºC) GC/MS.

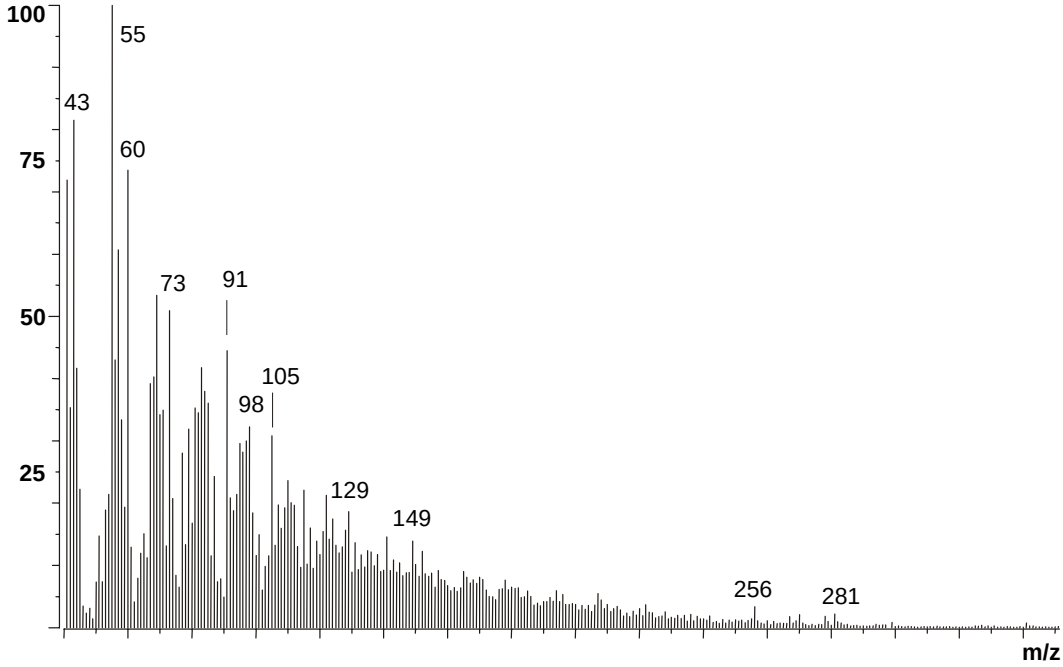


Figure 24. Curie-point pyrolysis (610 ºC) GC/MS summation mass (t_R 2-40 min.) of 25-year-old dried lead white pigmented linseed oil (CCI).

compounds are also observed in the DTMS spectra. Other types of compounds detected are alkylated benzenes pyrolysis products and other substituted benzene products (Compounds 1, 7, 8, 14, 25, 33) which give fragment ions at either m/z 77, 91, or 105. These ions are observed in the high temperature region of the DTMS runs of aged linseed oils. Furthermore, a number of compounds are identified (Compounds 40, 44, 49, 57-60) which are thought to be impurities originally absent in fresh oil (paint). These compounds can originate from different sources since they are used as plasticizers in a variety of modern materials and often part of the laboratory atmosphere. In a DTMS spectrum the phtalate esters adsorbed on the sample can be identified by m/z 149, which is a phtalic anhydride formed upon EI induced fragmention [356]. A comparison between the 16 eV DTMS data and that of 70 eV Py-GC/MS can be obtained by summation of the mass spectra of all scans recorded between 2 and 29 minutes. The high intensity peaks of both C16 and C18 fatty acids have not been incorporated because of their dominant and disturbing presence in the summation spectrum. As can be seen in Figure 24 a mass spectrum is obtained for the Py-GC/MS measurement which looks similar compared to previously shown DTMS spectra, although the relative amount of low molecular weight fragment ions seems higher. The fact that 70 eV EI ionisation has been used, is an important factor that will lead to more abundant fragment ions of low mass. It should also be noted that a large part of (fragment) ions of higher molecular weight of the unresolved envelope is missing. This suggests that a fraction of the already small amount of pyrolysed material is be filtered out due to retention in the chromatographic inlet system. Close inspection of the overall spectrum and the identified compounds confirms the presence of the typical (fragment) ions, and the compounds that have been ascribed to them, as previously discussed for the DTMS data of oil paint.

4.4 Discussion

From the examples presented in this chapter it is clear that DTMS can be used as an analytical fingerprinting technique for the presence of oil based paint and related materials in a paint sample. Although the information that can be obtained does not give a 100% confirmation of the exact structural properties of the material present which is the case for (pyrolysis)-GC/MS, sufficient information can be obtained on the presence of certain structural elements on the basis of characteristic m/z values alone. One should be careful however to not misinterpret the m/z's observed since it cannot be excluded that non-oil paint materials will give identical masses. Furthermore, it has been shown that different types of oil paint constituents will give identical fragment ions. Examples of this have been shown for m/z 98, which was observed in the mass spectrum of ricinoleic acid, azelaic acid, metal carboxylates, triacylglycerols, heated linseed oil, and aged linseed oil. Two other examples are m/z 256 and 284, and their acylium ions, m/z 239 and 267, that showup in metal carboxylates, TAGs, and aged oil paints. However, due to the thermal separation step some additional information on the sample investigated can be gained. The combination of both information

sources makes it possible to monitor with DTMS the transition of low molecular weight TAGs to cross-linked paint, the formation of degradation products due to hydrolysis and oxidation, and the formation of metal carboxylates upon curing and ageing.

A high number of non-characteristic low molecular weight breakdown products are formed due to pyrolytic degradation of the larger oil networks. This contrasts with the specific fragments observed for neat polymeric material, which consists of only one or a few repeating building blocks [355]. The complexity of the oil paint system and its life cycle, as depicted schematically in Figure 1, makes it difficult to obtain structural information on the cross-linked networks. However, fragment ions originating from alkylated benzenes (m/z 91 and 105), that were shown to be derived from the oil network are an indication of the cross-linked nature of the oil-based paint. Fatty acids, either present as ester or metal carboxylate, can be released from the network (Figure 1c) accompanied with the uptake of hydrogen from the residual networks, resulting in an increase of the aromatic nature of the residue.

From the results presented it is clear that a big advantage of the DTMS technique is that both the organic as well as certain inorganic materials can be measured at the same time and in some cases even their composites. It should however be kept in mind that the profile obtained on evaporation and/or pyrolysis of the paint sample depends on a number of factors. This can lead to slight shifts in the appearance time windows of the different compound classes. Therefore one should always examine a number of specific masses commonly observed in oil paints and relate that information to the different appearance time windows.

4.5 Conclusions

Direct temperature resolved mass spectrometry is a quick and informative microanalytical tool for the analysis of paint samples based on drying oils. The presence of volatile low molecular weight material, the oxidation products thereof and cross-linked fractions can be identified within oil paint based on the appearance of specific masses within certain temperature windows. It has been shown that free fatty acids, cross-linked material, metal carboxylates and triacylglycerols could be differentiated on the basis of their appearance time and mass spectrum. However, due to the formation of a large number of oxidised breakdown- and/or cross-linking products in aged oil paint systems upon autoxidation, detection of specific structures that are formed is hampered. On the other hand, it is possible to semi-quantify the ratio of low- and high molecular weight material based on the TIC. Information on typical processes taking place within the paint system such as hydrolysis and cross-linking can be obtained in this way, although it should be kept in mind that inorganic material may contribute to the overall signal observed.

Chapter 5

5a. Identification of non-cross-linked compounds in methanolic extracts of cured and aged linseed oil based paint films using GC/MS

Methanolic extracts of paint samples of different composition and age were qualitatively investigated by GC/MS using an on-column injector after off-line methylation or trimethylsilyl derivatisation, and on-line thermally assisted (trans)methylation with tetramethylammonium hydroxide (TMAH) using Curie-point pyrolysis GC/MS. The combination of these three analytical strategies led to the identification of typical oxidation products of unsaturated fatty acids by interpretation of their mass spectrum. Some of the identified compounds have not been reported before. Both the off-line and on-line GC/MS strategy show series of short chain fatty (di)acids and C16 and (oxidised) C18 fatty acids. The big advantage of the on-line pyrolysis-GC/MS approach is that chemical work-up is minimal and very quick. With this technique both the carboxylic acid functionalities and hydroxyl groups are methylated. Young paint films are shown to contain relatively more oxidised C18 fatty acids and less diacids compared to older paints, which is indicative for the on-going oxidation processes within the paint. After trimethylsilylation, monoacylglycerols are detected indicative for hydrolytic processes, which reflect the relative distribution of the most prominent silylated fatty acids present. Relatively more C16 and C18 monoacylglycerols are found in young paints, whereas older paints contain higher amounts of monoacylglycerols of diacids.

5.1 Introduction

It is known since the 11th and 12th century descriptions by Theophilus that certain vegetable oils (linseed, poppy and walnut) are capable of forming an elastic and insoluble paint film [3]. These oils, which are still used today for painted works of art or in a modified form in 20th century household alkyd paints, are

called drying oils because of their ability to dry chemically, i.e. to cross-link to a semi-solid.

The chemical drying of oil is a result of auto- and/or photoxidation [52, 108] of the doubly- and triply unsaturated fatty acids moieties (linoleic- and linolenic acid, respectively) that are present in high amounts in these oils (> 65%) [292]. The subsequent free radical chain reactions give rise to a variety of cross-linked materials [124, 125, 295] and degradation products that partially are lost by evaporation [135, 138, 141].

Furthermore, partial hydrolysis of ester bonds of the triacylglycerols (TAGs) occurs, leading to free fatty acids. Part of these free acids will react with pigments or driers present in the paint and metal salts will be formed [217]. This leads to a paint system consisting of a solvent removable fraction (the so-called "mobile" phase) and a cross-linked polymeric system (the "stationary" phase) [28, 29]. The mobile phase includes oxidised- and de-esterified fatty acids, glycerol, mono-, di- and triacylglycerols and oligomeric structures. Since every oil paint has its own characteristic formulation, history of storage, restoration and cleaning and age, the ratio and chemical composition of both phases will be different.

The process of film formation has been subject of elaborate analytical chemical investigations. Information on the fundamental mechanisms is available on cross-linking, the different types of cross-links, the types of functional groups present in the polymeric system and the size of the cross-linked material. These studies include (high performance) size exclusion chromatography [115, 123], different types of mass spectrometry [124], (Fourier transform) infrared spectroscopy [220, 252, 256], and swollen- or solid state ^{13}C –NMR [221, 250]. However, these methods only provide general information on the structure and size of the high molecular weight material and almost no detailed structural information due to complexity of the material and insufficient resolution of the analytical techniques applied.

The analysis of the low molecular weight fraction of oil paint samples gives more detailed structural information. One of the common analytical techniques for the analysis of this fraction is gas chromatography, often combined with off-line chemical derivatisation [10, 13, 14, 20, 22, 24].

Surprisingly, the authors of these studies on paint samples only used reagents capable of methylating carboxylic acid groups. Other polar functional groups, like hydroxy-, keto or epoxy groups that are expected to be formed upon oxidation seemed overlooked or at least have not been reported. Furthermore, triacylglycerols nor their hydrolysis/oxidation products seem to be present, whereas in tempera based paints these compounds were identified [28]. Only recently, two keto substituted oxidised fatty acids were reported to be present in aged oil paint [27] and simultaneous analysis of fatty acids, mono-, di- and triacylglycerols present in extracts with high temperature GC/MS [25] was reported. No oxidised acylglycerols were detected despite the age of the paint sample (18^{th} century). The authors however did report the presence of fully saturated triacylglycerols, which is remarkable and may be explained by interesterification or contamination since these compounds are not present in fresh

linseed oil [36, 37]. In another recent study an extended range of (oxidised) mono- and diacylglycerols were identified in solvent extracts of relatively young oil paints by LC/MS [26].

In this chapter, the results are described of three different analytical strategies to qualitatively characterise the extractable fraction of an oil paint sample. As extraction solvent methanol was chosen because of its high polarity and the ability to extract oxidised fatty acids, acylglycerols and polar oligomeric material. Methylation of all free carboxylic acid groups using trimethylsilyl-diazomethane is one of the two off-line derivatisations applied. Other functional groups are not derivatised under the conditions used. Methylation of hydrolysed oil paint or oil paint extractables has been used most often in conservation science [10, 14, 20]. The results are compared to off-line trimethylsilylation (TMS) of the extract using bis(trimethylsilyl)trifluoroacetamide. This derivatisation reagent transforms free carboxylic acid groups and hydroxyl groups of both acylglycerols and oxidised fatty acids into their TMS derivatives [357]. This method is occasionally used for the analysis of paint samples [246].

On-line pyrolysis and the subsequent introduction of the pyrolysate into a chromatographic system has been shown to be suitable for the analysis of the organic part of alkyd and oil paint samples [244, 245, 247, 358] without extensive chemical work-up. Only the addition of low amounts of aqueous or methanolic solutions of quarternary ammonium salts like tetramethylammonium hydroxide (TMAH) is needed for the on-line derivatisation [245, 359-361]. This reagent transmethylates all ester bonds and methylates all free acids and partially also hydroxyl groups, depending on the pK_a [362]. Although both low and high molecular weight materials can be investigated using this method, in this case only the extractable fraction was analysed in order to compare the results with the two previously mentioned methods.

In this study the results of the analyses of extracts of a relatively young 5-year old stand oil film without pigmentation, a 26-year old lead white pigmented oil paint and 373-year old paint material of an unknown composition are reported. These three samples were selected from a much larger survey study. The main objective of this study is to obtain a better qualitative chemical picture of the composition of these paint films by identification of most of the compounds present in the extractable fraction and to relate the results to the age of the paint material. It may be clear that a more systematic study is needed to identify quantitative differences and changes of ageing oil paint systems in more detail [13, 22, 363].

5.2 Experimental

5.2.1 Chemicals

Tetramethylammonium hydroxide pentahydrate (minimum 97%), and (trimethylsilyl)diazomethane (TMS-diazomethane; in hexane, 2 M) were obtained from Aldrich (Zwijndrecht, The Netherlands). Deuterated tetramethylammonium hydroxide pentahydrate (98%) was supplied by Cambridge Isotope Laboratories (Andover, MA, USA). Bis(trimethylsilyl)trifluoroacetamide (BSTFA; ≥ 99%) was purchased from Fluka Chemie (Zwijndrecht, The Netherlands). Cremnitz white (basic lead carbonate or lead white) was supplied by Old-Holland Classic Oilcolours (Driebergen, The Netherlands).

5.2.2 Paint Films/Samples

A stand oil (linseed oil that has been prepolymerised by heating to 280 °C) (Talens, Apeldoorn, The Netherlands) film of about 0.5 mm thickness was made by spreading out oil on a glass slide. This test paint was stored under room conditions on the window-sill at our institute for 5 years. This typical sample was chosen since oils used to be prepolymerised quite often according to historical sources [43].

The lead white pigmented film was made in 1973 by H.-C. von Imhoff by grinding basic lead carbonate (Schmincke) with linseed oil (Mühlfellner-Rupf, Zürich) in such a ratio that a workable paint was obtained. The paint was applied on primed linden wood and was hung up at the Canadian Conservation Institute (CCI), Ottawa, under room conditions. Samples were taken be careful scraping off the paint layer and homogenised prior to use.

The old paint was sampled from a fragment of a canvas painting from 1626 made by an unknown artist, and supplied by the Rijksmuseum (Amsterdam, The Netherlands). The (restoration) history of this painting is unknown. The paint system consists of 6 layers with a total thickness of 0.3 mm, including a red ochre ground and a varnish layer. The third and fourth layers contain azurite with smaller amounts of black and white particles. The second layer contains lead white. The paint mixture was obtained by carefully scraping off the mixture of thin paint layers. The powder was homogenised prior to analysis by extensive grinding.

5.2.3 Methylation derivatisation procedure (off-line)

An extract of the paint sample was obtained by repetitive immersion of the paint film in methanol. After evaporation of the combined extracts to dryness, for every mg of sample 40 µl benzene and 10 µl methanol is added. The dissolved sample is methylated by addition of 5 µl of a 2.0 M TMS-diazomethane solution in hexane. After 5 minutes the reaction mixture was dried under a gentle stream of nitrogen and immediately dissolved in DCM, either with or without hexadecane as internal standard (50 ng/µl). It is important not to dry too long or under extreme conditions in order to avoid loss of relatively volatile components as much as possible.

5.2.4 Trimethylsilylation derivatisation procedure (off-line)

The extractable material was collected as previously described. The solvent was dried under a gentle stream of nitrogen and dissolved in 250µl hexane (for every mg of extractable material). Subsequently, 13 µl/mg of BSTFA was added and the mixture was kept at 75 °C for 60 min. Every 15 minutes the reaction vial was thoroughly shaken. Immediately after evaporation to dryness the residue was dissolved in DCM, either with or without hexadecane as internal standard (50 ng/µl).

5.2.5 GC/MS with on-column injection

1 µl of derivatised sample was directly injected into a SGE BPX5 column (25 m, 0.32 mm i.d., 0.25 µm film thickness) using an AS 800 on-column autoinjector (Fisons Instruments). The GC/MS system and conditions are the same as described in 2.3 unless otherwise stated. In a number of cases more than one sample of the same paint was prepared and analysed in order to test reproducibility. For the paints investigated similar chromatograms were obtained with minor differences in the relative intensities.

5.2.6 Curie-point Py-TMAH-GC/MS

About 15 µl of the extracted material was applied onto a rotating 610 °C Curie-point wire and 3-4 µl of a 2.5% aqueous solution of TMAH was added before the sample was dried *in vacuo*. The ferromagnetic wire was inserted in a glass liner, flushed with argon to remove air and subsequently placed into the pyrolysis unit. Curie-point pyrolysis was performed with a FOM 5-LX pyrolysis unit [345]. The ferromagnetic wire was inductively heated for 6 s in a 1 MHz Rf field to its Curie-

point temperature (610 °C). Pyrolysis fragments were flushed (splitless) into a SGE BPX5 column (25 m, 0.32 mm i.d., 0.25 µm film thickness) mounted in a Carlo-Erba gas chromatograph (series 8565 HRGC MEGA 2) which was coupled directly to the ion source of a JEOL DX-303 double focussing (E/B) mass spectrometer via a home built interface which was kept at 280 °C. Helium was used as carrier gas at a flow rate of approximately 2 ml/min as regulated with a CP-CF 818 pressure/flow control box (Fisons Instruments). The initial temperature of the gas chromatograph was 50 °C, which was maintained for 2 minutes. The oven temperature was programmed with a ramp of 6 °C to an end temperature of 320 °C (50(2)-6-320). Ions were generated by electron impact ionisation (70 eV) or chemical ionisation using isobutane at a pressure of 10^{-3} Pa in the ionisation chamber (180 °C), accelerated to 3 keV, mass separated and postaccelerated to 10 keV before detection. The mass spectrometer was scanned from m/z 40-700 with a cycle time of 1 s. A Jeol MP-7000 data system was used for data acquisition and processing. The compounds were identified based on their 70 eV electron impact mass spectrum [348, 364, 365].

5.3 Results and discussion

5.3.1 Off-line methylation combined with on-column injection and GC/MS

The GC/MS total ion current trace (Figure 1) of the methylated methanol extract of the 5-year old stand oil film shows short chain fatty acids (C7-C10), diacids (C7-C11), saturated long chain fatty acids (C16-C18, C20-22), a cyclic C18 fatty acid and some unsaturated and/or oxidised C18 fatty acids (see Table 1 for full list). These compounds could be identified by interpretation of their 70 eV mass spectrum or comparison with published spectra in literature or mass spectral databases. In case of low intensity peaks or overlapping peaks the mass spectra were obtained using selected ion monitoring, (background)subtraction, and comparison with spectra of neighbouring homologues, when part of a series. The identified compounds all arise from the initial triacylglycerols either via hydrolysis or oxidative degradation. Esterified carboxylic acid groups from acylglycerols or cross-linked low molecular weight materials are not (trans)methylated with the derivatisation technique and therefore will not pass the chromatographic system used. The ratio of palmitic- to stearic acid (P/S) of 1.3, based on peak areas, is well within the range for linseed oil based paints [10]. The doubly and triply unsaturated fatty acids, originally present in high amounts, are not detectable anymore, whereas the relative amount of monounsaturated C18 fatty acids is still reasonably high. Upon autoxidation, the monounsaturated fatty acids are less

Table 1. Identified methylated compounds in the methanolic extracts of oil paint samples after off-line methylation in combination with on-column GC/MS or on-line (trans)methylation using Curie-point pyrolysis GC/MS (on basis of 70eV EI spectra).

No.	Molecular weight	Compound
1	108	methoxybenzene
2	116	pentanoic acid, methyl ester
3	120	1,3-dimethoxy-2-propanol
4	134	1,2,3-trimethoxy-propane
5	120	2,3-dimethoxy-propanol
6	130	hexanoic acid, methyl ester
7	132	propanedioic acid, dimethyl ester
8	94	hydroxybenzene
9	142	heptenoic acid, methyl ester
10	144	heptanoic acid, methyl ester
11	158	2-ethyl hexanoic acid, methyl ester
12	144	butenedioic acid, dimethyl ester
13	146	butanedioic acid, dimethyl ester
14	166	silane, trimethylphenoxy
15	160	-methyl butanedioic acid, dimethyl ester
16	136	benzoic acid, methyl ester
17	156	octenoic acid, methyl ester
18	158	octanoic acid, methyl ester
19	160	pentanedioic acid, dimethyl ester
20	172	nonanoic acid, methyl ester
21	174	hexanedioic acid, dimethyl ester
22	186	decanoic acid, methyl ester
23	188	heptanedioic acid, dimethyl ester
24	202	octanedioic acid, dimethyl ester
25	194	1,2-benzenedicarboxylic acid, dimethyl ester
26	216	-methyl octanedioic acid, dimethyl ester
27	230	,. -dimethyl octanedioic acid, dimethyl ester
28	214	dodecanoic acid, methyl ester
29	216	nonanedioic acid, dimethyl ester
30	232	-methoxy octanedioic acid, dimethyl ester
31	230	-methyl nonanedioic acid, dimethyl ester
32	226	hexadecane (internal standard)
33	244	,. -dimethyl nonanedioic acid, dimethyl ester
34	228	tridecanoic acid , methyl ester
35	230	decanedioic acid, dimethyl ester
36	246	-methoxy nonanedioic acid, dimethyl ester
37	244	-methyl decanedioic acid, dimethyl ester
38	242	tetradecanoic acid, methyl ester
39	244	undecanedioic acid, dimethyl ester

40	260	-methoxy decanedioic acid, dimethyl ester
41	256	pentadecanoic acid, methyl ester
42	258	dodecanedioic acid, dimethyl ester
43	268	hexadecenoic acid, methyl ester
44	270	hexadecanoic acid, methyl ester
45	272	tridecanedioic acid, dimethyl ester
46	256	hexadecanoic acid
47	284	heptadecanoic acid, methyl ester
48	296	octadecenoic acid, methyl ester (cis/trans)
49	298	octadecanoic acid, methyl ester
50	290	octadecatetraenoic acid, methyl ester (9(-o-propylphenyl)-nonanoic acid, methyl ester)
51	296	octadecadienoic acid, methyl ester
52	284	octadecanoic acid
53	326	8-methoxy-9-octadecenoic acid, methyl ester
54	326	11-methoxy-9-octadecenoic acid, methyl ester
55	326	9-methoxy-10-octadecenoic acid, methyl ester 10-methoxy-8-octadecenoic acid, methyl ester
56	312	9,10-epoxy-octadecanoic acid, methyl ester
57	312	4-oxo-octadecanoic acid, methyl ester
58	312	9-hydroxy-10-octadecenoic acid, methyl ester 10-hydroxy-8-octadecenoic acid, methyl ester
59	312	9-oxo-octadecanoic acid, methyl ester 10-oxo-octadecanoic acid, methyl ester
60	326	icosanoic acid, methyl ester
61	310	9-hydroxy-10-octadecenoic acid + :1, methyl ester 10-hydroxy-8-octadecenoic acid + :1, methyl ester
62	358	9,10-dimethoxy-octadecanoic acid, methyl ester
63	354	docosanoic acid, methyl ester
64	368	tricosanoic acid, methyl ester
65	382	tetracosanoic acid , methyl ester
66	396	pentacosanoic acid, methyl ester

reactive compared to the doubly- and triply unsaturated fatty acids [173]. Since this paint is only 5 years old, reasonably thick compared to traditional paint layers, and no driers or pigments are present that speed up the oxidation, they have not reacted away and are still detectable. The doubly and triply unsaturated C18 fatty acids however already have reacted away, partially by Diels-Alder cyclisations upon heating during the prepolymerisation, giving rise to cyclic C18 fatty acids and carbon-carbon linked oligomers of triacylglycerols [115, 153, 180], or are converted to oxidised products or incorporated into the cross-linked polymeric fraction. The short chain fatty acids found are cleavage products formed upon oxidation of the initial unsaturated fatty acids [135, 141]. Most of the acid groups of these fatty acids probably are formed upon oxidation of intermediate aldehydes,

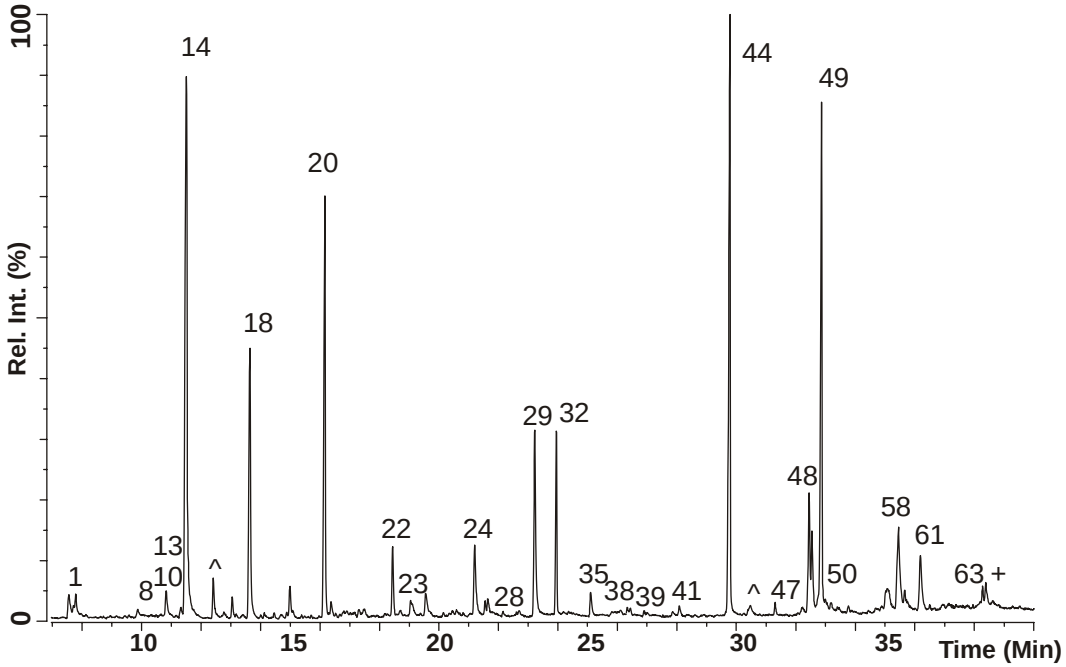


Figure 1. Total ion chromatogram of a methanolic extract of a 5-year old stand oil film after methylation and GC/MS analysis using on-column injection. Temperature program: 50(5)-6-320 (See Experimental section for further explanations). Peaks labelled ^ and + are identified as polysiloxanes and diisoctylphtalate, respectively.

although some of these acids may arise from hydrolysis of degradation products formed via a different pathway [127, 141]. Other degradation products that are formed upon oxidation of unsaturated fatty acids, like short chain aldehydes, ketones, alkanes and alcohols, are volatile and are lost from the paint film by evaporation, giving the drying paint its typical smell. Furthermore, (highly) volatile compounds that will remain within the paint will be lost upon drying during sample preparation. The diacids are typical compounds from oxidised unsaturated fatty acids. It is thought that the relative ratio of the C8 (suberic acid; compound 24) and C9 (azelaic acid; compound 29) diacids gives an indication whether the oil has been prepolymerised by heat treatment, although it has never been proven scientifically. Due to isomerisation of the original double bond systems relative higher amounts of diacids other than azelaic acid are formed. In this case the oil has been heated and a ratio of suberic acid to azelaic acid of 0.4 is found. The cyclic C18 fatty acids are formed by cyclisation of linolenic acid upon heating [166]. These types of compounds are present in trace amounts (*e.g.* compound 50, MW = 290), and no absolute structures could be derived on the basis of their mass spectrum other than an indication of the presence of an aromatic ring. This is also a proof for heat-treatment of the oil. Two oxidised mono- and two doubly unsaturated C18 fatty acids (compounds 58 and 61, respectively) are not well resolved, which hampers structural identification. However, it is clear that these compounds contain a hydroxyl group as can be deduced from their mass spectra (not shown: molecular ions at m/z 312 and 310, respectively, and the loss of water

18, leading to fragment ions m/z 294 and 292). Based on retention time, the doubly unsaturated compounds most probably contained another hydroxyl group that is eliminated upon EI ionisation, after separation on the GC column. Glycerol, mono-, and diacylglycerols, which can be present in the paint sample due to hydrolytic processes, are not detectable with this analytical procedure.

The chromatogram of the methylated extract of 17th century paint material (Fig. 2, Table 1) shows basically the same compounds but in different relative quantities. No attention has been paid to the varnish constituents of this paint since this falls outside the scope of the chapter. The oil is identified, based on a P/S ratio of 1.4, as a linseed oil. The ratio of the suberic- to azelaic acid of 0.2 points to an oil, which has not or hardly been heat-treated prior to usage. No cyclic fatty acid structures were detected. Short chain fatty acids are present in relatively low amounts, partially because they must have evaporated from the paint film, or have been removed by previous restoration treatments. Moreover relatively increased amounts of hydrolysed fatty acids and diacids are expected. Almost no monounsaturated and/or oxidised C18 fatty acids are detected since these must have reacted away to a large extent in time.

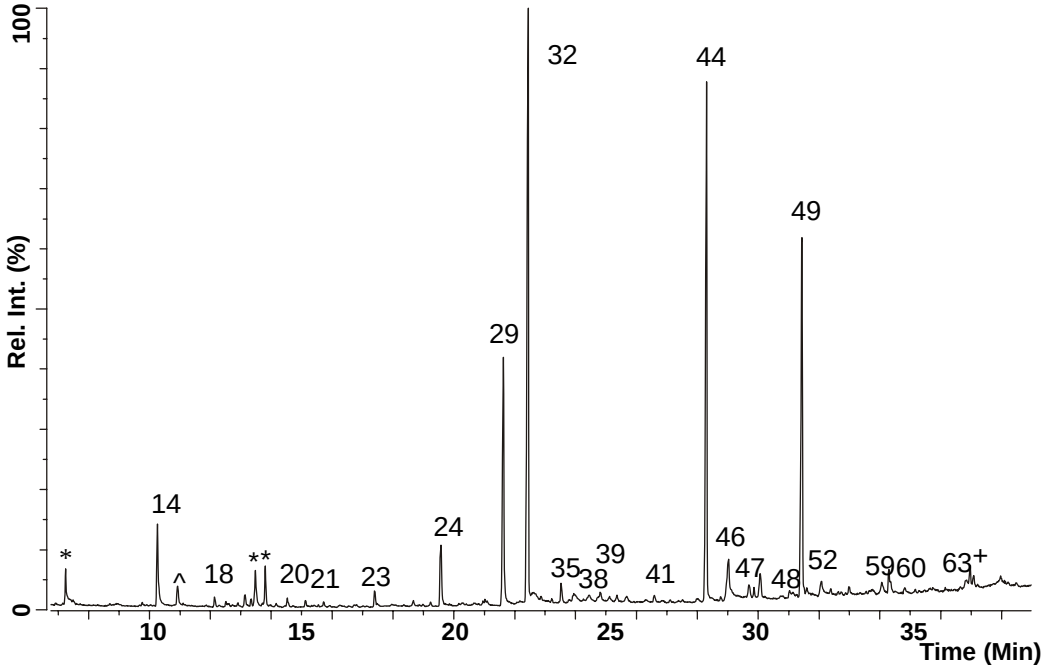


Figure 2. Total ion chromatogram of a methanolic extract of 17th century paint after methylation and GC/MS analysis using on-column injection. Temperature program: 50(2)-6-320. Peaks labelled * are presently unidentified, whereas peaks labelled ^ and + are ascribed to polysiloxanes and diisoctylphtalate, respectively.

5.3.2 Off-line trimethylsilylation combined with on-column injection and GC/MS

The two methanol extracts described in 5.3.1 were also analysed with GC/MS in combination with on-column injection after trimethylsilylation. Now all free carboxylic- and hydroxyl groups are trimethylsilylated, whereas ester bonds remain intact. The P/S ratios of 1.3 and 1.5, and C8/C9 diacid ratios of 0.5 and 0.3, for the 5-year old stand oil and the 17th century paint are in good agreement with the results obtained with the methylation procedure for this extract. The TIC of the analysis of the stand oil extract (Fig. 3, Table 2) shows several new peaks that were not detected in the experiment with methylation only. First, co-eluting with the C8 short chain fatty acid is trimethylsilylated glycerol (compound 5, Table 2). Secondly, new compounds are the monoacyl glycerols of suberic-, azelaic-, sebacic-, palmitic- and stearic acid (compounds 32, 34, 38, 40/42, and 44/46, respectively; see Table 2.). Both the 1- and 2-substituted monoacyl glycerols of the C16 and C18 fatty acids are found. Two of the three original ester bonds have been hydrolysed in this case. Since the oil is reasonably young and oxidation is still proceeding, smaller amounts of monoacylglycerols of diacids, relative to those of saturated fatty acids, are detected. Di- and traces of triacylglycerols are expected to be present as well but are not chromatographable with the GC system used. The oxidised unsaturated fatty acids (compounds 35-37), which also were observed in the previous experiments (compounds 58, Table 1), and their monoacylglycerols (compound 47) could now be positively identified on the basis of the mass spectrum. As in the methylation experiment, short chain fatty acids are present in high amounts and no monoacylglycerols of these compounds are detected. This clearly indicates that their acid group has been formed to a large extent upon oxidation and was not esterified to glycerol originally. Two series of new compounds were identified based on their mass spectra: silylated -, and -hydroxy diacids (compounds 18, 19, 22, 23, 26, 27). The -hydroxy diacids most problably are derived from dihydroxy fatty acids whereas the -hydroxy diacids, only present in trace amounts, most probably originate from epidioxides, both formed upon autoxidation [96, 97].

Table 2. Identified compounds in the methanolic extracts of oil paints after off-line trimethylsilylation and on-column GC/MS (on basis of 70eV EI spectra).

No.	Mol. Mass	Compound
1	202	heptanoic acid, TMS ester
2	236	glycerol, 1,3-bis[(TMS)oxy]
3	214	octenoic acid, TMS ester
4	216	octanoic acid, TMS ester
5	308	glycerol, 1,2,3-tris[(TMS)oxy]

6	262	butanedioic acid, di-TMS ester
7	230	nonanoic acid, TMS ester
8	276	pentanedioic acid, di-TMS ester
9	244	decanoic acid, TMS ester
10	290	hexanedioic acid, di-TMS ester
11	304	heptanedioic acid, di-TMS ester
12	226	hexadecane (internal standard)
13	390	1,2-benzenedicarboxylic acid, diethyl ester
14	282	4-[(TMS)oxy)]benzoic acid, TMS ester
15	318	octanedioic acid, di-TMS ester
16	332	nonanedioic acid, di-TMS ester
17	300	tetradecanoic acid, TMS ester
18	406	octanedioic acid, -OTMS ether, di-TMS ester
19	406	octanedioic acid, -OTMS ether, di-TMS ester
20	346	decanedioic acid, di-TMS ester
21	314	pentadecanoic acid, TMS ester
22	420	nonanedioic acid, -OTMS ether, di-TMS ester
23	420	nonanedioic acid, -OTMS ether, di-TMS ester
24	360	undecanedioic acid, di-TMS ester
25	328	hexadecanoic acid, TMS ester
26	434	decanedioic acid, -OTMS ether, di-TMS ester
27	434	decanedioic acid, -OTMS ether, di-TMS ester
28	374	dodecanedioic acid, di-TMS ester
29	342	heptadecanoic acid, TMS ester
30	388	tridecanedioic acid, di-TMS ester
31	354	octadecenoic acid, TMS ester
32	464	octanedioic mono-TMS ester, 2,3-bis(TMS)oxy propyl ester
33	356	octadecanoic acid, TMS ester
34	478	nonanedioic mono-TMS ester, 2,3-bis(TMS)oxy propyl ester
35	442	9-octadecenoic acid, 8-TMS ether, TMS ester
36	442	9-octadecenoic acid, 11-TMS ether, TMS ester
37	442	10-octadecenoic acid, 9-TMS ether, TMS ester 8-octadecenoic acid, 10-TMS ether, TMS ester
38	492	decanedioic mono-TMS ester, 2,3-bis(TMS)oxy propyl ester
39	384	icosanoic acid, TMS ester
40	474	hexadecanoic acid, 1,3-bis(TMS)oxy propyl ester
41	532	octadecanoic acid, 9,10-bis[(TMS)oxy], TMS ester
42	474	hexadecanoic acid, 2,3-bis(TMS)oxy propyl ester
43	398	docosanoic acid, silyl ester
44	502	octadecanoic acid, 1,3-bis(TMS)oxy propyl ester
45	500	octadecenoic acid, 2,3-bis(TMS)oxy propyl ester
46	502	octadecanoic acid, 2,3-bis(TMS)oxy propyl ester
47	588	10-octadecenoic acid, 9-TMS ether, 2,3-bis(TMS)oxy propyl ester 8-octadecenoic acid, 10-TMS ether, 2,3-bis(TMS)oxy propyl ester

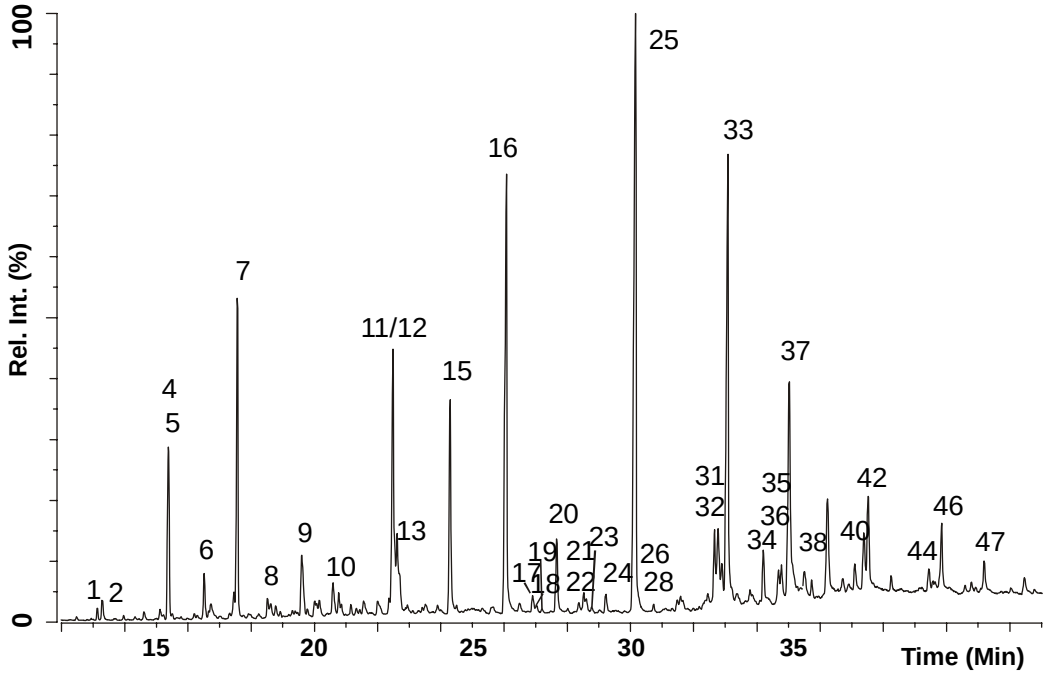


Figure 3. Total ion chromatogram of a methanolic extract of 5-year old stand oil film after trimethylsilylation and GC/MS analysis using on-column injection. Temperature program: 50(2)-6-320.

Most of the other compounds identified were also observed in the previous analyses of the methylated extracts. The main difference between the two analytical methods is the relative amount of diacids detected, which is higher in the case of the silylation experiment. Apart from hypothesising larger response factors of trimethylsilylated diacids relative to saturated fatty acids derivatives when compared with methylated compounds, there is no explicit explanation for the origin of this difference.

This last phenomenon was also observed for the 17th century paint (compare Fig. 2 and 4). Again relatively low amounts of short chain- and oxidised (unsaturated) C18 fatty acids are found. One oxidation product however, is present in moderate amounts: the completely silylated derivative of 9,10-dihydroxy octadecanoic acid (compound 41). This compound is often detected when analysing old oil paint layers. Although this paint is 373 years old, still intact ester bonds were detected in the form of monoacylglycerols. The relative amount of monoacylglycerols of the diacids is higher relative to those of the saturated C16 and C18 fatty acids, which were only present in low amounts (compounds 42 and 46). This can be explained by the ongoing oxidation of the unsaturated (and oxidised) fatty acids, which leads to higher amounts of diacids. In addition, an increased number of their monoacylglycerols will be detected due to ongoing hydrolysis.

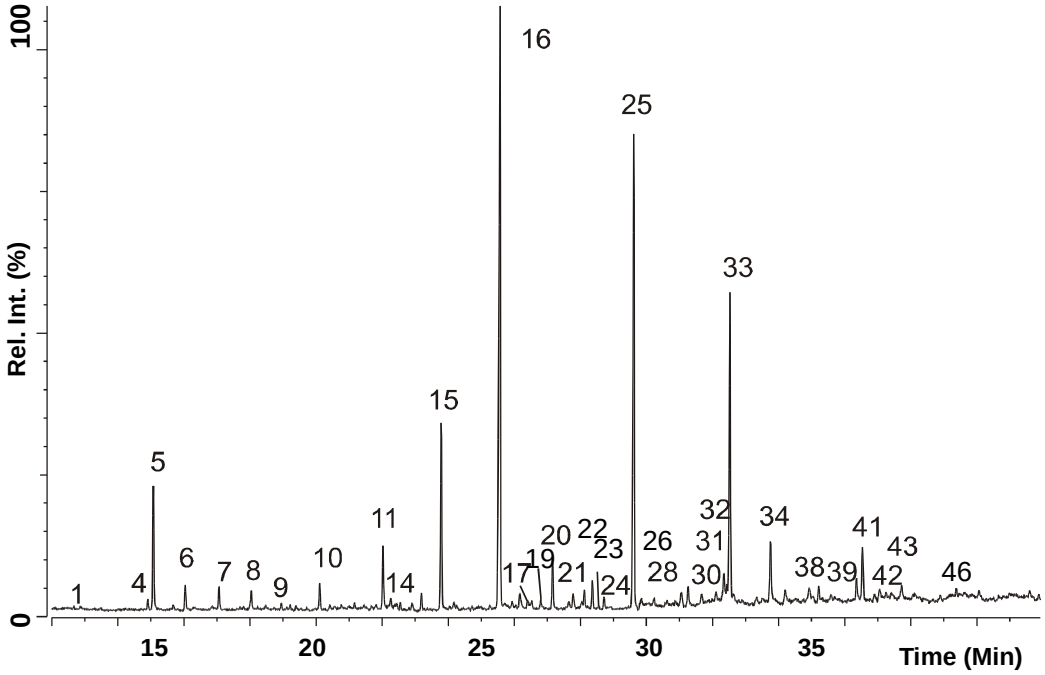


Figure 4. Total ion chromatogram of a methanolic extract of 17th century paint after trimethylsilylation and GC/MS analysis using on-column injection. Temperature program: 50(2)-6-320.

5.3.3 On-line Curie-point pyrolysis-GC/MS

In Figure 5 the TIC of a Cu-Py-GC/MS analysis of a 5-year old stand oil film is shown. The same compounds as in 3.1 and 3.2 are identified (see Table 1) but previously unidentified material as well. Compounds 3-5 all are methylated derivatives of glycerol, which was liberated during the transmethylation reaction of the acylglycerols. Since this film still contains high amounts of extractable tri-, di- and monoacylglycerols, next to (trans)methylated fatty acids, glycerol is detected in relatively high amounts. The relative abundance of the short C7-C10 fatty acids, formed upon oxidative degradation, is relatively lowered compared to the methylation/silylation experiments because of the additional transmethylation of the esterified compounds present in the extract.

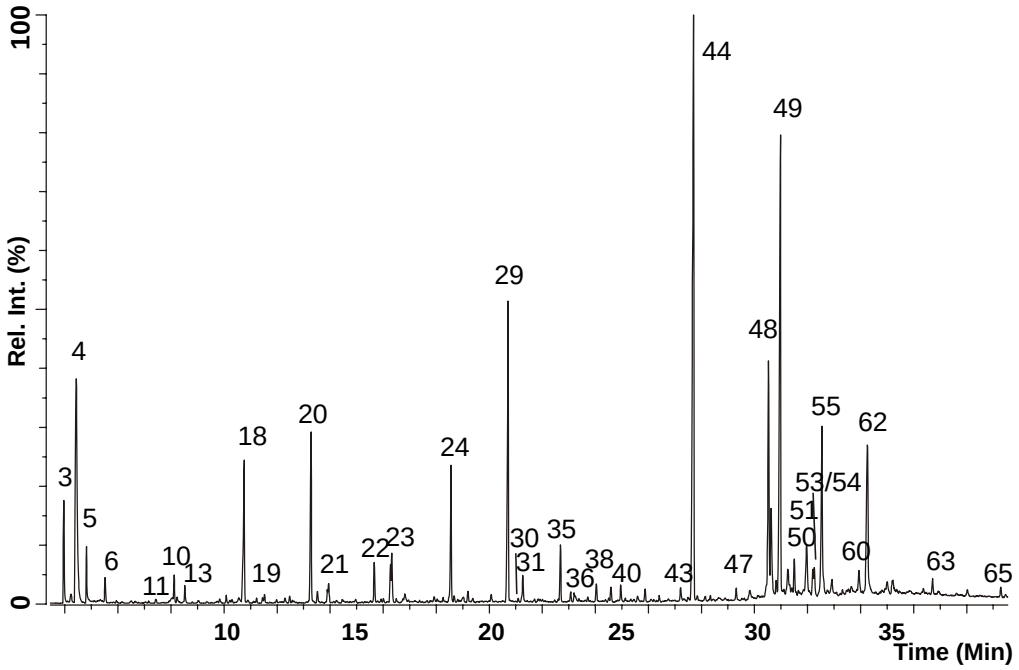


Figure 5. Total ion chromatogram of a methanolic extract of a 5-year old stand oil film after Curie-point pyrolysis assisted with on-line (trans)methylation using TMAH. Temperature program: 40(6)-6-320.

A series of unsaturated short chain fatty acids is detected as well. These are not seen in the methylation and silylation experiments and are supposed to be formed upon pyrolytic elimination from the extracted (cross-linked) oligomeric material in the extract [336, 352, 366]. Another series of new compounds identified are -methylated- and ,-dimethylated diacids. These are by-products formed by reaction of the TMAH derivatisation reagent with the diacids as was proven for pure azelaic acid reference material [345]. A second series of compounds that can be found in the chromatogram are the -methoxylated C8-C10 diacids (compounds 30, 36 and 40), which were also detected after trimethylsilylation of the extract. The -methoxylated diacids were not detected.

The (trans)methylation procedure results in new mass spectra that not have been reported before to our knowledge. In Figures 6a-c the EI spectrum, the EI spectrum using deuterated TMAH reagent and the CI spectrum using isobutane are depicted for 2-methoxynonanedioic acid dimethyl ester (36). Here α-cleavage next to the methoxy group gives rise to the most intense fragment ions (m/z 187) followed by two times the loss of methanol (m/z 155 and 123, respectively). The molecular mass of m/z 246, found by chemical ionisation using isobutane (Figure 6c), is in agreement with the proposed molecular structure.

Compounds 53, 54, 55a and b are identified as octadecenoic acid methyl esters substituted with a methylated hydroxy group. These types of compounds were also

visible in both the methylated and silylated extracts and the combination of these three measurements led to the positive identification. As an example, the mass spectrum of 8-methoxy-9-octadecenoic acid methyl ester (compound 53) is depicted in Figure 7. In the 70 eV EI mass spectrum (Fig 7a) the most intense ion m/z 183 is formed by α-cleavage next to the methoxy group. An -cleavage next to the double bond gives rise to fragment ions of m/z 213. The result of the experiment with deuterated TMAH supports the proposed structure. An increase of 6 amu was observed for the -cleavage fragment next to the methoxy group, whereas the other fragment ion only increased with 3 amu (Figure 7b). Chemical ionisation using isobutane showed the molecular weight of the compound to be m/z 326 (Figure 7c). Other oxygenated fatty acids identified include 9,10-epoxy octadecanoic acid methyl esters (56) and 9,10-dimethoxy octadecanoic acid methyl esters (62). Similar to the methylation and silylation experiment the cyclic C18 fatty acid was also detected with Py-TMAH-GC/MS. It could now be identified as 9(-o-propylphenyl)-nonanoic acid, methyl ester, because of the more specific fragment ions visible. Besides this particular fatty acid, trace amounts of other isomeric aromatic C18 fatty acids were detected as well. With the previous two methods only free fatty acids and some monoacylglycerols are monitored. On-line derivatisation of the methanol extract with tetramethylammonium hydroxide, assisted by 610 °C Curie-point pyrolysis, however, transmethylates esterified fatty acids as well. Therefore, a more quantitative picture can be obtained of the composition of the extract. Furthermore, hydroxyl groups, including those of glycerol, are (partially) methylated which leads to better chromatographic separation and MS identification. The only materials that cannot be analysed straightforward this way are the cross-linked oligomeric compounds formed upon oxidative or heat induced polymerisation.

The P/S and C8/C9 diacid ratios of the extract were 1.3 and 0.4, respectively. These values are identical to the results obtained with the off-line derivatisation procedures.

A methanolic extract of a 26-year old lead white pigmented linseed oil paint sample has been analysed by Py-TMAH-GC/MS as well in order to have a complementary result in between the very young and very old paint. However, it should be reminded that these three samples have a different composition and history. The same compounds are found as in the extract of the 5-year old film,

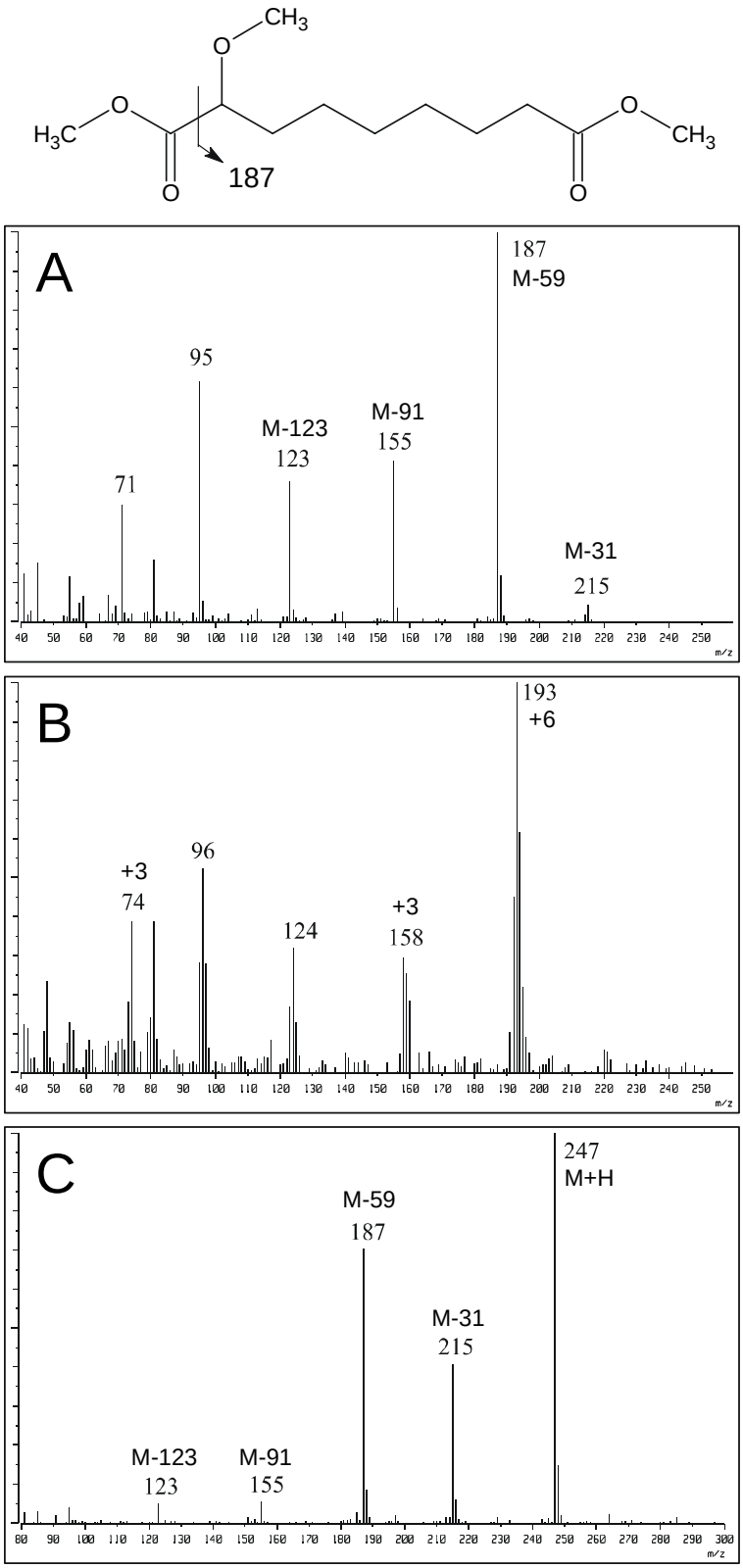


Figure 6. Mass spectrum of nonanedioic acid, -methoxy, dimethyl ester using (a) 70eV EI; (b) 70 eV EI and deuterated TMAH, and (c) chemical ionisation using isobutane.

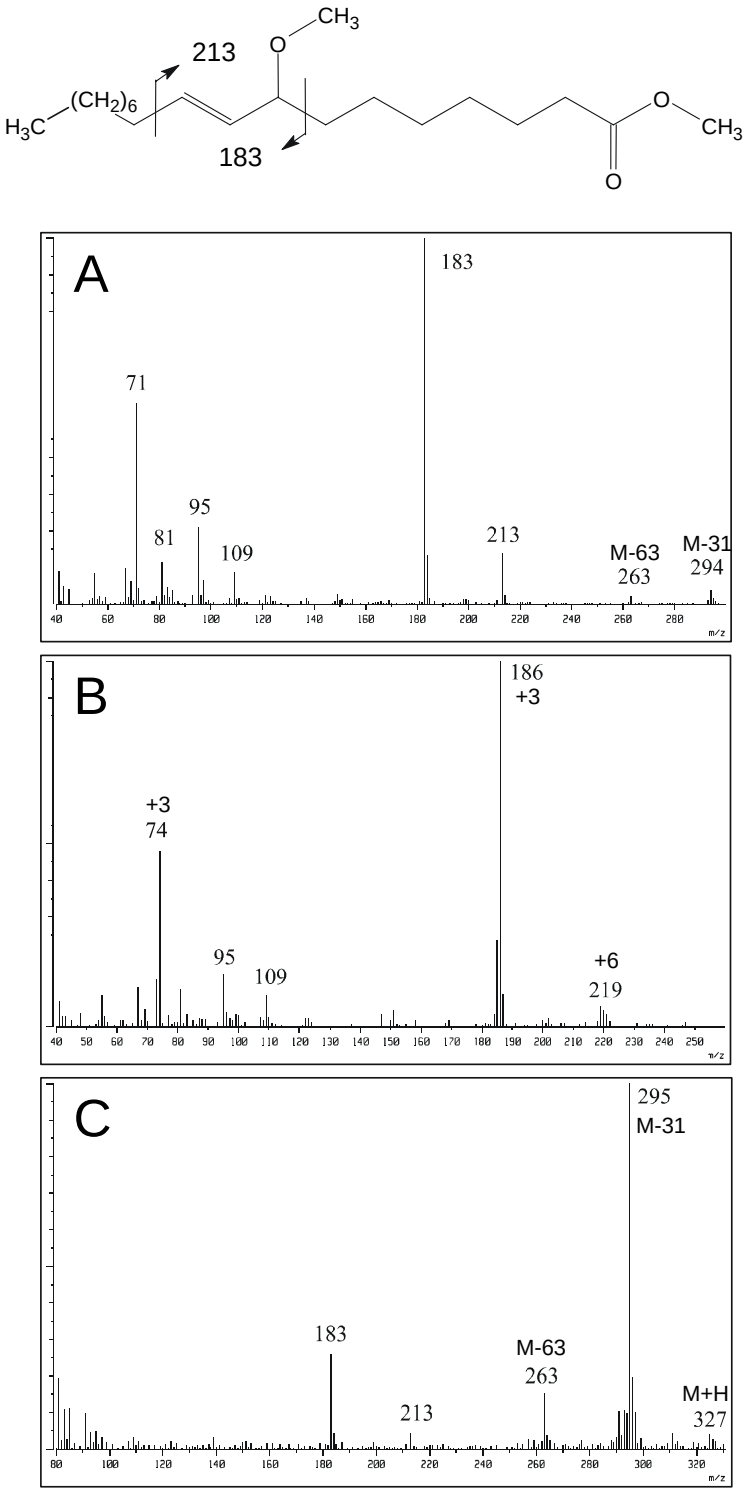


Figure 7. Mass spectrum of 8-methoxy-9-octadecenoic acid, methyl ester using (a) 70eV EI; (b) 70 eV EI and deuterated TMAH, and (c) chemical ionisation using isobutane.

except for the cyclic fatty acids (see Figure 8, Table 1). The P/S ratio of 1.4 is within the range of linseed oil. Cyclic C18 fatty acids were not detected and the ratio of C8 to C9 diacid of 0.3 is an analytical confirmation that the oil has not been heat-bodied [367]. As expected, only minor amounts of monounsaturated C18 fatty acids are detected in the extract. These species have reacted away upon oxidation and are partially incorporated in the oil network. The relative amount of oxidised C18 compounds is still high and comparable to the 5-year old paint. The relative amount of diacids, however, is somewhat lower. Formation of lead soaps with these diacids is expected, and more favourable relative to the fatty acids containing one acid group. This leads to lower amounts of extractable fatty diacids. The relative amounts of short chain fatty acids are lower compared to the previous analysis and low amounts of glycerol derivatives are detected. Analysis of the paint residue by Py-TMAH-GC/MS after extraction showed that relatively high amounts of glycerol still are present. It is therefore suggested that due to a high degree of polymerisation only low amounts of acylglycerols can be extracted. This is to be expected for a lead white pigmented oil film since lead is known for its positive effect on the polymerisation [39, 58]. Furthermore, the lead soap formation with carboxylic acid groups present in the oligomeric material is also expected to result in reduced extractability of glycerol containing material, as long as the number of hydrolysed ester bonds is low. Most of the glycerol will be bound to the networks in this latter case.

The analysis of the methanol extract of the 17th century paint is presented in Figure 9 (see also Table 1). The same types of compounds are found when compared to the other extracts investigated. Both the P/S and C8/C9 diacid ratios obtained are comparable to the numbers measured with the previous described off-line strategies. Again the main difference is the relative amount of the different ageing products detected. Glycerol cannot be detected anymore. Hydrolysis of the ester bonds, in combination with evaporation and the (unknown) restoration history has led to the loss of glycerol from the paint. Oxidised C18 fatty acids are present in relative low amounts, whereas the diacids are detected in relatively high quantities compared to the younger paint samples. This can be ascribed to the ongoing oxidative degradation of the paint material, in combination with evaporative losses.

A comparison of the results obtained with the three analytical strategies applied in this study shows that for the major components present, similar qualitative information is obtained. The palmitic to stearic acid and suberic to azelaic acid ratios of each extract were identical for all three methods. The off-line methylation procedure, despite the fact that it only takes 15 minutes to derivatise the sample, is of the three methods least favourable. Only free fatty acids are monitored and polar groups on the fatty acids are not derivatised, which has a negative influence on the chromatographic separation. Trimethylsilylation, however, is capable of derivatising hydroxy groups and chromatographically

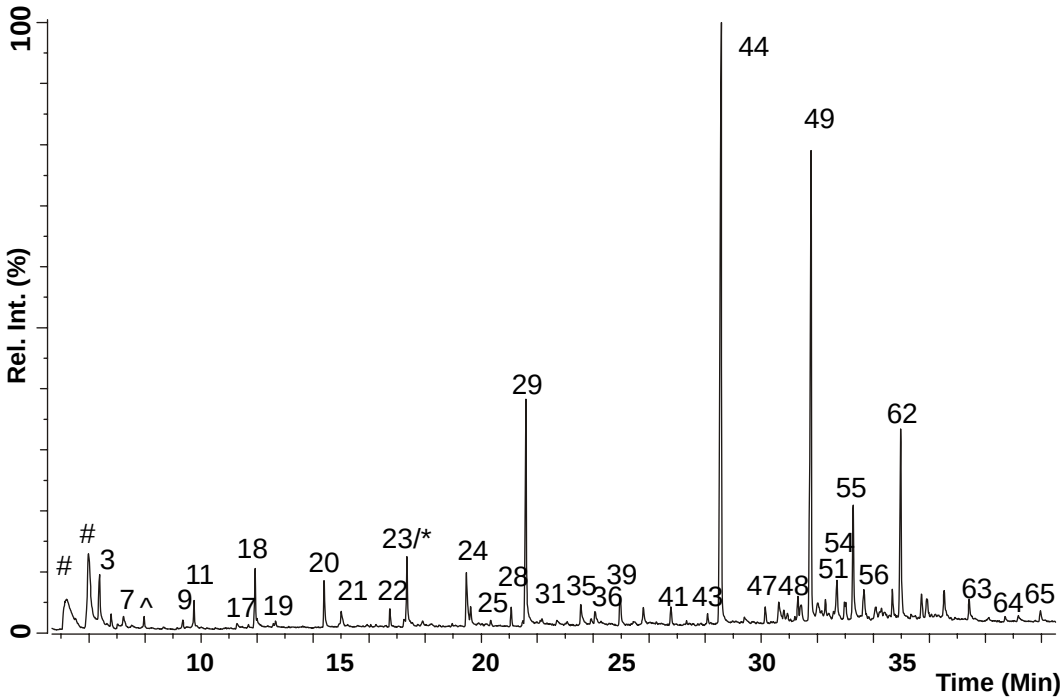


Figure 8. Total ion chromatogram of a methanolic extract of a 26-year old lead white pigmented linseed oil paint after Curie-point pyrolysis assisted with on-line (trans)methylation using TMAH. Temperature program: 50(2)-6-320. Peaks labelled # and ^ are identified as TMAH reagent derived by-products and polysiloxanes, respectively. The peak labelled 23/* contains an unidentified compound.

resolved oxidation products can now be identified on the basis of their mass spectrum. Furthermore monoacylglycerols are chromatographable and their relative abundance can give information to what extent hydrolytic processes have taken place. A disadvantage is the longer reaction time needed, the drying steps that may lead to loss of volatile low molecular weight when not carefully performed and the fact that di- and triacylglycerols and other esterified oligomeric materials are not included in the analysis. This would require high temperature columns or an LCMS technique [298, 310]. The pyrolysis-TMAH-GC/MS technique is of the three strategies the only one capable of analysing all the extractable non-cross-linked compounds. Both free- and esterified fatty acids are methylated and can be detected whereas for the methylation and trimethylsilylation experiments only free fatty acids (or monoacylglycerols in the case of a TMS-derivatisation) are monitored. In this sense the on-line (trans)methylation technique is comparable to the “classic” saponification with KOH, followed by extraction and methylation. A disadvantage, however, of the Py-TMAH approach is the formation of by-products, which are not seen with the other methods mentioned. On the other hand, there are three big advantages. First, the chemical work-up is much quicker and easier, and secondly, there is no loss of material due to extraction and drying steps. Last but not least, due to the methylation capabilities of TMAH also hydroxyl groups are derivatised, which makes separation and identification of oxidised fatty acids favourable.

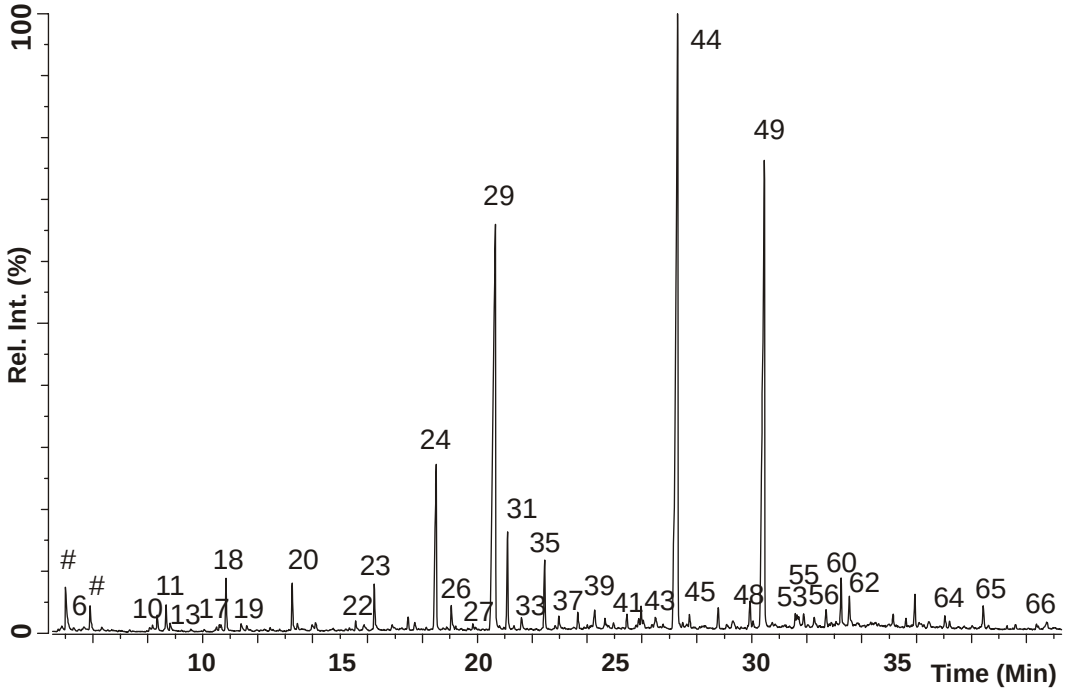


Figure 9. Total ion chromatogram of a methanolic extract of 17th century paint after Curie-point pyrolysis assisted with on-line (trans)methylation using TMAH. Temperature program: 50(2)-6-320. Peaks labelled # are identified as TMAH reagent derived by-products.

5.4 Conclusion

There are numerous factors that influence the chemical composition of an oil paint. Not only age plays a role but also other important factors like the kind of oil and the processing methods, the type and amount of pigment, the environmental conditions, and the (restoration) history of the paint. It is not known whether certain synergistic effects will play a role and how all these factors together will influence the development of the oil paint system.

However, there are many common features in the development of oil paints. In the extracts of paint films of different ages, always products can be found from de-esterification of the triacylglycerols and/or degradation of the oxidised fatty acids. In young paint samples oxidised C18 fatty acids, short chain fatty acids and glycerol are found in relatively high amounts. Some of the more polar compounds identified with both trimethylsilylation using on-column injection combined with GC/MS and Py-TMAH-GC/MS are reported for the first time. Most of these compounds are lost from old paint films due to oxidative degradation, evaporation from the surface of the paint and(/or perhaps) repetitive cleaning procedures. Diacids, the relatively stable end products of the oxidation of unsaturated fatty acids, are relatively more prominent in aged paint samples.

Both the off-line trimethylsilylation and methylation strategy are suitable for the analysis of the extractable non-cross-linked fraction with GC/MS in combination with on-column injection. Trimethylsilyl derivatisation not only gives information on free (oxidised) fatty (di)acids but also makes it possible to identify monoacylglycerols. The relative amount of these species gives semi-quantitative information on the degree of hydrolysis of the paint. Other esterified fatty acids, however, cannot be monitored. Py-TMAH-GC/MS on the other hand (trans)methylates all esterified and free fatty acids present in the extract and the totality of non-cross-linked material can be detected very easily in this way.

5b. Unwanted alkylation during direct methylation of fatty (di)acids using tetramethyl ammonium hydroxide reagent in a Curie-point pyrolysis unit

The formation of -methylated and ,. -dimethylated (di)acids during on-line (trans)methylation with tetramethylammonium hydroxide (TMAH) in a Curie-point pyrolysis unit is studied. The reaction of TMAH with nonanedioic -, hexadecanedioic- and octadecanoic acids is investigated to determine the contribution of the solvent system and the amount of TMAH added to this phenomenon. The results obtained clearly show a different behaviour of diacids in methanolic solutions compared to aqueous systems. At least three different reaction products were identified for the acids when using a methanolic solution of TMAH. Different relative amounts of by-products were formed when varying the amount of TMAH reagent relative to the normal methylated reaction product. A mechanism is postulated for their formation and a hypothesis is drawn up to explain the observed differences in reactivity for both solvents.

5.5 *Introduction*

The direct methylation of carboxylic acids and their esters using tetramethyl ammonium hydroxide (TMAH) in combination with heat is already known since 1963 by the work of Robb and Westbrook [359]. It is thought that this *in-situ* (trans)methylation takes place in two steps: the strong alkaline TMAH first deprotonates the more acidic functional groups (pK_a < 12) , eventually formed after saponification, to form a tetramethyl ammonium salt. This salt is subsequently thermally decomposed in the hot injection port of the gas chromatograph and a methyl ester is formed together with trimethylamine. Other compounds, besides carboxylic acids, that have been derivatised in this way include phenols, sulfonamides and heterocyclic nitrogen compounds. Other functional groups known to be affected are aliphatic –OH and $–NH_2$, although they are not necessarily completely deprotonated due to their weaker acidity (pK_a 16-25) [362]. Initially the sample-reagent combination was injected as an aqueous or methanolic solution in the hot injection port. Nowadays, the reagent is also used in combination with pyrolysis GC(/MS). The classes of compounds investigated with this technique include synthetic polymers, bio(macro)molecules, fats, oils, coals, kerogens, humic acids and microorganisms [336, 360, 368].

Several aspects involved in the methylation reaction have been investigated in more detail. The solvent methanol was found to play a modest role as it also contributes to the transmethylation of aromatic polyesters by methanolysis and not only via hydrolysis/pyrolytic methylation [369]. This was also observed by Venema et al. (1995) for polyaramide samples. However, it was concluded from their work on mono- and dibasic aliphatic- and aromatic acids that the direct methylation with methanol, catalysed by TMAH, did not play an important role in the case of these model compounds [370]. The effect of the temperature on the conversion efficiency depends on the type of sample investigated and the configuration of the analytical set-up. For saturated aliphatic acids and – diacids it was found that a pyrolysis temperature of 300 to 600 ºC did not influence the conversion [370], whereas for polyunsaturated fatty acids the optimum was determined to be 350 ºC [371]. Octadecyl octadecanoate, a C36 wax ester, was "pyrolysed" at 358 and 610 ºC and a clear difference was observed in the gas chromatograms [361]. In the latter case hydrolysis-methylation was the most dominant process leading to higher conversions, whereas evaporation took place at the lower temperature as well.

The work described in this section is part of a larger study on the organic chemistry of chemically dried linseed oil based paint systems. It was in particular inspired by the finding of -methylated and -methoxylated diacids that were previously unidentified in pyrolysis-TMAH gas chromatograms of linseed oil paint samples. Initially, these compounds were thought to originate from the polymeric networks upon pyrolysis/methylation with TMAH since they could not be found with traditional on-column GC/MS after methylation of fatty acids, diacids and their oxidation products [328]. However, tests done on reference material indicated that some of these compounds can be formed even during the on-line derivatisation. Recently, the same phenomenon was reported by Asperger et al. [372] who identified -methylated aliphatic C17 and C18 fatty acids after pyrolytic methylation of these reference materials. Unwanted methyl incorporation when applying TMAH for on-line methylation has been observed for other classes of compounds as well [373-375].

This chapter reports of the influence of several parameters on the -methylation of fatty acids and diacids. First, the type of solvent is monitored since this proved to be an important parameter before. Previous oil paint analyses were performed using an aqueous TMAH solution and almost no side reactions were observed. However, when using methanolic solutions because of the advantageous shorter drying times, increased amounts of unwanted products were observed. A second factor tested is the amount of TMAH reagent relative to the material investigated. A mechanistic explanation is postulated to explain the formation of -methylated fatty acids and diacids and the differences observed between the different analytical conditions.

5.6 Experimental

5.6.1 Material

Tetramethylammonium hydroxide pentahydrate (minimum 97%), stearic acid (> 99%) and nonanedioic acid (approximately 98%) and hexadecanedioic acid (96%) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Deuterated tetramethylammonium hydroxide pentahydrate (98%) was supplied by Cambridge Isotope Laboratories (Andover, MA, USA). Stand oil (prepolymerised linseed oil) was purchased from Talens (Apeldoorn, The Netherlands) and lead white was obtained from Old-Holland Classis Oil Colours, Driebergen, The Netherlands. A film of about 0.5 mm thickness was made by spreading out the pigment/oil mixture on a glass slide. This test paint was stored under room conditions for 5 years.

5.6.2 Curie-point Py-TMAH-GC/MS

Curie-point pyrolysis was performed with a modified FOM 4-LX pyrolysis unit [376]. In this new set-up (FOM 5-LX) an new inlet system has been incorporated (see Fig. 1) which makes it is possible to flush the glass-liner in a cool zone to remove any traces of air prior to insertion into the heated zone of the pyrolysis unit. A big advantage of this set-up is that it is possible to pull back the glass-liner after pyrolysis from the heated zone into the cool zone again. This is useful since multiple step pyrolysis experiments can be done easily on the same sample with increasing temperatures without major loss of sample in between two runs.

An aliquot of 5 µl of a methanolic solution of the reference material was applied onto a rotating Curie-point wire and 2, 4, or 8 µl of a 2.5% aqueous (268.6 nM/µl) or methanolic (217.6 nM/µl) solution of TMAH were added before the sample was dried *in vacuo*. The absolute molar amounts of nonanedioic-, octadecanoic- and hexadecanedioic acid applied, either mixed or as single compound, were 0.236, 1.45, and 1.31 nM, respectively. The ferromagnetic wire was inserted in a glass liner and subsequently placed into the pyrolysis unit. The sample was flushed with helium for 45 s, lowered and after 35 s the ferromagnetic wire was inductively heated for 6 s in a 1 MHz Rf field to its Curie-point temperature (610 °C). After two minutes, the glass-liner was withdrawn from the heating zone. Pyrolysis fragments were flushed into a SGE BPX5 column (25 m, 0.32 mm i.d., 0.25 µm film thickness) mounted in a Carlo-Erba gas chromatograph (series 8565 HRGC MEGA 2) which was coupled directly to the ion source of a JEOL DX-303 double focussing (E/B) mass spectrometer via a home built interface which was kept at 180 °C. Helium was used as carrier gas at a flow rate

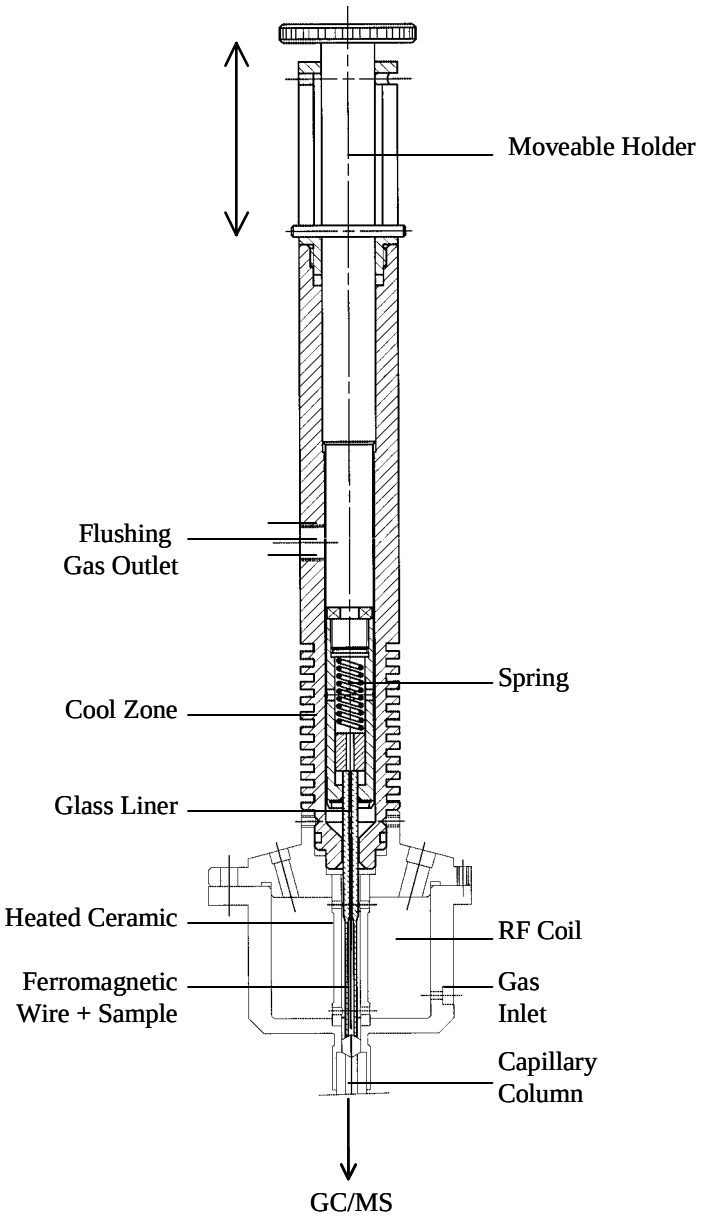


Figure 1. Design of the FOM 5-LX pyrolysis injector.

of approximately 2 ml/min as regulated with a CP-CF 818 pressure/flow control box (Fisons Instruments). The initial temperature of the gas chromatograph was 50 °C, which was maintained for 2 minutes. The oven temperature was programmed at a ramp of 6 °C to an end temperature of 320 °C (50(2)-6-320). Ions were generated by electron impact ionisation (70 eV) or chemical ionisation using isobutane at a pressure of 10^{-3} Pa in the ionisation chamber (180 °C), accelerated to 3 keV, mass separated and postaccelerated to 10 keV before detection. The mass spectrometer was scanned from m/z 40-700 with a cycle time of 1 s. A Jeol MP-7000 data system was used for data acquisition and processing.

5.7 Results and Discussion

5.7.1 On-line (trans)methylation of a linseed oil paint sample

Figure 2 depicts the typical chromatogram of a pyrolysis GC/MS run of a dried linseed oil paint pigmented with lead white, which was analysed after (trans)methylation with a 2.5 % methanolic solution of TMAH. The identified compounds, based on their 70eV electron impact mass spectrum, can be found in Table 1. Most of these compounds are detected when analysing aged oil paint samples [10, 13, 244]. They arise from the breakdown of the initial highly

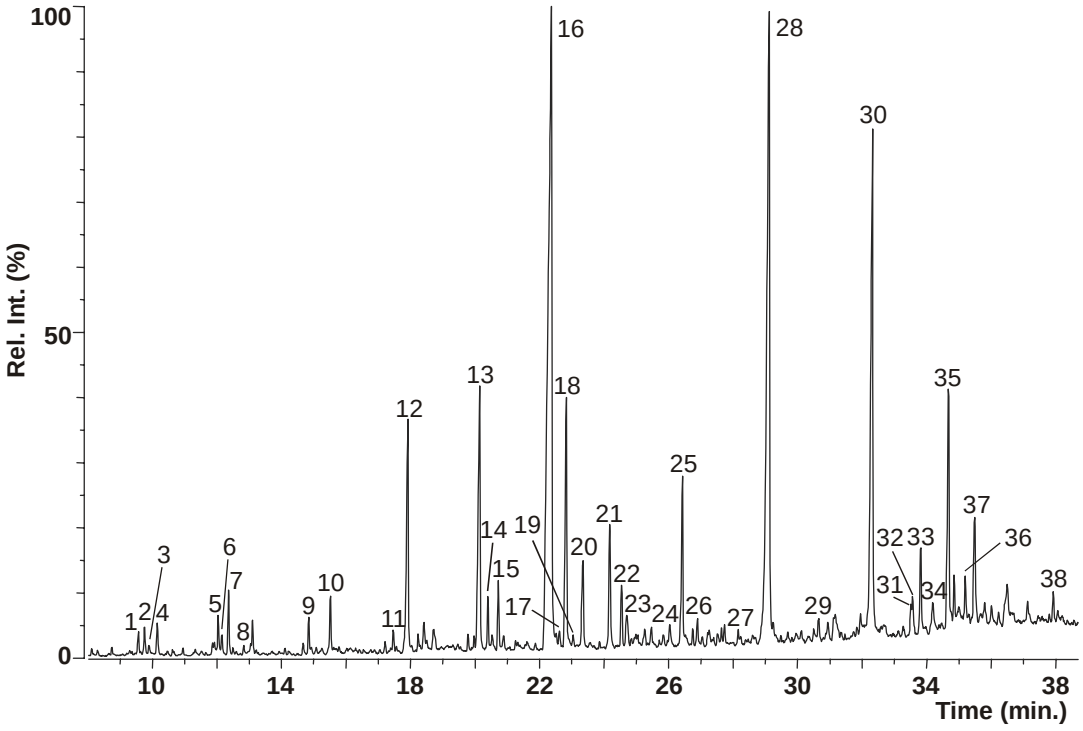


Figure 2. TIC of lead white pigmented linseed oil paint after 610 ºC Curie-point pyrolysis assisted with on-line methylation using 2.5 % methanolic TMAH. Temperature program: 50(2)-6-320. Peak numbers correspond to Table 1.

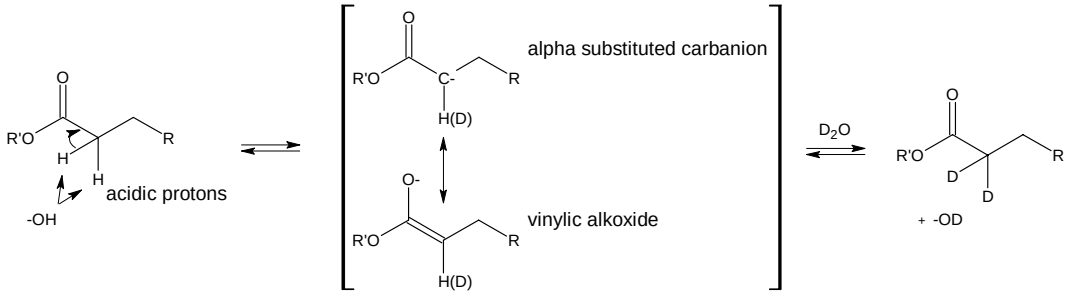
unsaturated triacylglycerols upon autoxidation. However, the identity and origin of some of the compounds was not clear and it was questionable whether they were present as such in the paint film. In this case it was uncertain whether compounds 18 and 20b were ethyl or propyl esters, respectively, or branched analogues of nonanedioic acid. In order to verify this, the analysis was repeated using A. a solution of deuterated TMAH reagent and B. chemical ionisation with isobutane.

Table 1. Identified compounds in the chromatogram of a lead white pigmented linseed oil paint sample after 610 ˚C Curie-point pyrolysis-TMAH-GC/MS

Peak No.	M.W.	Compound
1.	142	heptenoic acid, methyl ester
2.	144	heptanoic acid, methyl ester
3.	144	butenedioic acid, dimethyl ester
4.	146	butanedioic acid, dimethyl ester
5.	156	octenoic acid, methyl ester
6.	158	octanoic acid, methyl ester
7.	158	pentenedioic acid, dimethyl ester
8.	160	pentanedioic acid, dimethyl ester
9.	172	nonanoic acid, methyl ester
10.	174	hexanedioic acid, dimethyl ester
11.	186	decanoic acid, methyl ester
12.	188	heptanedioic acid, dimethyl ester
13.	202	octanedioic acid, dimethyl ester
14.	194	1,2-benzenedicarboxylic acid, dimethyl ester
15.	216	-methyl octanedioic acid, dimethyl ester
16.	216	nonanedioic acid, dimethyl ester
17.	232	-methoxy octanedioic acid, dimethyl ester
18.	230	-methyl nonanedioic acid, dimethyl ester
19.	242	,. -dimethyl nonenedioic acid, dimethyl ester
20. a	228	-methyl nonenedioic acid, dimethyl ester
21. b	244	,. -dimethyl nonanedioic acid, dimethyl ester
22.	230	decanedioic acid, dimethyl ester
23.	246	-methoxy nonanedioic acid, dimethyl ester
24.	244	-methyl decanedioic acid, dimethyl ester
25.	244	undecanedioic acid, dimethyl ester
26.	260	-methoxy decanedioic acid, dimethyl ester
27.	256	pentadecanoic acid, methyl ester
28.	258	dodecanedioic acid, dimethyl ester
29.	270	hexadecanoic acid, methyl ester
30.	284	heptadecanoic acid, methyl ester
31.	298	octadecanoic acid, methyl ester
32.	326	8-methoxy-9-octadecenoic acid, methyl ester
33.	326	11-methoxy-9-octadecenoic acid, methyl ester

34.	326	9-methoxy-10-octadecenoic acid, methyl ester 10-methoxy-8-octadecenoic acid, methyl ester
35.	312	9-oxo-octadecanoic acid, methyl ester 10-oxo-octadecanoic acid, methyl ester
36.	312	9-epoxy-octadecanoic acid, methyl ester
37.	326	icosanoic acid, methyl ester
38.	358	9,10-dimethoxy-octadecanoic acid, methyl ester
39.	354	docosanoic acid, methyl ester

Figures 3a presents the EI spectrum of nonanedioic acid dimethyl ester (16) obtained when using TMAH. The 70 eV mass spectrum of this compound has been extensively described by Ryhage [348]. In Figure 3b the mass spectrum of the same compound is depicted when using deuterated TMAH reagent for the methylation. The fragment ions m/z 74, 143, 156 and 185 that still should contain one deuterated methyl group, are expected to increase in mass with 3 amu, as indicated in Figure 3b. However, from the mass spectrum it is clear that all ions, including those that aren't expected to contain a deuterated methyl group, have obtained a higher mass. This indicates that some H-D exchange has occurred. When looking in detail at the observed fragment ions in this spectrum and other spectra of the same compound (not shown), it can be clearly seen that for most of the fragment ions the exchange of up to four protons atoms has occurred on the carbon chain in between the carboxylic acid groups. The only exception are the ions originally found at m/z 55, 59, 74, 83, 111, and 143 which only exchanged up to a maximum of two hydrogens. This indicates that the H-D exchange is taken place preferentially on the -positions, since these particular fragment ions are the only ones having one -carbon with only 2 exchangeable protons. This process can be understood when the acidity of the protons next to the carbonyl group is considered. Protons on the alpha position of a carbonyl group are acidic (pK_a=19-20) in contrast to the protons beta, gamma, delta and so on, which are not acidic (pK_a=40-50) [377, 378]. Strong bases like tetramethylammonium hydroxide can abstract acidic alpha protons from carboxylic acids to yield resonance-stabilised enolate anions. These intermediates can pick up deuterium as is depicted in Scheme 1.

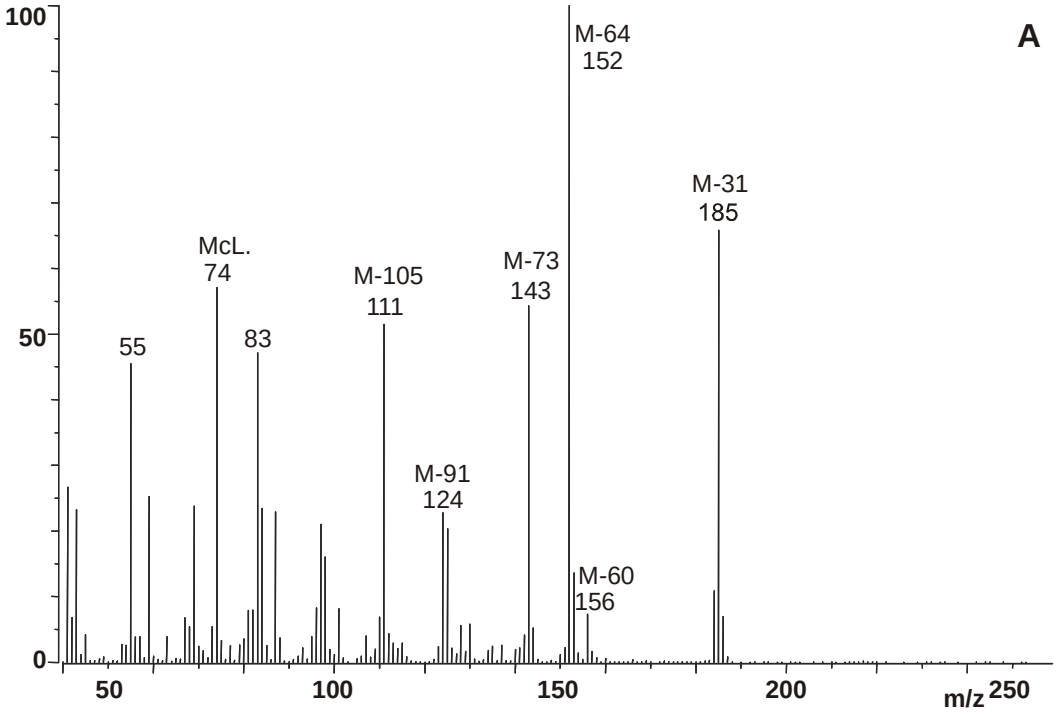


Scheme 1. Proton–deuterium exchange at the methylene -position of the carbonyl functional group.

Table 2. Characteristic fragment ions in the 70 eV mass spectra of identified diacids

m/z	Fragment(s) (lost)
$[M-31]^+$	$\bullet OCH_3$
$[M-59]^+$	$\bullet COOCH_3$
$[M-60]^{+\bullet}$	$COOCH_3 + H$
$[M-64]^{+\bullet}$	$2 \times CH_3OH$
$[M-73]^+$	$\bullet CH_2\text{-}COOCH_3$
$[M-91]^+$	$\bullet COOCH_3 + CH_3OH$
$[M-105]^+$	$\bullet CH_2\text{-}COOCH_3 + CH_3OH$
74	$[CH_3OC(OH)CH2]^{+\bullet}$ [a]
88	$[CH_3OC(OH)CH_3]^{+\bullet}$ [a]
102	$[CH_3OC(OH)C(CH_3)_2]^{+\bullet}$ [a]

[a] These ions are formed upon a McLafferty rearrangement [356].



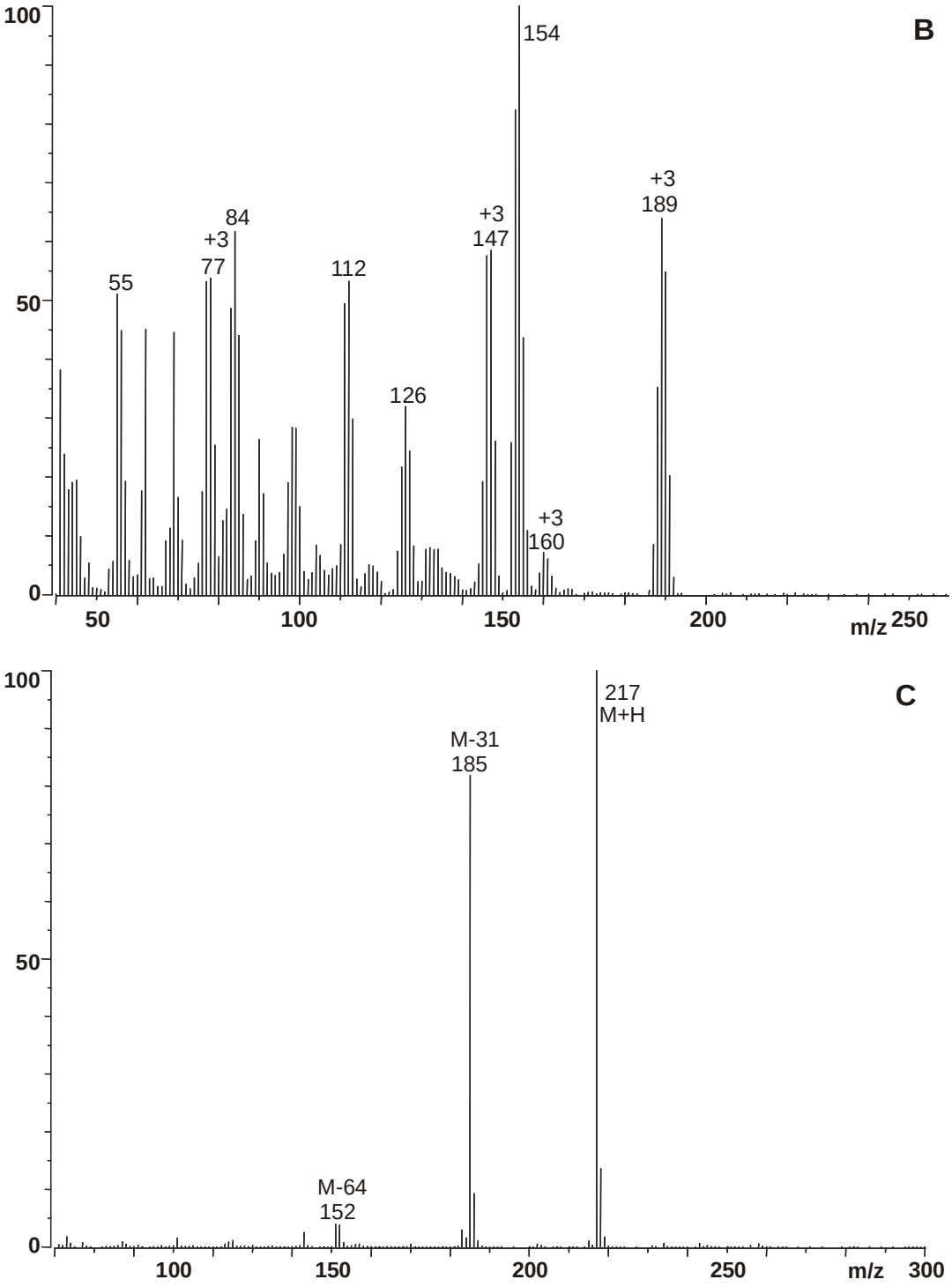
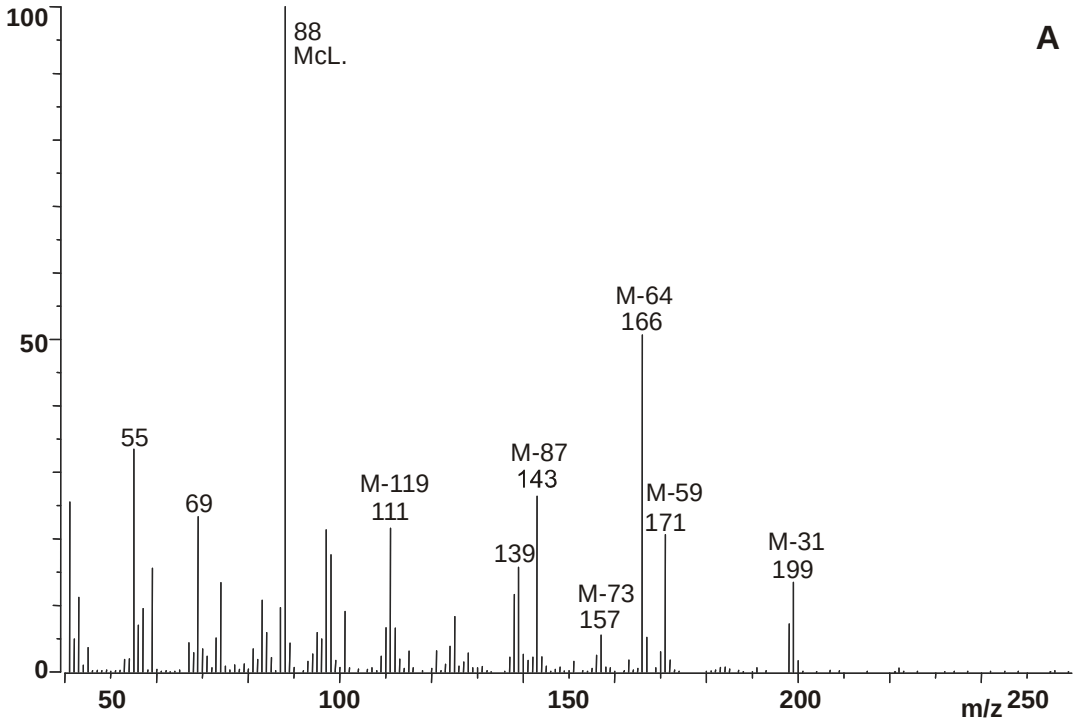


Figure 3. Mass spectrum of nonanedioic acid, dimethyl ester using (a) 70eV EI; (b) 70 eV EI and deuterated TMAH, and (c) chemical ionisation using isobutane.

The mass spectrum obtained under CI conditions using isobutane, is depicted in Figure 3c. Next to the protonated molecular ions, the most prominent fragment

ions are observed that were visible in the normal 70 eV spectrum as well, e.g. m/z 185, 152, 143 and 111.

In Figure 4a-c the same type of mass spectra are depicted for the unknown derivative of nonanedioic acid dimethyl ester (18). In the 70eV mass spectrum (Fig. 4a) it can be seen that almost all ions have increased with 14 amu compared to the spectrum shown in Figure 3a, including the ion that has been ascribed to a McLafferty rearrangement. The ethyl, methyl ester of nonanedioic acid, that has a molecular weight of m/z 230, could be ruled out because this compound is expected to show fragment ions both at m/z 185 ($[M-45]^+$), 199 ($[M-31]^+$) and m/z 152 ($[M-78]^+$) (see Table 2), which was not observed. This indicates that an extra methyl group has been introduced on one of the -carbons rather than that an ethyl ester has been formed upon derivatisation. This is conclusion is supported by the increased intensity of the fragment ion m/z 171, $[M-59]^+$, formed upon -cleavage next to the carbonyl group and leading to the loss of the carboxylic acid group. The experiment with deuterated TMAH shows that the additional methyl group is derived from the deuterated TMAH since certain fragments have increased with at least 6 amu (m/z 94, 177, and 205) whereas others only increased 3 amu (m/z 142, 146, and 169). This observation supports the idea that the methyl group is incorporated on the -position during the on-line derivatisation and is not formed



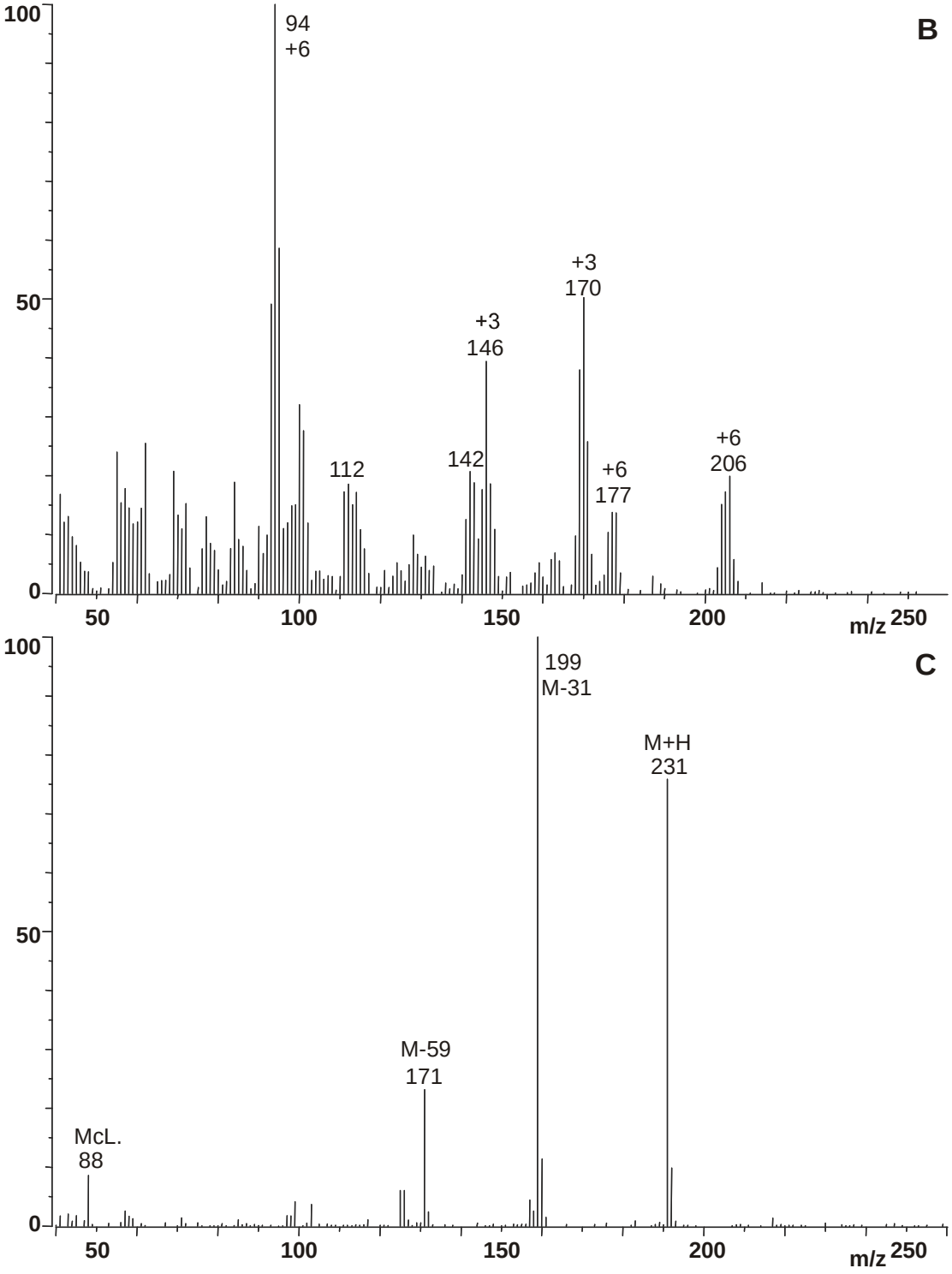


Figure 4. Mass spectrum of nonanedioic acid, -methyl, dimethyl ester using (a) 70eV EI; (b) 70 eV EI and deuterated TMAH, and (c) chemical ionisation using isobutane.

upon rearrangement of cross-links within the paint film. Again it is clear that some H-D exchange has occurred, although it seems to a lesser extent when compared to Figure 3b. An -hydrogen is replaced by a methyl group which reduces the number of exchangeable hydrogens by one. The molecular mass was verified by chemical ionisation using isobutane and determined to be 230 (see Fig. 4c).

In some cases, we have observed a very pronounced formation of - and ,- methylated diacids upon Curie-point pyrolysis-TMAH-GC/MS analysis of oil paint samples and the reaction products are of even higher intensity than the expected product. Furthermore, the incorporation of an extra methyl group has also been observed next to the other acid group of the diacid at the '-position, giving a triply substituted diacid (unpublished results). In a few cases even normal long chain saturated fatty acids showed incorporation of an extra methyl group, as was observed by Asperger [372] as well. Unfortunately due to the unknown (absolute) composition of both the organic and inorganic fraction of oil paint samples and the amount of sample actually applied on the wire, it cannot be immediately deduced which factors play an important role in the unwanted methylation reaction.

5.7.2 On-line (trans)methylation of fatty (di)acid standards

The structure of the -methylated diacids is elucidated above. The question remains how these compounds are formed and under what conditions. Therefore the on-line methylation of three reference compounds (nonanedioic acid, hexadecanedioic acid and octadecanoic acid) was investigated using a variety of derivatisation conditions. The last two compounds were chosen to examine the influence of the presence of two acid groups within one molecule and the length of the carbon chain, respectively. All compounds were dissolved in methanol and analysed in the same way as the oil paint sample. The chromatogram in Figure 5 depicts the result of the pyrolytic methylation of a mixture of different amounts of the reference materials with TMAH reagent dissolved in methanol. It is clear from this figure that there's a difference in behaviour of the materials. For both diacids at least three peaks (compounds 1-5 and 11-15; see Table 3) of reaction products are observed with relatively high intensities compared to the expected dimethylated reaction products, whereas for the C18 fatty acid only trace amounts of unwanted by-products are seen. Closer inspection of the mass spectra shows that next to the dimethyl esters of -methylated diacids also ,- dimethylated products are present. Besides two unsaturated analogues are found as well (compounds 3,4 and 13,14). The mass spectrum of the ,- dimethylated nonanedioic acid - and hexadecanedioic acid, dimethyl ester are depicted in Figure 6 and 7, respectively. Small mass peaks of both the molecular ions and fragment ions due to the loss of a methoxy group are visible. The McLafferty rearrangement ion is found at m/z 102 and typical fragment ions are observed that were reported above for the normal diacid (Table 2). The mass spectra of the monounsaturated analogues (not depicted) look very similar and only differ by two mass units for almost all

fragment ions except for the McLafferty rearrangement ion and the $[M-59]^+$, which has become the more favourable $[M-60]^+$. However, the retention times of the -. methylated unsaturated compounds 4 and 13 are higher than would be expected based on the retention times of the saturated reaction products, especially the unsaturated -methylated dimethyl analogue of nonanedioic acid. It should be said that it was difficult to obtain a pure spectrum of compounds 4 and 13 due to co-elution with the saturated ,. -dimethylated compound.

Table 3. Identified compounds in the chromatogram of a mixture of nonanedioic-, octadecanoic- and hexadecanedioic acid after 610 ˚C Curie-point pyrolysis-TMAH-GC/MS.

Number	M.W.	Compound
1	216	nonanedioic acid, dimethyl ester
2	230	-methyl nonanedioic acid, dimethyl ester
3	242	,. di-methyl nonenedioic acid, dimethyl ester
4	228	-methyl nonenedioic acid, dimethyl ester
5	244	,. di-methyl nonanedioic acid, dimethyl ester
6	270	hexadecanoic acid, methyl ester
7	?	unidentified contamination
8	298	octadecanoic acid, methyl ester
9	312	-methyl octadecanoic acid, methy ester
10	326	,. -dimethyl octadecanoic acid, methyl ester
11	314	hexadecanedioic acid, dimethyl ester
12	328	-methyl hexadecanedioic acid, dimethyl ester
13	340	,. di-methyl hexadecenedioic acid, dimethyl ester
14	326	-methyl hexadecene acid, dimethyl ester
15	342	,. di-methyl hexadecanedioic acid, dimethyl ester

The formation of the -methylated fatty acids can be explained by considering the acidity of the -protons next to the acid groups. Their pK_a value of 19-20 [377] makes it possible that they react with the strongly basic TMAH, giving rise to alpha-substituted carbanions (See Scheme 2). The TMAH salt, which will have a slightly higher pK_a, comparable to esters, will behave behave in the same way. These intermediates will form singly -methylated (di)acids upon an S_N2 reaction with the TMAH. After the reaction of one of the protons there is a second proton which can undergo the same reaction again and an ,. -dimethylated (di)acid with a tertiary carbon atom will result. Although the incorporation of the first methyl group is thought to decrease the rate of proton removal of the

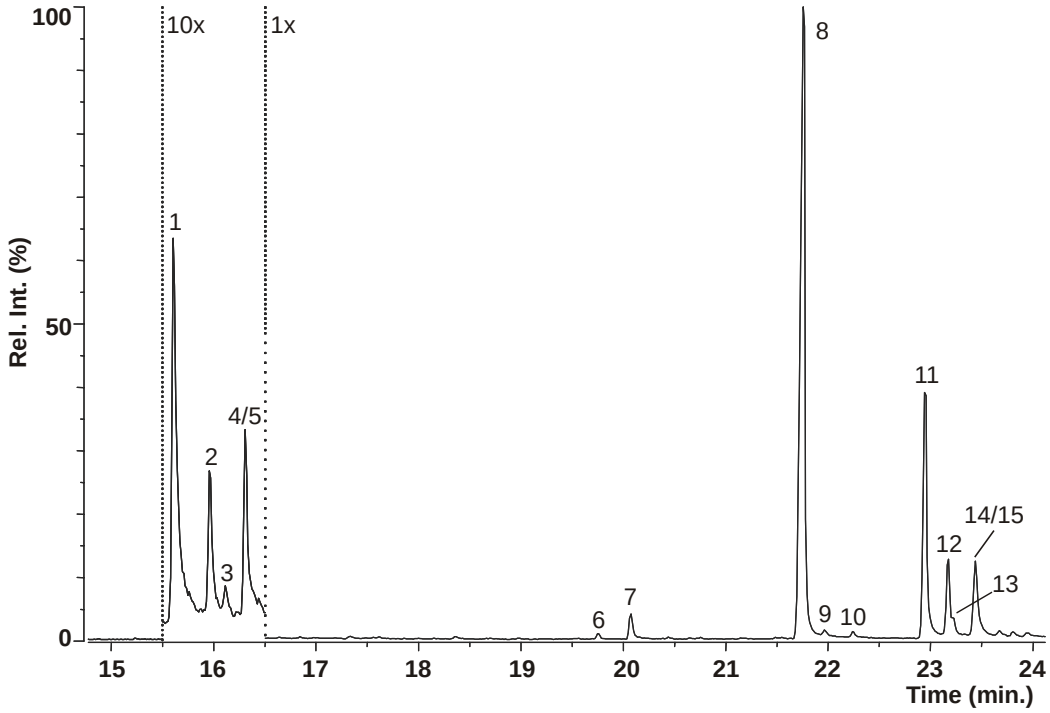


Figure 5. TIC of a mixture of nonanedioic-, octadecanoic- and hexadecanedioic acid after 610 ºC Curie-point pyrolysis assisted with on-line methylation using a 2.5% methanolic TMAH solution. Temperature program: 50(2)-10-320. Peak numbers correspond to Table 3.

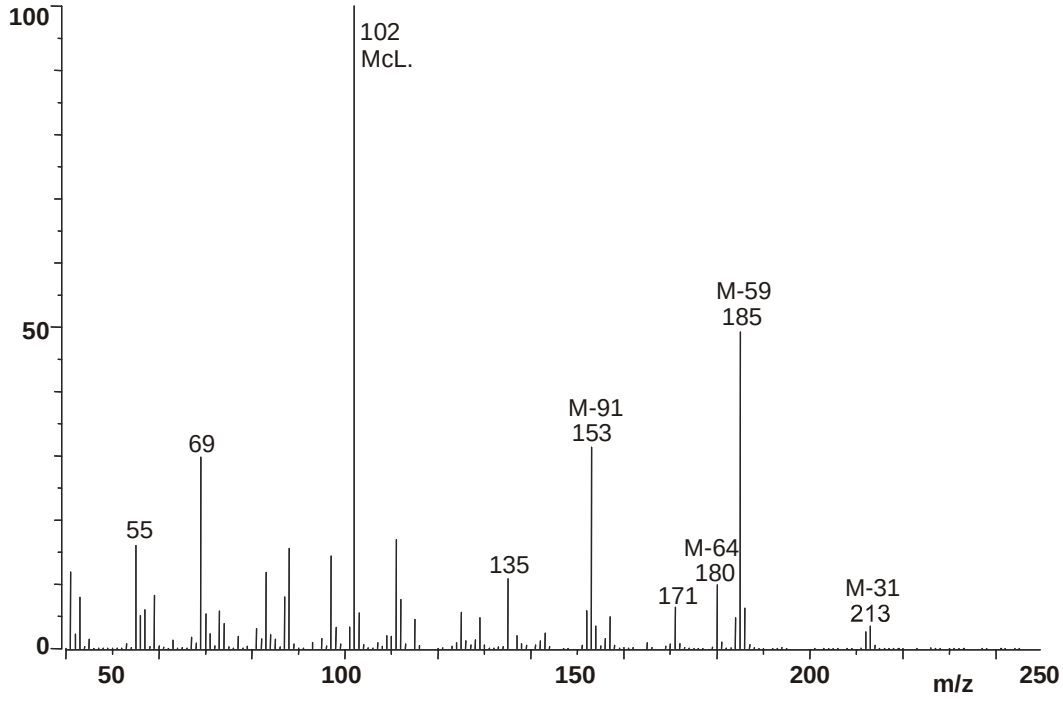


Figure 6. 70 eV EI mass spectrum of ,. -dimethyl nonanedioic acid, dimethyl ester.

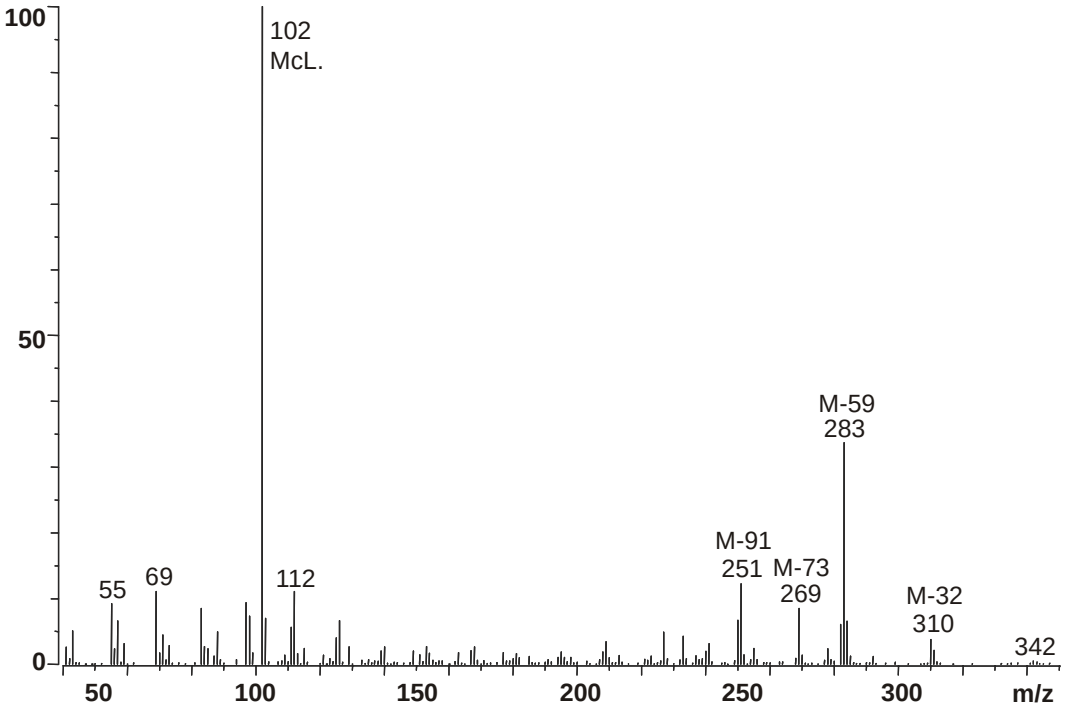
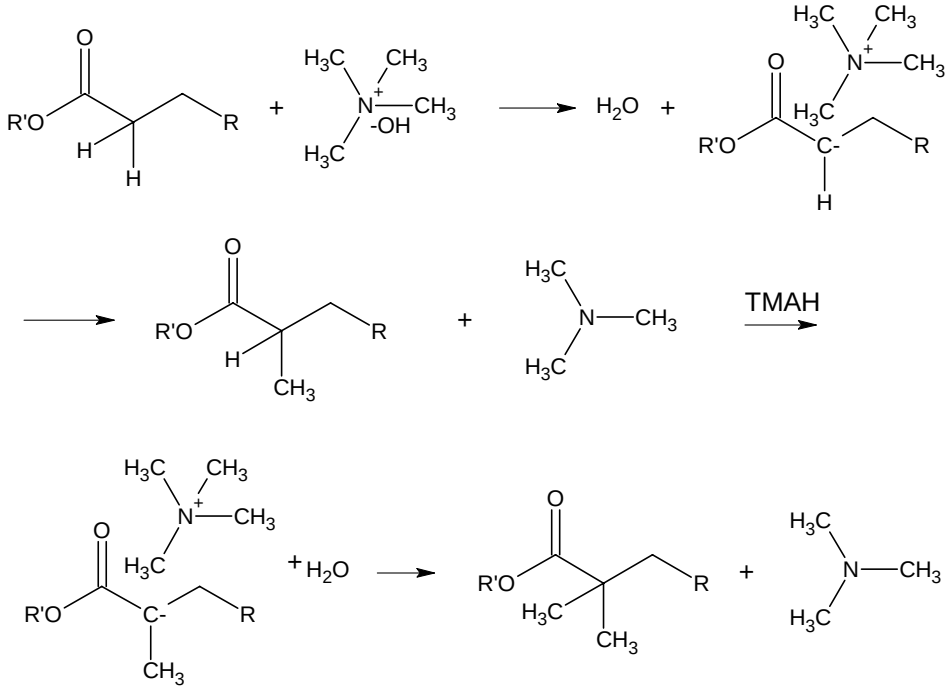


Figure 7. 70 eV EI mass spectrum of ,. -dimethyl hexadecanedioic acid, dimethyl ester.

Scheme 2. Postulated mechanism of formation of -. and ,. -methylated diacids.



remaining -hydrogen and subsequently would lead to a preferential removal of one of the '-hydrogens next to the other acid group this is not observed. The fact that the ,. -dimethylated reaction products are formed is due to acid-base proton transfer between the alkylated product and the unreacted enolate anion. Once the methylated species is formed it finds itself in solution with a relatively high concentration of a strong base (the remaining enolate anion) that can remove another -proton, giving a new enolate ion that is further methylated. The formation of the unsaturated analogues is not completely understood but experiments using different pyrolysis temperatures showed increasing amounts of these species when using higher temperatures, whereas the overall amount of by-products didn't change to a large extent (results not shown). The description of the formation of the (.). methylated species, however, doesn't account for the enhanced relative amounts of derivatives of the diacids compared to the normal fatty acids. We conclude from the observations above (Fig. 5) that the presence of two acid groups plays a role rather than the length of the carbon chain. Py-TMAH-GC/MS analyses of three other fatty diacids with a carbon chain length of 6, 8, and 10, respectively, gave the same type of reaction products (results not shown). In most standard organic chemistry books also the unusual reactivity of malonic acid (C4 diacid) is reported when considering -methylation. This is, however, a special case due to a six-membered resonance stabilised structure that can be formed, giving -protons with a pK_a of approximately 9.

In a subsequent experiment TMAH was dissolved in deuterated methanol to see whether it was the methanol that led to the increased incorporation of the -. methyl groups. No peaks were observed in the mass spectra that could be attributed to the incorporation of a methyl group having a mass of 18 (-CD_3). When the experiment was repeated using a deuterated TMAH salt in normal methanol, the opposite was found: all methyl groups had a mass of 18 (-CD_3), indicating that methanol itself does not participate in the methylation reaction.

As stated before, it was found that the solvent plays an important role in the -methylation reaction observed in oil paint samples. This is also immediately clear when the results of experiments with the reference materials are looked upon (Table 4). The relative percentages of the non -. and ,. -methylated derivatives are depicted for the three reference compounds when analysed with Curie-point pyrolysis using a 2.5 % TMAH solution in both water and methanol, respectively. The data were obtained by determining the peak area of the normal reaction product and dividing it by the sum of the peak areas of all major peaks derived from the starting material observed. Whereas in water percentages were found for all three compounds varying between 96,7 and 100%, in the case of methanolic solutions they were around 98% for the octadecanoic acid and much lower (35-60%) for the diacids. This suggests that in methanol the acidity of the -protons is increased which are subsequently relatively easy to remove. It is thought that this is due the formation of some kind of aggregate of the diacid and the TMAH salt in methanol, although no direct evidence is present for this suggestion. Apparently, the monocarboxylic acid cannot form such a structure.

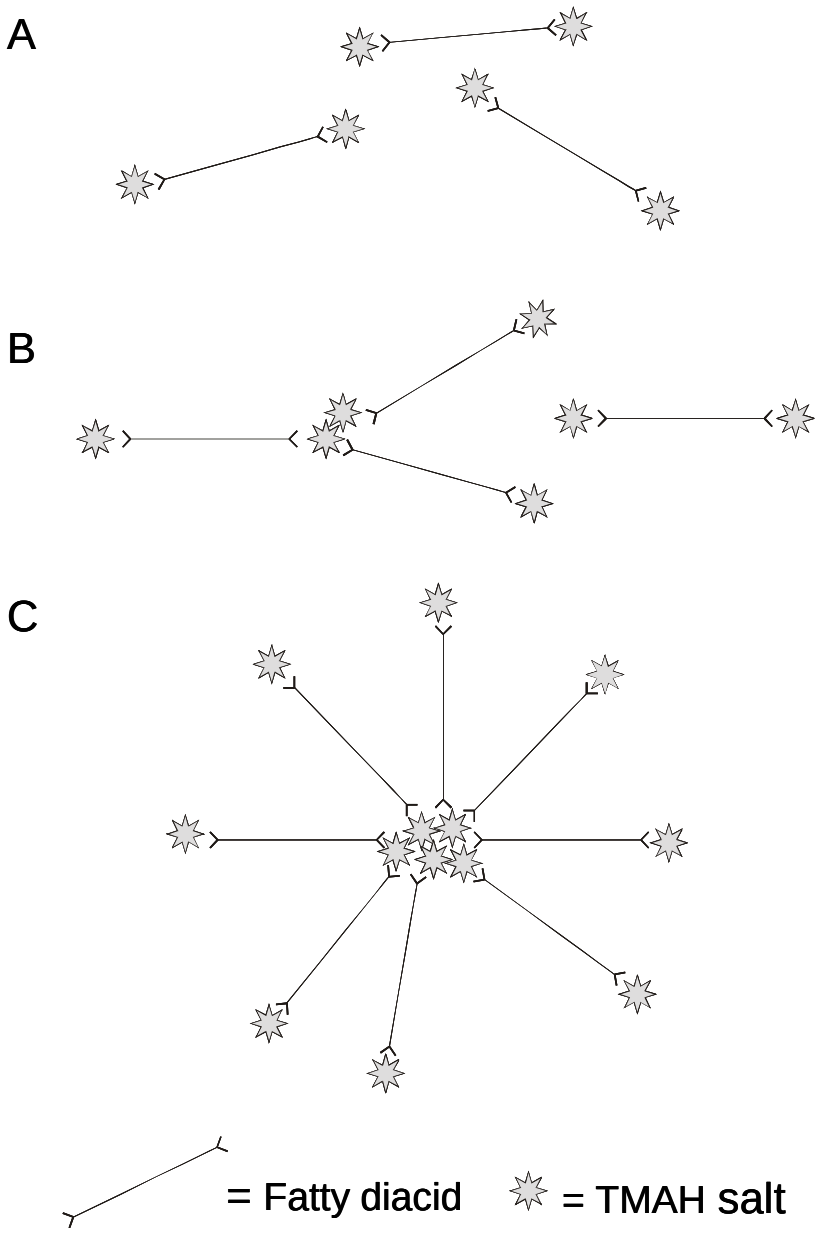
Since other research groups use TMAH for the on-line methylation of lipids or fatty acids as well but with different concentrations and relative amounts of TMAH to analyte and since the formation of -methylated fatty acids hardly has been reported, the influence of the relative amount of TMAH was tested. The amount of TMAH was both doubled and halved relative to the amount of the experiments previously described. The result is rather surprising as can be seen in Table 4. For the analyses of the diacids done with half the amount of aqueous reagent drastically increased amounts of by-products were found, whereas with methanol these had decreased. The opposite was observed for higher amounts of TMAH. Almost complete conversion was obtained for all experiments with the aqueous solution in this case. The methanolic solution however led to slight increase of -methylated species.

Table 4. Results of the study on the effects of several parameters involved in on-line (trans)methylation of C9 diacid, C16 diacid, and C18 using a 2.5% TMAH solution in combination with 610 ºC Curie-point pyrolysis-GC/MS

Amount TMAH (ul)	Solvent	Drying Time (min)	C9 DIFAME[a] (%)	C16 DIFAME (%)	C18 FAME[b] (%)
2	water	12	65,4 (2,9)[c]	65,6 (3,4)	99,4 (0,2)
2	methanol	12	78,8 (3,3)	77,6 (4,1)	99,5 (1,4)
4	water	12	99,1 (1,6)	98,7 (1,1)	99,8 (1,7)
4	methanol	12	60,2 (5,6)	63,1 (8,9)	99,0 (0,3)
4	water	12(5)[d]	98,1 (n.d.)[e]	97,3 (n.d)	99,7 (n.d.)
8	water	17	100 (0,3)	99,4 (1,8)	99,1 (1,3)
8	methanol	12	48,4 (n.d.)	51,0 (n.d.)	98,0 (n.d.)
8	methanol	17	43,7 (3,3)	45,0 (4,4)	98,2 (0,2)

[a] DIFAME = fatty diacid, dimethyl ester.
[b] FAME = fatty acid, methyl ester
[c] In brackets the standard deviation is given. All measurements were done in triplicate at least.
[d] The mixture of reference compounds was applied onto the pyrolysis wire and dried within 5 min. Subsequently aqueous TMAH reagent was added and the sample was dried for another 12 min.
[e] n.d. = not determined (single measurement

It is our hypothesis that co-ordination chemistry in the solvent-analyte mixture determines the reactivity of TMAH with respect to the reactants (see Scheme 3). In the case of high amounts of aqueous TMAH reagent with respect to the reactant, the diacids are co-ordinated by TMAH forming a salt in such a way that -methylation is much less favourable (Scheme 3a). When the amount of aqueous TMAH is lowered, it is more likely that part of the TMAH co-ordinates with different acid groups in the interior of an aggregate and as a result of this co-



Scheme 3. Postulated hypothesis to explain the difference in reactivity of aqueous and methanolic TMAH solutions. (a) Linear combination of TMAH salt and fatty diacids in water at high TMAH concentration; (b) Lowering the amount of added aqueous TMAH reagent leads to formation of aggregates, and (c) In methanol aggregates are thought to be present. Lowering the amount of methanolic TMAH reagent leads to a lower amount of micelles.

ordination the acidity of the -protons is increased (3b). This gives rise to higher amounts of -methylated diacid derivatives. In methanol the situation is different. A phase separation is postulated to occur forming micelles with TMAH at the centre (3c). As the concentration of TMAH is lowered, less micellar structures will be formed and subsequently less –methylation can be expected. This mechanism proposed rationalises the data in Table 4, although it should be stressed there is no direct evidence available yet. Given the popularity of this relatively easy derivatisation method it is important that further studies will be conducted to elucidate the formation of these by-products and to find ways to prevent them.

5.8 Conclusion

The data presented above clearly demonstrated that TMAH is not only capable of (trans)methylating the acid groups of fatty (di)acids, but can also react with acidic protons on the -position, giving rise to different species of unwanted alkylated fatty acids. The (unwanted) reactivity of the diacids was shown to be higher in methanolic systems and less concentrated aqueous solutions. It is our believe that differences in the coordination chemistry of the TMAH and the reactant leads to different acidities of the alpha-hydrogens, hence the formation of the unwanted by-products. The use of water as solvent for the TMAH reagent, although being more time-consuming upon drying, therefore is advised for the analysis of carboxylic acids when using Py-GC/MS.

Chapter 6

Determination of the degree of hydrolysis of oil paint samples using a two-step derivatisation method and on-column GC/MS

An elegant method is described for the determination of the degree of hydrolysis of (unsaturated) triacylglycerols, and their oxidation products, present in linseed oil based paints. The analytical strategy, a transethylation of esterified fatty acids followed by a trimethylsilylation of free fatty acids and their salts, is first tested on reference materials comparable or identical to the compounds present in fresh and aged oil paints. Reproducibility and repeatability are examined on free- and methylated fatty acids, a sodium- and lead salt and several triacylglycerols. Unwanted trimethylsilylated products could be observed for the triacylglycerols and other esterified fatty acids up to a maximum of 6 %. Free fatty acids and their salts are shown to be completely trimethylsilylated but incomplete derivatisation was observed for azelaic acid. Glycerol, liberated upon transethylation, was (partially) trimethylsilylated. However, the recovery was not uniform and the results could not be used for quantitative determination of the amount glycerol in the paint sample. Studies on the influence of the pigments indigo, lead white, and prussian blue and glycerol showed that for lead white and indigo doped reference material divergent results were obtained. The addition of these last compounds led to increased amounts of hydrolysed products up to 37%. For the other compounds, no adverse influence on the analytic results is onserved. The repeatability of the determination of the degree of hydrolysis within oil paint systems was tested on a number of paints. It is shown for 5-year old test paints with a relatively homogeneous composition that the method is reproducible. For less defined paints consisting of multi-layered systems it is shown that the position and way of sampling can have a significant influence on the spread in the analytical result. This is caused by the large variations that are possible in the sampled material in these inhomogeneous systems. The degree of hydrolysis of an oil paint is taken as the average of the values obtained for azelaic-, palmitic- and stearic acid, based on the results presented. Overall, it is observed that the relative amount of hydrolysed fatty (di)acids increases in time. Surprisingly, in contrast to

the results obtained on reference material, for all lead white pigmented paints lower degrees of hydrolysis were found relative to other paints from that particular set.

6.1 Introduction

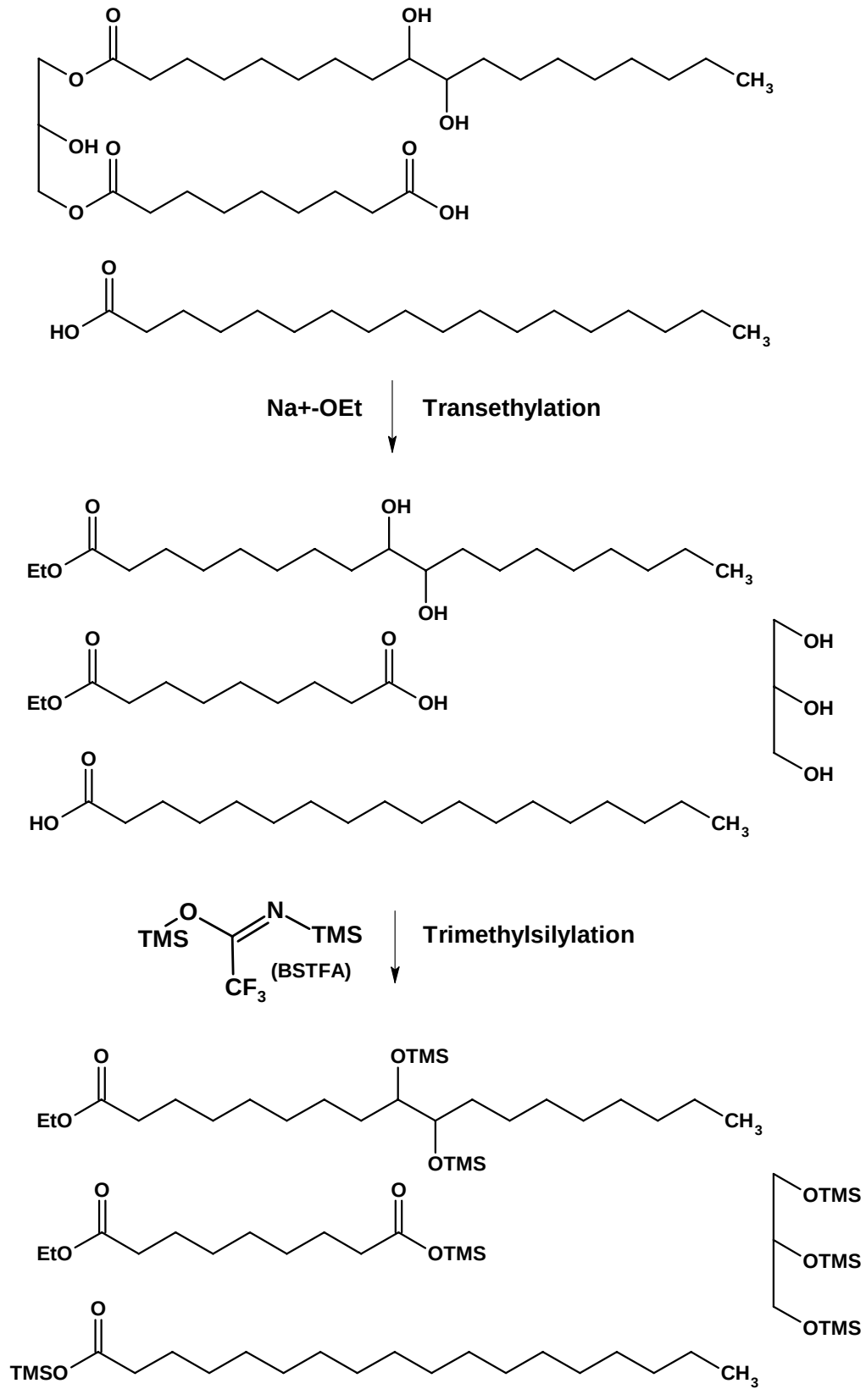
Drying oils like linseed, walnut and poppyseed have been widely used as binding medium for paintings. When fresh, these viscous oils consist of relatively apolar mixtures of triacylglycerols (TAGs) with different fatty acid profiles. Only a small percentage of these fatty acids (FAs) is fully saturated (6-9%) whereas most of the other FAs are polyunsaturated (68-90%)[292]. This high amount of unsaturated FAs, highly susceptible to oxidation, makes it possible for the oil to form a dried film. In this case drying is not the evaporation of a solvent but chemical drying, which involves the cross-linking of TAGs to high molecular weight substances [124, 295]. Due to the chemical environment in paintings, these "binding" substances are not stable end products. Further chemical changes in the oil paint film involve hydrolysis of the ester bonds, formation of new oxygen containing functional groups, oxidative cleavage of the fatty acid hydrocarbon chains, and metal ion coordination of the fatty acid group of the cross-linked material and non cross-linked fractions.

A relatively good understanding of the process of curing exists as a result of detailed chemical studies of alkyd paint [123, 125]. The transition of the initial "polyester" to other forms of polymeric systems in ageing traditional oil paint is less understood. It is likely that the different processes partly overlap in time. On the basis of literature on ionomers and linseed oil based ceramer coatings it has been speculated that the polymer end groups react with or are partially adsorbed on the inorganic particles [262, 278, 379]. As part of our studies on the formation of ionomeric systems in historical paints, in this study a new analytical strategy is developed to monitor the process of de-esterification of the initial TAGs of oil paint systems. A first description of these processes and preliminary chemical data have been reported by Boon et al. [28] and Van den Berg et al. [29].

Already since the '60s oil paint samples have been analysed using a combination of chemical work-up and subsequent gas chromatography analysis [10]. Initially this was done to identify the type of oil used for painting based on the determination of the ratio of saturated palmitic- and stearic acids which, at that time, were thought to remain unchanged within the paint film upon ageing. First, the paint sample was completely hydrolysed in order to analyse liberated (oxidised) fatty acids. After extraction into an organic layer, a washing step and acidification the fatty acids were methylated and analysed. Later on (conservation) scientists became interested in the way the fatty acids are present within the paint film [22]. After immersion of the paint sample in an organic solvent the extractable fraction, consisting of free fatty acids, acylglycerols and smaller cross-linked networks, were separated from the metal salts and high molecular weight materials which were thought to be insoluble. Both fractions were analysed as previously

described and information was obtained on the amount and types of compounds in both fractions [14, 25, 248]. Especially the solvent extractable fraction is of great interest because this is the fraction that theoretically can be partially removed upon solvent cleaning of oil paintings. More recently the relevance of these immersion studies was questioned by White and Roy, who did a study on the effect of solvent cleaning of old master paintings [27]. They concluded that when cleaning of old paintings "is in the hands of trained conservators" the amount of extractable material will be that low that it is not expected that serious damage is caused. Koller et. al. [20] extended the analytical research and developed a multiple-step extraction procedure to further specify the different fractions within an oil paint. In the first two steps hexane and methanol are used to extract the non polar- and polar fraction, respectively. In a third extraction chloroform is applied to remove glycerol esterified fatty acids whereas metal salts are released in the last step by treatment of the remaining paint with a 10% methanolic solution of oxalic acid. Although the idea of separation of the different fractions prior to analysis is very interesting there can be some doubts about the effectiveness and selectivity of the extractions. Esterified fatty acids or metal salts are not completely insoluble in the solvents used for extractions and therefore are likely to have been removed partially. Besides, a disadvantage of these aforementioned analytical strategies is the laborious procedure, with extraction and washing steps that can lead to loss of material. Furthermore, it cannot immediately be deduced from these analyses what the ratio of esterified to hydrolysed material is. This information is useful since it gives an insight into the processes occuring in the paint system. A new analytical strategy is explored in this chapter that consists of a two-step analytical procedure, carried out in one vial without preceding separation and that only involves one transfer step. It consists of a transethylation reaction to turn all ester bound fatty acids into their ethylated analogues, followed by a second step to derivatise all free fatty acids and salts thereof into trimethylsilyl esters (see scheme 1). The first derivatisation focuses on the (oxidised) fatty acids of the initial triacylglycerols of the oil that are still esterified to the glycerol backbone. By using an ethanolic solution of basic sodium ethoxide for the solvolysation, the original ester groups can be replaced by an ester of the new alcohol [380-382]. At the same time, the reagent will not be able to esterify free fatty acids. It should be noted that this reaction requires anhydrous conditions because the presence of water may lead to irreversible hydrolysis of the ester bonds, giving rise to lower yields. This ethanolysis reaction is still a popular way of making esters from triacylglycerols and related fats, enabling their fatty acid composition to be determined [383]. The second step in chemical work-up is a classical trimethylsilylation using bis(trimethylsilyl)trifluoroacetamide (BSTFA), with the addition of 1% trimethylchlorosilane (TMSC) [357]. In order to dilute the sample inert hexane was chosen because of its ease of removal by evaporation. Solubilisation of the analyte(s) in hexane prior to derivatisation is not absolutely necessary since this may occur as trimethylsilylation proceeds.

The combination of both derivatisations has been tested for both reproducibility and repeatability on reference materials that resemble the compounds that can be found in oil paints, as well as reconstructed linseed oil based paints and real oil paint samples.



Scheme 1. Two-step derivatisation methodology.

6.2 Experimental

6.2.1 Materials

Azelaic acid and sodium palmitate (both 98%), margaric acid, trimyristate, tripalmitate, sodium ethoxide 21wt% solution in denatured ethanol (all approximately 99%), and 14-methyl-hexadecanoic acid were obtained from Sigma-Aldrich Chemie Bv., Zwijndrecht, The Netherlands. Indigo and bis(trimethylsilyl)trifluoroacetamide, containing 1% trimethylchlorosilane, were purchased from Fluka, Zwijndrecht, The Netherlands. Glycerol (>98%) was bought from Merck, Amsterdam, The Netherlands. The pigment prussian blue ($Fe_4[Fe(CN)_6]_3$) was manufactured by L. Cornelissen & Son, London whereas Cremnitz White (lead white; basic leadcarbonate) was produced by Old-Holland Classic Oil Colours, Driebergen, The Netherlands.

6.2.2 Preparation of lead(II)stearate

Lead(II)stearate was freshly prepared in the following way: 80 ml p-xylene (Acros, Geel, Belgium, >99%), together with 0.98 g lead oxide (Sigma-Aldrich, 99.999%) was de-aerated, 2.00 g stearic acid (Sigma-Aldrich, 99%) was added, the system was de-aerated again and maintained under a nitrogen flow. The mixture was heated with an oil bath to an end temperature of 138 °C at which the mixture was refluxed for 15 min. The hot solution was decanted over a glass filter in another round bottom flask to remove remaining unreacted lead oxide. This was also done under a stream of nitrogen. The obtained solution was gradually cooled so that crystals could precipitate. After cooling the solution to 0 °C, the crystals were collected on a glass filter, and washed with demineralised water and freshly distilled ethanol and acetone, respectively.

After washing and drying of the crystals in a rotary evaporator, the total amount of silver-white crystals was 2,58 g (95%). The melting point was 117/8 °C, which agreed with melting points (110-125 °C) mentioned in the literature [341-343]. An infrared spectrum was recorded to see if residual free fatty acids were present. The spectrum was identical to a reference spectrum of lead stearate [205, 344] and no peaks indicative for the presence of free fatty acids were found.

6.2.3 Reconstructed oil paints

Stand oil, a linseed oil that is prepolymerised by heating, was obtained from Talens, Apeldoorn, The Netherlands and Cremnitz White (basic lead

carbonate) was purchased from Old-Holland Classic Oil Colours, Driebergen, The Netherlands. The oil paint (70:30 w/w; oil/pigment) was applied on glass plates in 1994 with a thickness of approximately 0.5 mm and aged at room temperature under normal daylight conditions in our institute until samples were taken.

The 27-year old pigmented paints were made by H.C. von Imhoff with cold-pressed linseed oil (Mühlfellner-Rupf, Zurich, Switserland) that was allowed to stand in flat dishes of 4 mm height for 3 weeks. After the skin had been removed the oil was mixed with the pigment until a workable paint was obtained. The paints were applied on presized and primed lime wood and aged under normal conditions. This collection of paints is presently stored at the Canadian Conservation Institute (CCI), Ottawa, Canada.

Four different samples of alkali refined linseed oil paints were donated by the National Gallery of Art, Washington. These are part of a large collection of test paints made by Nathan Stolow and presently stored at the NGA. The investigated paints are dated 1965 (lead white: acid refined linseed oil (arlo) 85:15, barium sulphate: arlo 74:26, and iron oxide:arlo 81:19) and 1954 (lead white:arlo 85:15). They all were applied on a glass support and naturally aged to date. Leaching studies have been done on these paints in the late ‘60s by Stolow and Rogers [22].

The Getty Conservation Institute, Los Angeles, CA, USA, supplied us with relatively young 5-year-old naturally aged paint films made of blown linseed oil only and with the pigments yellow ochre (76% w/w), vine black (70% w/w), and lead white (62% w/w). These paints already have been investigated in more detail by Schilling [13].

6.2.4 Transethylation/Trimethylsilylation

For every mg of reference material or paint sample in a GC auto injector vial, 250 µl of a 0.01 M ethanolic sodium ethoxide solution was added. This amount of reagent is sufficient to accomplish complete transesterification of the esterified fatty acids present in a fresh oil paint sample, which contains 30% w/w pigmentation. Typical amounts of material analysed are in the order of 250 µg to 2 mg. The vial was flushed with dry nitrogen, sealed and placed in an oven at 75-80 ˚C. After 90 minutes the vial was taken out of the oven and allowed to cool to room temperature (RT). To neutralise the sample, 30 µl of a saturated ethanolic ammoniumchloride solution is added. After 20 minutes the ethanol is evaporated under a gentle stream of dry nitrogen and the residue is dissolved in hexane (250 µl per mg of sample). The resultant suspension is treated with 15 µl bis(trimethylsilyl)trifluoroacetamide, sealed and returned to the oven for a further 30 minutes. After cooling down (RT), and evaporation of the solvents the analytes are redissolved in dichloromethane, containing hexadecane as an internal standard (100 mg/l). The mixture is subsequently centrifuged and the upper layer is carefully separated from pigment particles and remains of the paint film and subsequently transferred into a new auto injector GC vial.

With the aid of an AS 800 on-column autoinjector (Fisons Instruments) 1 µl of derivatised sample was directly injected into a SGE BPX5 column (25 m, 0.32 mm i.d., 0.25 µm film thickness), mounted in a Carlo-Erba gas chromatograph (series 8565 HRGC MEGA 2). The gas chromatograph was directly coupled to the ion source of a JEOL DX-303 double focussing (E/B) mass spectrometer via a home built interface which was kept at 180 °C. Helium was used as carrier gas at a flow rate of approximately 2 ml/min as regulated with a CP-CF 818 pressure/flow control box (Fisons Instruments). The initial temperature of the gas chromatograph was 50 °C which was maintained for 2 minutes. The oven temperature was programmed at a ramp of 6 °C to an end temperature of 320 °C (50(2)-6-320). Ions were generated by electron impact ionisation (70 eV) in the ionisation chamber (180 °C), accelerated to 3 keV, mass separated and postaccelerated to 10 keV before detection. The mass spectrometer was scanned from m/z 40-700 with a cycle time of 1 s. A Jeol MP-7000 data system was used for data acquisition and processing.

6.3 Results and Discussion

6.3.1 Reference materials

In a first test triacylglycerols of myristic- and palmitic acid (C14 and C16 TAG, respectively) and a methylated stearic acid (C18 FAME) were subjected to the two-step derivatisation procedure. A typical result is shown in Figure 1. By examining the relative ratios of the ethylated and trimethylsilylated (TMS) derivatives an indication is obtained on the selectivity of this analytical method. It is clear that, besides the expected transethylated species, also a small percentage of trimethylsilylated compounds is found. For the efficiency of conversion of the esterified reference material into ethylated analogues it made no difference whether the fatty acids were esterified to glycerol or methylated. Based on relative peak areas the values 4.88, 4.11 and 3.04% were found for the silylated species, respectively. This is however only a relative amount and the absolute numbers are expected to deviate, based on the difference in response factors. These response factors have not been taken into account and therefore absolute quantification is not possible. Validation of the reliability of the method was done by checking for underivatised compounds after both reactions by direct temperature resolved mass spectrometry [329]. Furthermore, determination of the ratio of both derivatives and comparison of measured response factors also indicated that reactions were complete. In general between 0 and 6% of unwanted trimethylsilylated derivatives are observed for the esterified reference materials. The real percentage, however, is expected to be lower based on the higher response factors for TMS fatty acids

compared to alkylated fatty acids. Table 1 presents the averages of replicate measurements, performed on pure compounds and different mixtures of the reference materials. It should however be pointed out that for the C18 TAGs in some of the chromatograms significantly lower amounts of ethylated C18 FAs were detected as well as TMS derivatives of monostearoylglycerols, indicative for

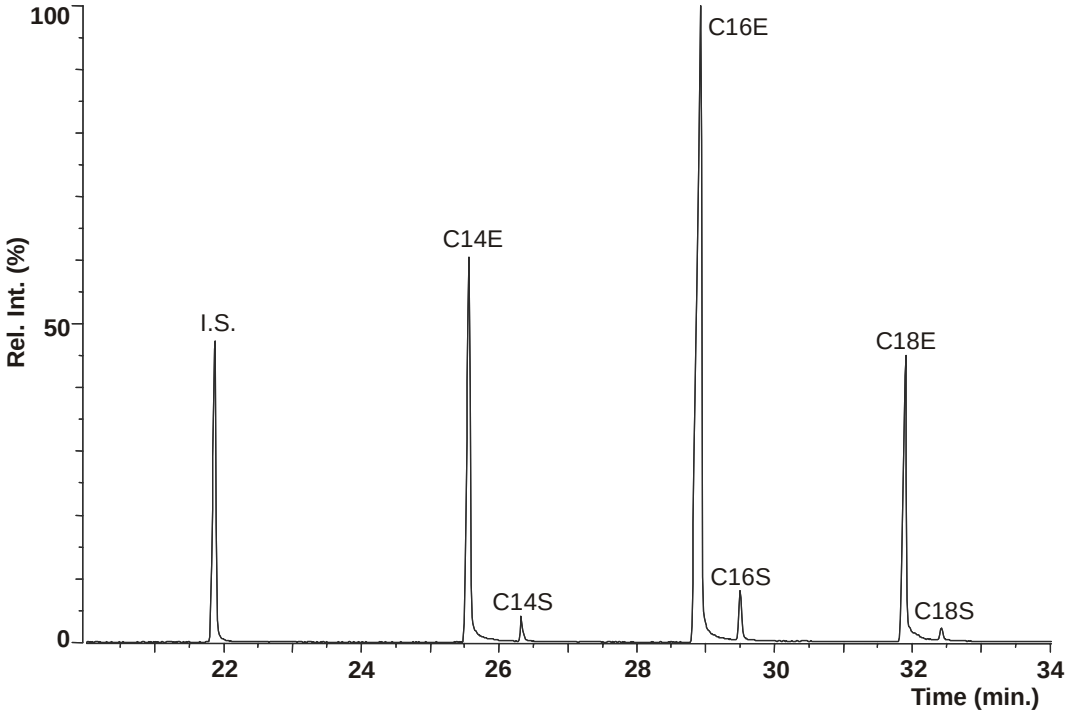


Figure 1. Partial TIC of a test mixture, containing trimyristine (C14 TAG), tripalmitine (C16TAG) and a methyl ester of octadecanoic acid (C18FAME), after transethylation (E) and trimethylsilylation (S) derivatisation.

the incomplete conversion of the TAGs. These results have not been included in Table 1. Hydroxy groups of glycerol liberated during the transethylation reaction of the TAGs, are trimethylsilylated in the next step. Three different derivatives can be observed in the chromatograms (not shown). Next to fully trimethylsilylated glycerol the two doubly trimethylsilylated isomers also are observed. The complete derivatisation is most probably prevented by steric hindrance of the bulky TMS groups. The presence, the relative ratios and the total amount of glycerol derivatives detected varies from analysis to analysis, whereas the amount of liberated fatty acids agreed with the amount of initial TAGs. Tests with varying lengths of the last drying step indicated there is no relation between the amount of derivatised glycerol detected and the time it takes to dry the sample. Although the source of this variation is not known yet, problems with solubility are suspected to be the cause.

A mixture of 14-methyl hexadecanoic acid (C16-14 FA), C14 TAG, and a C18 lead salt (C18 Pb) was analysed after chemical work up and it can be seen in Figure 2 for both the lead salt and the free fatty acid that the TMS derivative is the

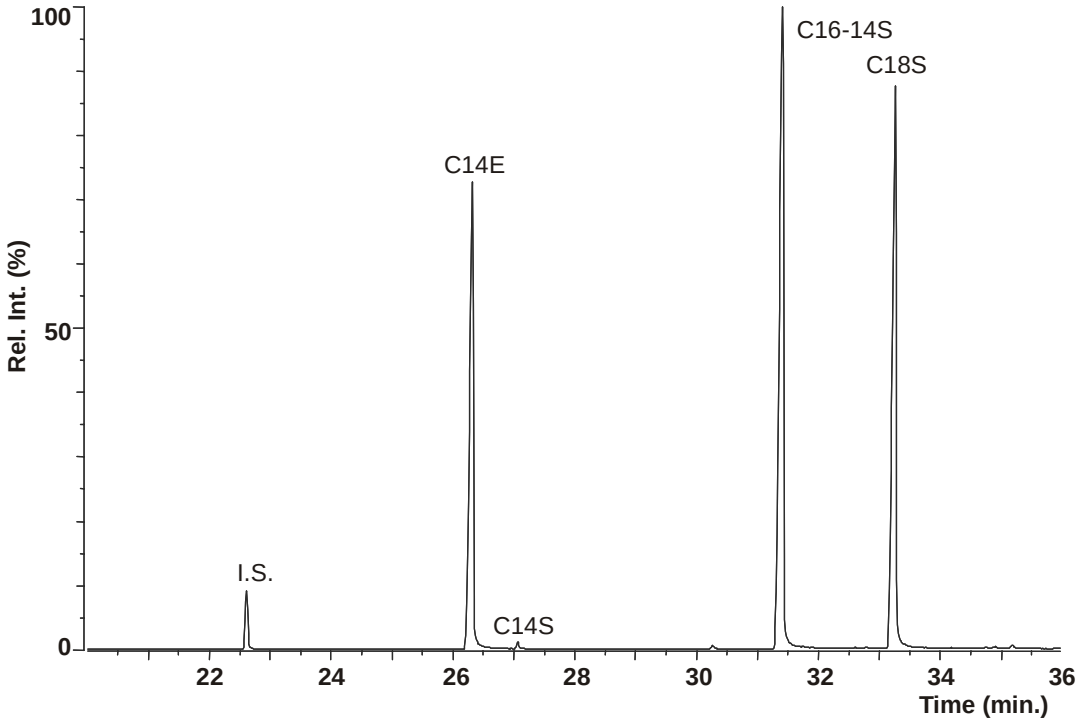


Figure 2. Partial TIC of a test mixture, containing trimyristine (C14 TAG), 14-methyl hexadecanoic acid (C16-14 FA) and a lead salt of octadecanoic acid (C18 Pb), after transethylation (E) and trimethylsilylation (S) derivatisation.

major product detected. Surprisingly, minute amounts of the ethylated derivative were detected despite the fact that the transethylation is not supposed to convert these type of fatty acids. The relative amount of TMS derivatives of the C14 FA is only 1.05 % in this case. For other analyses of the mixture of lead stearate and C14 TAG the relative amount of silylated fatty acids was higher and an average of 4.46% was obtained. The fact that both ethylated and TMS derivatised fatty acids are found suggests some interesterification has occurred between the analytes. Test experiments using margaric (C17) acid and sodium palmitate gave successful results that were shown to be repeatable (see Table 1). When testing the method on azelaic acid (C9 diacid) and mixtures of reference material containing C9 diacids, occasionally monosilylated derivatives were detected next to the doubly silylated product but only up to an average of 0.5 %. In one isolated extreme case the percentage of the monosilylated derivative amounted 1.9%.

Since the method was shown to work for the selected reference materials, the question remained whether organic and inorganic pigments and the presence of glycerol could possibly have an influence on the outcome of the two-step derivatisation method. Therefore three pigments (indigo ($C_{16}H_{10}N_2O_2$); 13-16% w/w, Cremser White ($2PbCO_3\ Pb(OH)_2$); 19-30% w/w, and Prussian Blue ($Fe_4[Fe(CN)_6]_3$); 16-23% w/w) and pure glycerol (8-14% w/w) were added to mixtures of the reference materials to be analysed. For glycerol and prussian blue no interference was observed for both the transethylation and trimethylsilylation derivatisations whereas for Cremser White and indigo large deviations were found

or the fraction of ethylated species (see Table 1). When glycerol was added, all its derivatives previously described were observed. Their recovery, however, was low in several instances just as was observed for the glycerol derived from TAGs in the previous experiments.

Table 1. Analytical results obtained on reference materials using the ethylation/trimethylsilylation method. C9DIFA = nonanedioic acid (azelaic acid), C16-14 = 14-methyl hexadecanoic acid, C14 TAG = triacylglycerol of C14 FA, C18:1 FAME = methyl ester of oleic acid, Gly = glycerol, Ind = indigo, LWh = lead white, PrBl = prussian blue.

Reference material	Number of analyses	Compound(s) included in tests	Average of relative % of ethyl derivative	Standard Deviation
C14 TAG	5	C18 Pb	95.54	2.14
C18:1 FAME	3	Gly	99.79	0.18
C18 TAG	9	Gly, PrBl	98.54	1.61
C18 TAG	3	LWh	65.34	2.24
C18 TAG	2	Ind	66.80	5.08
C9 DIFA	18	Gly, Ind, LWh, PrBl	99.57	0.50
C16 Na	3	C18 Pb	96.34	2.70
C16-14 FA	11	Gly, Ind, LWh, PrBl	99.00	1.32
C17 FA	3	-	99.00	0.19
C18 Pb	5	-	99.09	0.94

6.3.2 Reconstructed Oil Paints

Subsequently, it was checked whether the determination of the degree of hydrolysis of (pigmented) oil paint reconstructions would give a repeatable result. In order to do so a 5-year-old dried stand oil film (a linseed oil that has been prepolymerised by heating) was analysed. The results are depicted in Figures 3a,b and Table 2. Typical products identified include small fatty acids and diacids formed upon oxidative degradation of unsaturated C18 fatty acids. The fact that only TMS derivatives of C9 and C10 fatty acids and no ethylated species are found indicates that these fatty acids originate from the non-ester side of the oxidatively degraded C18 fatty acid. The diacids, however, are degradation products that include the original carboxylic group with which these acids were esterified to the glycerol backbone. Upon oxidation of the double bond system and subsequent degradation a second and free carboxylic acid group is formed. This acid group is always trimethylsilylated when derivatised.

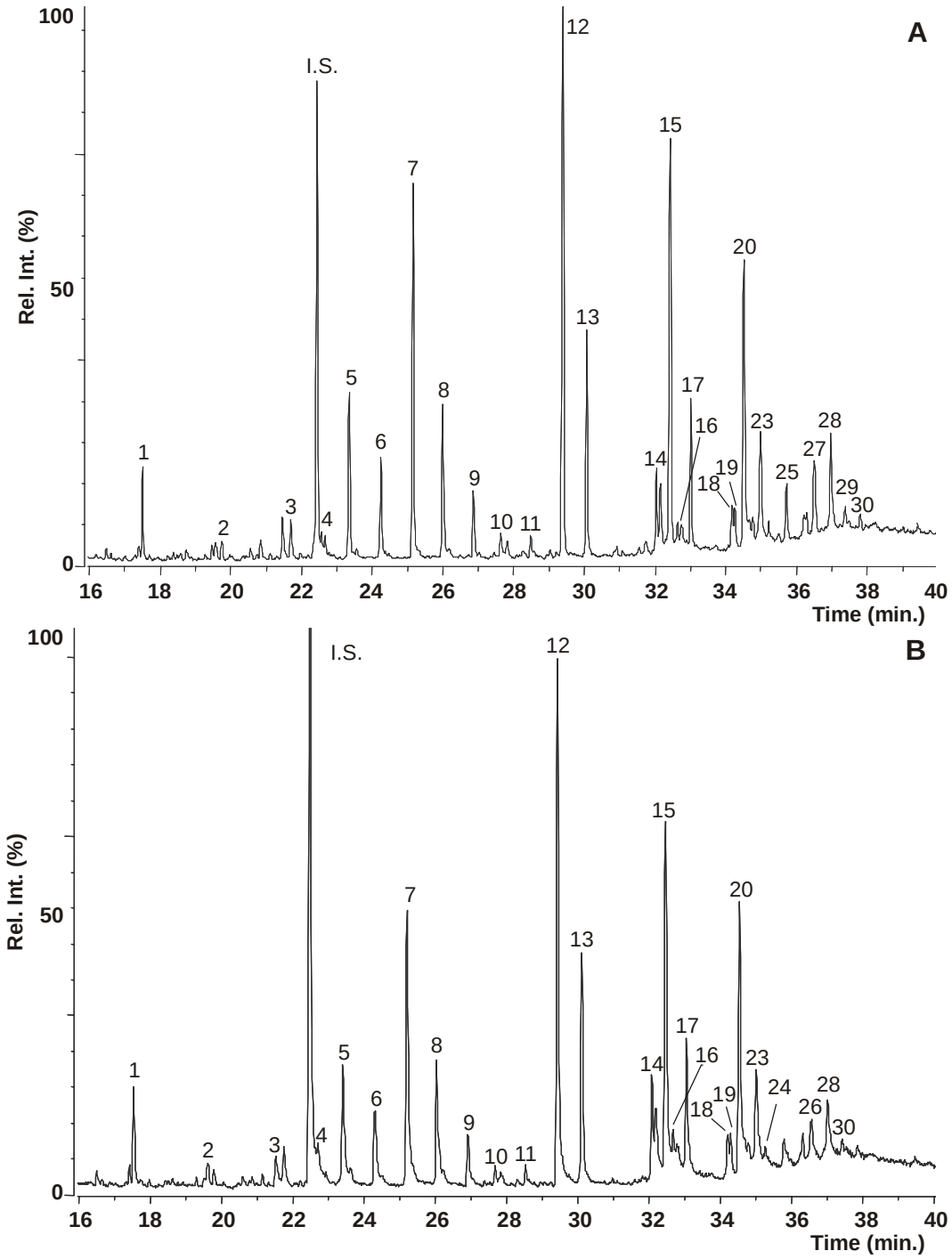


Figure 3 a,b. Two TICs of a 5-year-old stand oil film, after transethylation and trimethylsilylation derivatisation. The numbers correspond to Table 2.

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Table 2. Identified compounds (based on their 70 eV electron impact mass spectra) in aged oil paint systems after GC/MS analysis of their transethylation / trimethylsilylation products.

Label	Compound
I.S.	hexadecane (internal standard)
1.	nonanoic acid, TMS ester
2.	decanoic acid, TMS ester
3.	heptanedioic acid, ethyl TMS ester
4.	heptanedioic acid, di-TMS ester
5.	octanedioic acid, ethyl TMS ester
6.	octanedioic acid, di-TMS ester
7.	nonanedioic acid, ethyl TMS ester
8.	nonanedioic acid, di-TMS ester
9.	decanedioic acid, ethyl TMS ester
10.	decanedioic acid, di-TMS ester
11.	undecanedioic acid, ethyl TMS ester
12.	hexadecanoic acid, ethyl ester
13.	hexadecanoic acid, TMS ester
14.	octadecenoic acid, ethyl ester
15.	octadecanoic acid, ethyl ester
16.	octadecenoic acid, TMS ester
17.	octadecanoic acid, TMS ester
18.	9-octadecenoic acid, 8-TMS ether, ethyl ester
19.	9-octadecenoic acid, 11-TMS ether, ethyl ester
20.	10-octadecenoic acid, 9-TMS ether, ethyl ester 8-octadecenoic acid, 10-TMS ether, ethyl ester
21.	9-octadecenoic acid, 8-TMS ether, TMS ester
22.	9-octadecenoic acid, 11-TMS ether, TMS ester
23.	10-octadecenoic acid, 9-TMS ether, TMS ester 8-octadecenoic acid, 10-TMS ether, TMS ester
24.	eicosanoic acid, ethyl ester
25.	mixture of unidentified C18 oxidation products
26.	mixture of unidentified C18 oxidation products
27.	mixture of unidentified C18 oxidation products
28.	octadecanoic acid, 9,10-bis[(TMS)oxy], ethyl ester
29.	octadecanoic acid, 9,10-bis[(TMS)oxy], TMS ester
30.	docosanoic acid, ethyl ester

The transethylation and trimethylsilylation procedure results in mass spectra that not have been reported before. A typical mass spectrum of a diacid that was esterified prior to analysis (nonanedioic acid, ethyl TMS ester; compound 7) is shown in Figure 4. The molecular ion m/z 288 is hardly visible in contrast to the high intensity fragment ion at m/z 273 (M-CH_3). The fragment ion at m/z 243 is formed upon loss of the ethoxy group (-OC_2H_5). A typical even mass fragment ion

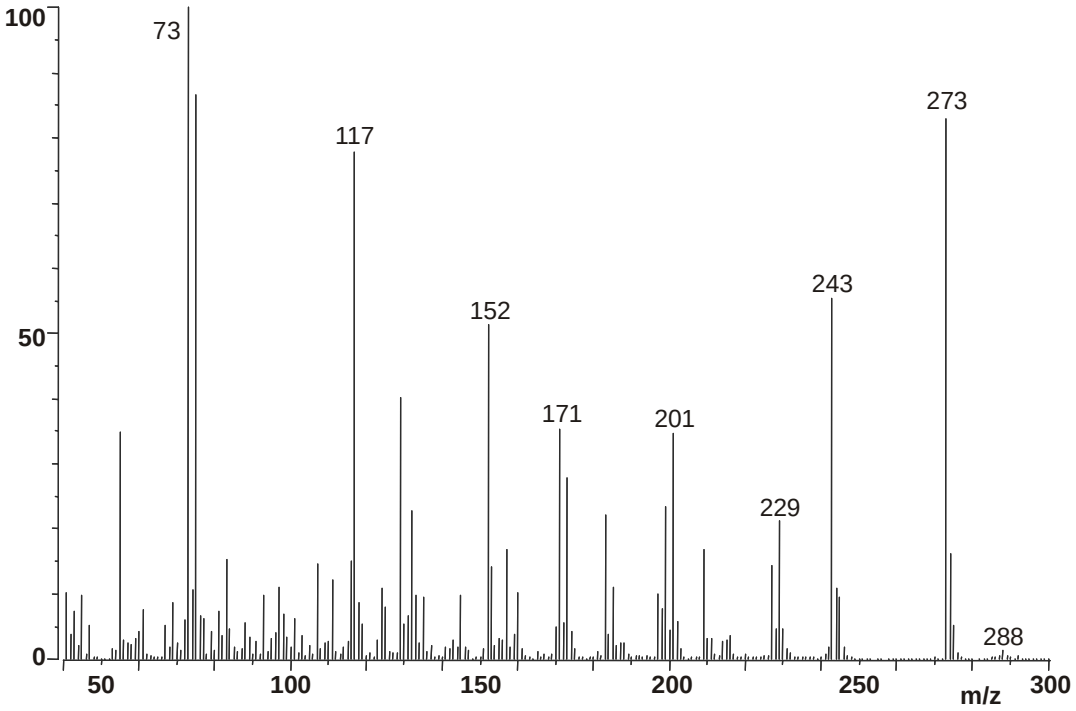


Figure 4. Mass spectrum of nonanedioic acid, ethyl TMS ester (compound 7) (70 eV EI).

of this particular diacid is observed at m/z 152 [348]. This is formed upon loss of both a trimethylsilanol and ethanol moiety. Another series of compounds identified is a range of monounsaturated, TMS ether containing C18 fatty acids of which both the ethyl and TMS derivative are detected (compounds 18-23). In Figure 5a,b and 6a,b the mass spectra of both derivatives are depicted of two compounds (compounds 20 and 23, respectively) of these series. In all cases the main fragment is formed upon -cleavage next to the –OTMS group and at the -position relative to the double bond. A second fragment of lower intensity is observed which can be ascribed to -cleavage next to the double bond. In all cases both the molecular ion (m/z 398 and 442, respectively) and a fragment ion due to loss of a methyl group (m/z 383 and 427, respectively) is observed. Also observable for some of the ethylated fatty acids is the loss of an ethoxy group, giving rise to a fragment ion at m/z 353. Furthermore, loss of m/z 31, leading to a fragment ion at m/z 411, is seen in all spectra of the TMS derivatives.

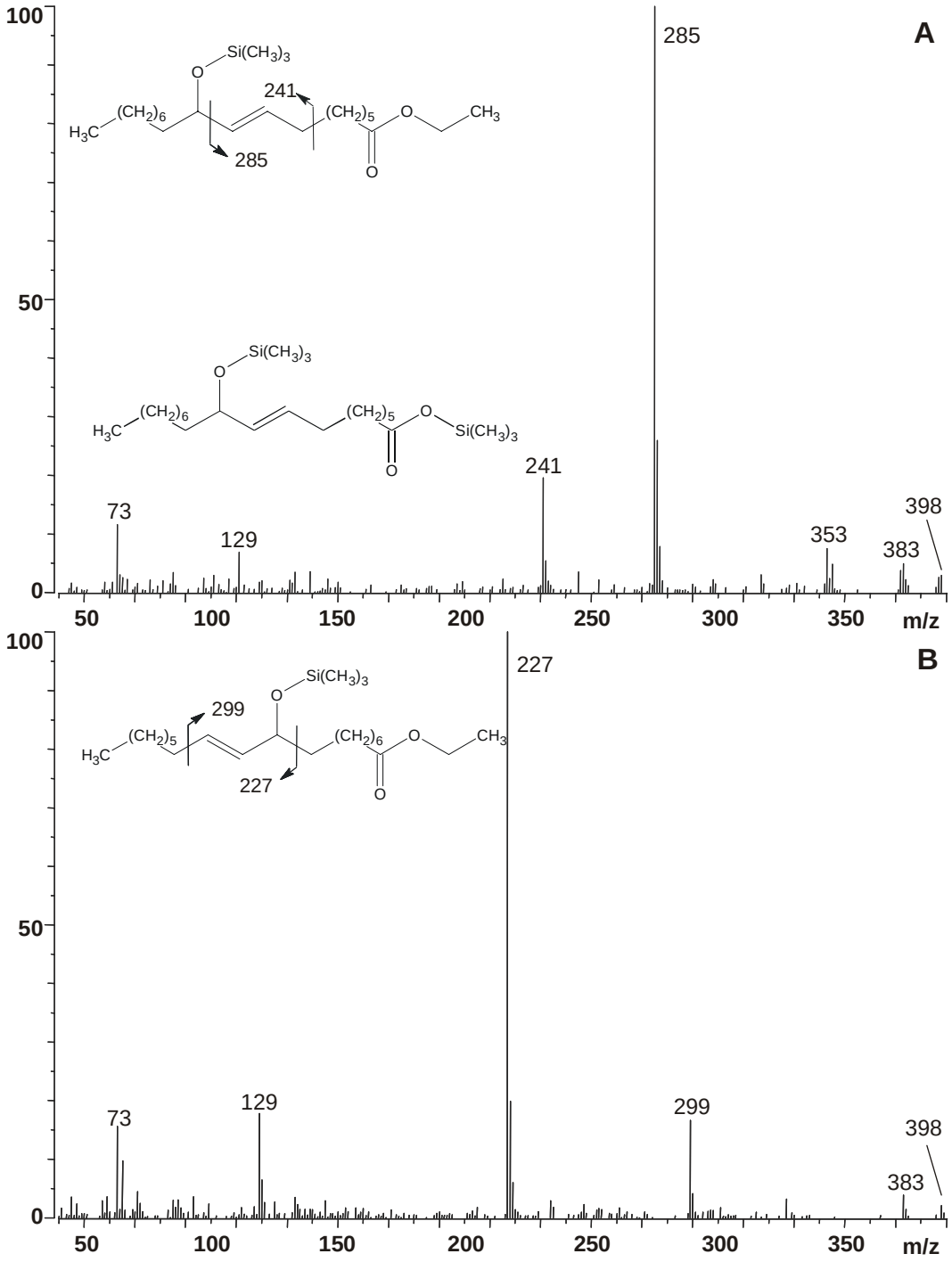


Figure 5 Mass spectrum of compound 20 (a) 10-octadecenoic acid, 9-TMS ether, ethyl ester, and (b) 8-octadecenoic acid, 10-TMS ether, ethyl ester (70 eV EI).

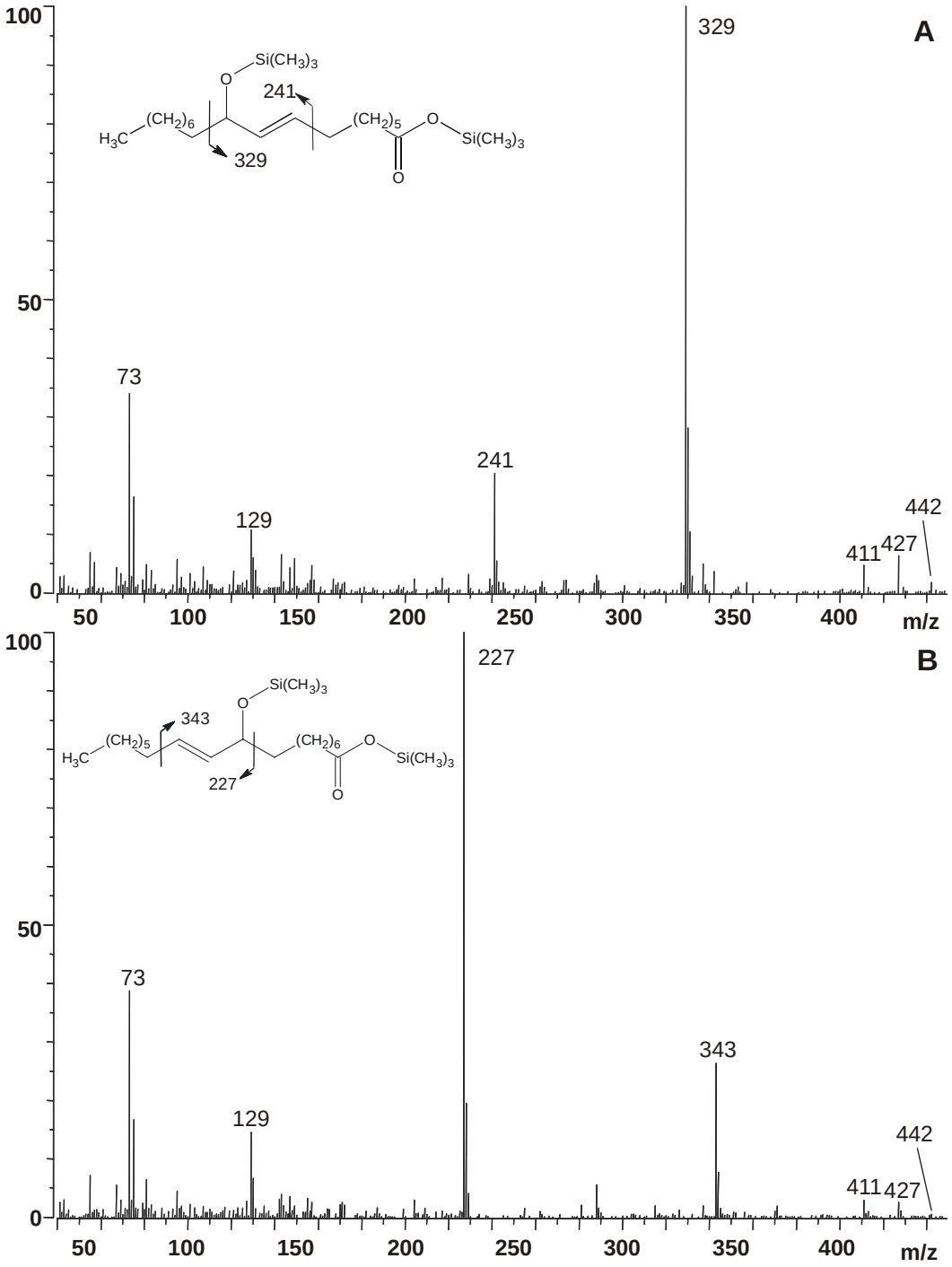


Figure 6 Mass spectrum of compound 23 (a) 10-octadecenoic acid, 9-TMS ether, TMS ester, and (b) 8-octadecenoic acid, 10-TMS ether, TMS ester (70 eV EI).

Comparison of the analytical results of two different samples of the same paint film, taken on different locations (Figures 3a,b) shows that similar results are obtained. Only a minor difference of less than 2% is found for the relative percentages of the two possible derivatisation products. These findings are depicted in Table 3, where the percentages for the 5 most abundant compounds can be found. Since this film is relatively young the degree of hydrolysis is still low: on average approximately 30%. The same was found for a 5-year-old pigmented stand oil paint film containing 30% (w/w) of lead white (LWH). Again, the results of two different samples were almost identical. The results obtained on the two paint systems show a large resemblance despite the presence of the lead white pigment (compare Figures 3a,b with Figure 7).

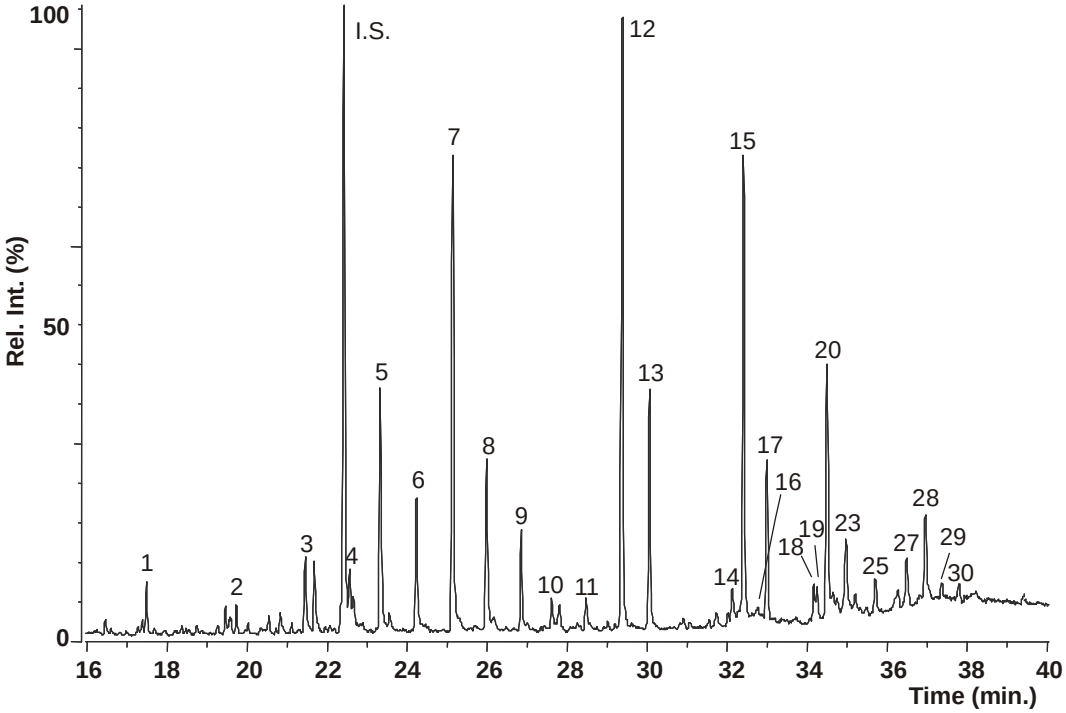


Figure 7. TIC of a 5-year-old stand oil film pigmented with 30% basic lead carbonate, after transethylation and trimethylsilylation derivatisation. The numbers correspond to Table 2

The only observable difference is the relative amount of both the diacids and C18:1 fatty acids. Due to the lead catalysed accelerated oxidation of the double bonds the last compounds have disappeared to a large extent, whereas the relative intensities of the diacids have increased a little. Surprisingly, the lead white pigmented stand oil had a somewhat lower degree of de-esterification for most of the abundant compounds present. It can be concluded from Table 3 that for these young paints the degrees of hydrolysis obtained for the diacids are generally higher than the ones measured for C16 and C18 fatty acids. Within the diacids themselves often a decrease is observed when going from C8 to C10. Sometimes, the values obtained for C10 diacids are higher but it is believed that this is caused by inaccuracy in the integration of the peaks, which are normally very small compared to those of C8

and C9 diacids. Another trend that is observed is the lower degree of hydrolysis found for the C18 fatty acids compared to C16 fatty acids. Both findings suggest there is a relationship between the length of the fatty (di)acid chain and the detected relative amount of the derivatives.

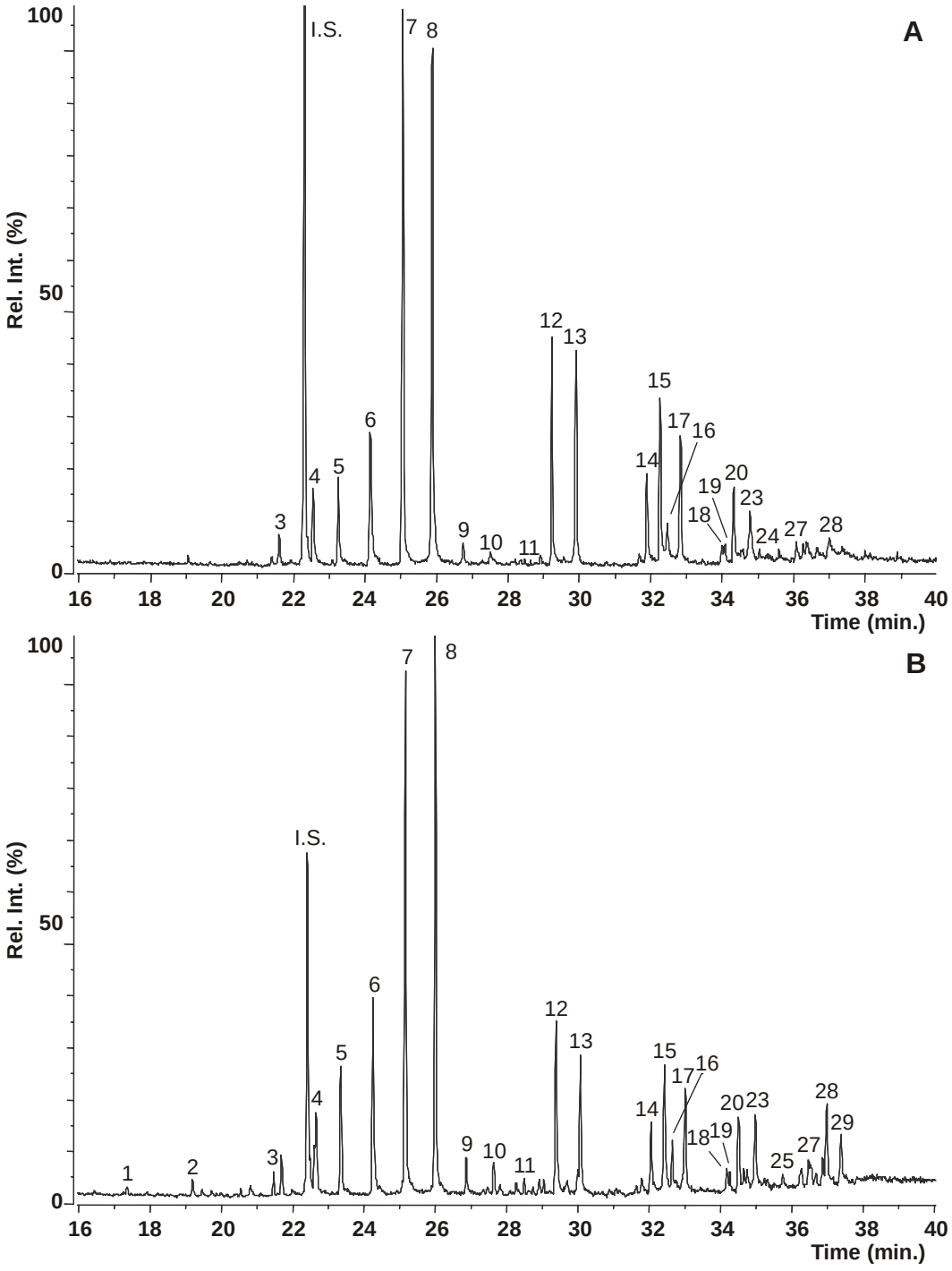


Figure 8a,b. Two TICs of a 27-year-old vine black pigmented oil film (CCI, Ottawa) after transethylation and trimethylsilylation derivatisation. The numbers correspond to Table 2.

In order to obtain a more realistic view on the analytical procedure the range of tested paints was extended. The first set of paints analysed originate from a collection of 27-year-old oil paint reconstructions presently at the Canadian Conservation Institute, Ottawa. These paints not only have a different historical background but also are made up of a three layered system including a primed lime wood board, a ground and a linseed oil paint on top. Paint samples were obtained by careful scraping of the top layer, meanwhile avoiding sampling the ground. The results for these samples were still reasonably reproducible as can be seen in Figures 8a and 8b, since most of the samples were collected as fine powders, which can be considered relatively homogeneous. It should be noted that the spread in two analyses on the same paint has increased somewhat compared to two paints reported before. This is confirmed by the chromatograms in Figures 8a,b for an ivory black pigmented oil paint. The calculated relative degrees of hydrolysis of the most important compounds present in this paint and the other paints investigated are depicted in Table 4. It is found for inorganic pigments containing metal ions that the relative amount of silylated derivatives is higher compared to the more organic pigments like vine black and indigo. This suggests that hydrolysis is promoted by the presence of metal ions, either through direct reaction with the ester bonds and/or indirectly via an increased amount of acidic degradation products. For indigo the percentages of the two fatty acids derivatives indicated a different behavior compared to the tests where pure pigment was mixed in with reference material: now a relatively low degree of hydrolysis was found. Remarkably, the lead white pigmented paint had a lower degree of hydrolysis again, compared to all other paints. Paints pigmented with the slightly acidic colorant alizarin deviated from the other organic pigments and a higher degree of hydrolysis was observed. The trends previously observed in Table 3 are in general also visible in Table 4, although the difference between the degrees obtained for C8 diacids and the long chain fatty acids is much more variable now, ranging from 1.1% for ultramarine to 34% for ivory black.

Table 3. Percentage of the area of trimethylsilylated derivatives relative to the total area of ethylated- and trimethylsilylated derivatives for the 5 most abundant compounds identified in 5-year old reconstructed stand oil paints. St. Oil/LWH = stand oil film pigmented with lead white.

Paint	C8 Di	C9 Di	C10 Di	C16	C18:1	C18
St.Oil /LWH 1	37.82	30.15	25.12	25.87	n.d.[a]	22.76
St.Oil / LWH2	36.77	28.26	29.98	27.56	22.00	24.87
Stand Oil 1	39.22	30.98	30.10	29.00	20.09	26.49
Stand Oil 2	41.34	33.47	36.04	29.56	19.99	28.36
Stand Oil 3	38.05	31.27	27.56	23.76	19.24	38.9

[a] n.d. = not detectable

A second set of paints investigated was made at the Getty Conservation Institute around the same time as the stand oil paints and is with its 5-year-old relatively young. These paints, however, were made with a much higher

concentration of pigments. The samples investigated (see Table 4) included blown linseed oil only and the oil mixed with 3 different pigments. All values found for the most abundant compounds are in the same range for this set, except for the C8 diacids, which are more hydrolysed (± 53%). Again, the lowest degree of de-esterification was recorded for the lead white pigmented paint. Although the percentage of lead white in the oil is comparable to the paint made in our institute (38% vs. 43%), the obtained degrees are somewhat lower (except for the C8 diacids). This is also observed for the unpigmented films. Most probably this can be ascribed to difference in both the type of oil and the storage conditions.

Table 4. Percentage of the area of trimethylsilylated derivatives relative to the total area of ethylated- and trimethylsilylated derivatives of the 5 most abundant fatty (di)acids in paint samples of different age, composition and history. Arlo = Acid refined linseed oil; LWH = lead white; IO = iron oxide; BS = barium sulphate; YO = yellow ochre.

Paint Sample	C8 Di	C9 Di	C10 Di	C16	C18
CCI Cremser White 1973	62.3	51.97	45.91	42.8	38.94
CCI Indigo 1973	64.60	32.96	20.75	47.3	43.12
CCI Ivory Black 1973 -1	72.83	50.35	43.22	49.72	41.03
CCI Ivory Black 1973 - 2	64.81	49.12	46.87	46.37	42.99
CCI Ivory Black 1973 - 3	70.8	49.97	42.02	49.24	42.07
CCI Vermilion 1973	66.51	48.6	38.35	63.95	57.01
CCI Ultramarine 1973	72.47	62.91	37.72	71.36	62.22
CCI Iron Oxide 1973 - 1	62.64	45.12	18.35	66.46	62.22
CCI Iron Oxide 1973 - 2	61.42	49.43	28.85	70.01	70.51
CCI Iron Oxide 1973 - 3	68.47	66.7	56.67	83.35	74.72
CCI Copper Carbonate 1973	63.11	60.16	60.5	79.38	67.41
CCI Alizarin 1973	66.84	72.58	61.04	62.08	70.16
CCI Cadmium Yellow 1973	70.79	65.75	53.62	82.88	77.67
Stolow Arlo/LWH 1965 - 1	33.77	23.64	26.59	29.63	26.59
Stolow Arlo/LWH 1965 - 2	41.56	34.68	35.00	40.19	33.03
Stolow Arlo/LWH 1954 - 1	68.94	68.12	62.35	74.09	70.18
Stolow Arlo/LWH 1954 - 2	69.14	66.94	53.1	71.50	72.61
Stolow Arlo/BS 1965 - 1	64.33	71.12	53.27	83.47	79.18
Stolow Arlo/BS 1965 - 2	63.29	73.23	54.56	75.17	76.5
Stolow Arlo/IO 1965 - 1	60.92	64.03	47.99	81.22	84.76
Stolow Arlo/IO 1965 - 2	58.15	59.37	50.10	82.72	83.30
Getty Blo/LWH 1994	51.8	18.88	14.6	18.04	16.30
Getty Blo/Vine Black 1994	56.17	20.95	n.d.[a]	19.61	17.29
Getty Blo 1994	53.74	25.74	22.9	23.79	20.94
Getty Blo/YO 1994	50.54	29.05	26.55	30.06	25.06

[a] n.d. = not detectable

The second half of Table 4 depicts the results obtained on reconstructions made by Stolow. All paints were analysed in duplo to check the repeatability. As can be seen there are only minor differences between both runs for all paints tested. Except for the acid refined linseed oil paint pigmented with lead white (1965), all samples show high degrees of hydrolysis, with the highest values for the iron oxide and barium sulphate pigmented films. The measured values for the two lead white paints differed significantly, which is to be ascribed to the fact that they differ 11 year in age and most probably have been stored under different conditions, especially in the curing stage.

In both Figure 1 and Table 1 it can be seen that upon transethylation of the esterified reference materials trimethylsilylated derivatives are found. As the tested materials were free of fatty acids (this was checked using direct probe insertion mass spectrometry) the origin is to be found in the analytical procedure. However, since only up to a maximum of 6% of TMS derivatised products are formed this can be taken into account when determining the degree of hydrolysis. Prior to the evaluation of this method it was thought that all fatty (di)acid within an oil (paint) sample would show the same degree of hydrolysis, independent of the length of the carbon chain. However, the results depicted in Figure 1, in combination with the results obtained for the test paints led to another insight. When going from C8 to C10 diacids and from C14 to C18 acids in the analyses, a decrease in the relative amount of TMS derivatives is measured. This can be ascribed to a second reaction, occurring during the transethylation step. It is known from other studies that under basic transesterification conditions the fatty acids have a tendency to become saponified [381]. Therefore a neutralisation step is required to suppress this process. In our case reaction times needed to completely transesterify the paint sample are much longer and neutralisation is only first carried out after 1.5 hours. It's likely that saponification has proceeded in the mean time. Especially for the short chain fatty acids this process was found to be pronounced [384, 385]. In Figure 3a,b, for example, for the C7-C9 diacids (compounds 3-8) it can be seen that the relative degree is decreasing very rapidly towards an end value in the same range as observed for the long chain fatty acids. This saponification effect however is not likely to be completely responsible for the large differences observed for the C7 and C8 diacids versus C9 diacids. Therefore it is suggested that for a fraction of the smaller C7 and C8 diacids both acid groups are formed upon oxidation of the oil paint.

As mentioned before, incomplete derivatisation and even complete failure was occasionally observed for the pure C18 TAGs. Bannon et. al. observed [385] that for a very hard fat like tristearin the reaction times needed for methanolysis are very long and unacceptable results were obtained. It is believed that insolubility or crystallisation of TAGs in the reaction mixture can account for this reduced reactivity, since triacylglycerols of unsaturated fatty acids are transethylated without a problem. C18 TAG has three different melting points, depending on the polymorphic state. It first melts at 55 ºC and than can solidify again. Its last melting point is at a temperature of 72 ºC [386]. For this reason and to increase the solubility of the TAGs in the reagent, a reaction temperature of 75-80 ºC was chosen. However, incomplete derivatisation was still observed occasionally. In

(aged) oil paint systems however, fats like tristearin are not expected to be present [36, 37].

The results obtained for the studies on the effect of lead white and indigo on the analytical procedure and the outcome of the analytical results for test paints are more questionable. The cause of this behavior is not understood and therefore additional studies on the interference of these and other pigments are necessary. It is of great importance that the method is reliable because in relation to the application of this method, the analysis of minuscule oil paint samples that can only be taken once, no errors can be allowed.

The finding that for all lead white pigmented paints relatively low degrees of hydrolysis are found within the sets of paints analysed is counter intuitive and does not agree with the observations for all other paints that are made of inorganic pigments containing metal ions. Furthermore this finding does not fit the facts observed for the lead white interference studies. It was expected that due to the formation of metal salts the degree of hydrolysis would have increased. This phenomenon may be related to the way the paint film dries and needs further investigation. The results of the test paint analyses show that the extent to which hydrolysis has occurred for the C9 diacid and the long chain fatty acids is in reasonable agreement. We therefore choose to take the average of these 3 values as a measure of the degree of hydrolysis of an oil paint film.

The values obtained for the test paints confirm the idea that in time the ester bonds within an oil paint system are not stable and that the degree of hydrolysis increases. The numbers obtained for the Stolow samples compare well with the results obtained by Stolow and Rogers in 1970 [22]. They found that the amount of leachable material was highest for the iron oxide and barium sulphate films, 62% and 52%, respectively. This would imply for these films that the polymerisation rate was low, and / or that de-esterification already had occurred to a large extent. Unfortunately it is not known at which relative humidity these paints were stored, another factor that may have contributed to the hydrolysis.

The somewhat increased spread in results measured for ivory black and iron oxide pigmented paints from the CCI collection can be explained by the way the samples were taken. For the tests paints made at our institute it was possible to sample the paint layer from top till bottom without leaving behind any material, since it was applied on a glass slide. In the case of the multi-layered systems of the CCI collection, it was tried to avoid sampling the ground. Inevitably, this procedure leads to a sampling of the paint with a variable thickness with respect to the ground and therefore some variation in composition can be expected. The samples from the Stolow collection were applied on glass on therefore the whole paint structure could be sampled, giving rise to less variation. The spread observed in the values obtained for inhomogeneous paints can be much larger than the reported 6% deviation and in case of sufficient oil paint material it is advised to do at least a duplicate measurement.

6.4 Conclusions

A two-step derivatisation procedure, consisting of a transethylation and a trimethylsilylation step, can be successfully used for the determination of the degree of hydrolysis of (aged) oil paint systems. Based on the results obtained on test paints, we suggest using the average of the relative percentages of C9 diacid, C16 and C18 fatty acid trimethylsilyl derivatives as a useful number to quantify the de-esterification. The method is shown to be reproducible for the various paints tested. However, it should be kept in mind that oil paint systems are generally inhomogeneous and spread in the outcome can be expected. Furthermore, interference of pigments with the analytical procedure may lead to a deviation in the degree of hydrolysis in some instances.

It has been seen that the degree of hydrolysis increases in time, with highest numbers found for metal ion containing oil paints. Lead white pigmented paints, however, had a relatively low degree of hydrolysis. The reason for this unexpected result is still unclear and needs further study.

Chapter 7

Studies on the composition and formation of bloom on primed canvas used by F. E. Church and on paint in works of art by F. Stella

The formation of patches of whitish, crystalline material observed on two unfinished canvases formerly in the possession of the painter F.E. Church and two modern works of art by Frank Stella is investigated in more detail using different analytical techniques. Two types of materials are observed. Bloom on the surface of the canvases is shown to consist of apolar long chain C16 and C18 saturated fatty acids and their lead soaps, independent of the type of paint and pigment present at the surface. This material is formed in the oil rich top layer pigmented with basic lead white. Formation of concentrated regions of lead soaps, so-called protrusions, is observed within this same layer as well. The composition of the top layer, in combination with the experimental grounds that are supposed to have been used is held responsible for the phenomena observed.

Observations and measurements are presented on a number of panels by F. Stella that show disturbing white patches. A red and blue paint, consisting of alizarin crimson and cobalt blue respectively, are sensitive to bloom formation. The crystalline bloom was identified as free C16 and C18 fatty acids, derived from the oil medium. The exact mechanism of their formation and migration within the paint is not clear. A more detailed study performed on one of the paints revealed a degree of hydrolysis of the paint of 40%. The absence of reactive pigments to trap free fatty acids, in combination with the high ratio of oil to pigment lead to an excess of potentially mobile fatty acids that can migrate to the surface of the oil paint.

7.1 Introduction

The appearance of paintings is sometimes altered by hazy, whitish patches on their surface, which often results in a loss of the aesthetic value of the work of art. Most of the time this surface phenomenon is observable on works of art ranging from the late 19th century and the 20th century, independent of the support.

This period coincidences with the increased incidence of new innovative painting materials, e.g. tube paint, and suggest a relation with modern painting materials and/or techniques.

The origin of the whitish patches has been ascribed to different processes in conservation literature, e.g. blanching by microfissures, deposition of foreign material, growth of micro-organisms (mould), light scattered from subsurface voids in the paint layer or fine particles on the paint surface [387]. The different surface phenomena observable have been reviewed recently by Skaliks [16] and included in a catalogue of pictures to illustrate the possible appearance. It is shown that a whole range of different organic and inorganic compounds could be identified on different works of art. In most of the cases the material found on the surface originates from the painting [16]. Examples that have been shown include the exudation of plasticizers from synthetic resins, the exudation of organic pigments, the flotation of pigments leading to hexagonal cells (Bénard cells) [6, 388], the exudation of wax-like materials [389] and the exudation of fatty acids and/or their soaps [17, 18, 387]. Inorganic compounds identified are mainly different types of sulphates and lead chloride [387, 390].

Two different terms have been used to describe the phenomena in art conservation. Efflorescence, a term used to describe the formation of soaps on walls or rocks in geology or architectural conservation, is one of these and has been used for both inorganic and organic compounds. The second term is bloom, which describes the formation of a white to bluish opaque or semi-opaque film on the surface and is preferentially used for organic compounds. This term also is used when describing the appearance of a white haze on chocolate, the so-called fat bloom [391-394]. The formation of so-called “ghost images”, deposits of (crystalline) organic compounds on the inside of protective glass plates in front of paintings, is related to the blooming phenomenon, as the compounds identified are similar to the, often crystalline, bloom observed on paintings [15-17]. In most cases free saturated fatty acids or their soaps could be identified. These are obviously originating from the binding media of paint or ground layers. However, to date little is known about the actual mechanism that causes the deposition of these, partially because of the large number of factors that seems to be involved. Nevertheless, it is possible to ascertain which factors may cause some paintings to exhibit bloom formation over others.

7.1.1 Theoretical considerations

Apparently in some cases the formation or better, the release of free fatty acids from the initial triacylglycerols by hydrolysis is accelerated in such a way that the paint cannot accommodate or trap these compounds sufficiently, resulting in a migration to the surface. The reason for this migration is still unclear: it may have to do with the contraction of the paint leading to syneresis, the expulsion of a liquid phase by the matrix of the gel-like paint system due to contractile forces, as

has been suggested by Williams [17]. The fact that bloom only occurs after the oil paint film has been dry for an appreciable time, when cross-linking has occurred to a sufficient extent to promote the syneresis or squeezing out of the bloom-forming material, supports this theory. On the other hand, drying oil paints already start to shrink quite early. If contraction is really the only driving force blooming is only expected after the film starts to shrink. Unfortunately, if the increasing polarity of the film would play a role (as well) (see below) it might coincide with the shrinking of the paint. The type of pigment present in the paint may influence the shrinkage process. Some paints will form harder films due to reaction of the carboxylic acid groups (including those of the free saturated fatty acids) with the pigment surface and therefore are less likely to shrink. At the same time, the pigments also will trap free fatty acids.

It is inferred from studies on paintings that show bloom formation that there are several pigments, which seem to facilitate blooming. The pigments reported are the blacks, sienna, ultramarine, (alizarin-)krapplack, cadmium yellow, cinnabar, toluidin red PR3, chrome oxide green, Hansa yellow, and Kassel brown. Pigments that seem to inhibit the formation of bloom are iron oxide, manganese black, umber, cobalt blue, Pb_3O_4 (red lead), lead white, zinc white, and chrome yellow [16]. In general, however, one should be careful when trying to correlate a pigment type with the possibility to form bloom because of the many variables involved. The quality and modification of the pigment can markedly change its properties in the paint film, as can the inorganic extenders and the type of binder additives. Besides, the time-dependent condition of the environment of the work of art is likely to be a major factor as well.

It has been observed for certain paintings that the presence of a barrier (another paint layer for instance) prevents the free FAs from migrating from a lower-lying layer to the surface. A varnish layer can have the same effect [16]. However, numerous examples are known where bloom formation is seen on top of the varnish or on top of a paint layer that itself doesn't show blooming unless painted over a paint that suffers from severe blooming [16, 395]. This is more pronounced when the varnish or paint layer is thin and doesn't occur in the spots where the layer is thicker. The influence of the thickness of the paint is clearly seen on a number of paintings where the bloom is more prevalent on top of the junctions of the threads and warps of the canvas. The paint layer is thinnest in these spots [16]. It also has been tried to prevent new blooming of a cleaned painting by the application of a varnish. However, in time the blooming reappeared at the surface. The painting "Horben" by Carl Schuster (Augustinermuseum Freiburg; Cat. No. 6) [16] even showed the accumulation of bloom underneath the varnish and the migration of material out of the cracking pattern in the varnish, which started to look like some kind of whitish grid.

Another reason why the FAs might migrate out of the paint is the polarity of the paint vs. the deposited compounds at the surface, in other words the incompatibility between the migrating compounds and the remaining materials in

the paint. Fatty acids that are not chemically or physically trapped within the paint might by driven out because of difference in polarity between the polar networks and the apolar saturated FAs, in other words, a “phase” separation occurs. The fact that, as far as known, no diacids have been observed in the bloom supports this theory.

Diacids are much more polar than the long chain saturated FAs because they have a relatively short apolar chain and two carboxylic acid groups instead of only one. They also have a higher melting point so they most probably don’t exist in a liquid state in the paint and hence are less easily been exuded. Their concentration in the paint may be low compared to the saturated FAs, especially in young films. Because of the two carboxylic acid groups, the chance is bigger that these types of molecules react with pigments/metals and are efficiently trapped.

Chocolate is another source of knowledge on blooming. Normally, in the production process the chocolate is tempered, a process which should produce the largest number of the smallest possible crystals of the so-called beta-form. These crystals have the highest melting points of the different crystalline forms that can occur in the chocolate. If this is not done, beta-prime crystals are formed which may later on crystallise to much larger beta crystals, appearing on the surface as a white hazy substance. A good temper should give a maximum contraction of the chocolate to improve the resistance to fat migration from the bulk. In chocolate however, the bloom does not consist of free fatty acids but triacylglycerols.

In the beginning of the introduction it already has been mentioned that additives of synthetic materials sometimes migrate to the surface. The term bloom in polymer science is not used for this phenomenon but it is described with the following three terms: migration, bleed or exudation – the transmission of a material from within a plastic film to its surface or to another neighbouring material. The solubility of additives in polymeric material is determined by the properties of the additive and the interactions with the polymer. Normally when a system reaches saturation the material will start to crystallise. A semi-crystalline polymer at temperatures below the melting point is extremely viscous and it may not be possible for the additive to form a precipitate phase, either because diffusion to nuclei is too slow or because the growth of a crystal in the polymer matrix requires deformation of the polymer, which is costly in terms of free-energy. In case the crystallisation cannot occur the additive will be present as a supersaturated system and migration to the surface may be energetically more favourable. If the additive is in a concentration below saturation and the polymer is exposed to air, then the only means of loss at the surface is evaporation. This will cause depletion of the concentration at the surface and sets up a concentration gradient. Further loss only can occur by diffusion along this gradient to replenish the surface from the bulk. Conversely, if the polymer is supersaturated it may be lost from the surface by blooming. Provided that there is no restriction on the nucleation of crystal formation on the polymer surface, blooming of the additive effectively fixes its concentration immediately at the surface and keeps it equal to the saturation

solubility of the additive in the polymer. The growth rate of the bloom will then be diffusion controlled. If the concentration of the material crystallising becomes lower than the saturated concentration the crystallisation will stop and evaporative processes will take over. A similar mechanism also may apply for the formation of bloom and ghost images. It has been tried to reduce the loss of additives by reaction with a long alkyl chain to reduce volatility. The effect is a good way to reduce loss by volatilisation but is much less effective as a protection against blooming. A last remark concerns the loss mechanisms of the additives in general: they are highly sensitive to the geometry of the sample; loss is much more rapid from thin films than from thick films. As said above, this has also been seen for paints; the pattern of the canvas is often seen in the bloom. The crossings of fibres are clearly marked by the bloom on top. The paint film is thinner in these cases.

The situation just described for the polymers seems a valid reason for the crystals to appear at the paint surface. The actual mechanism of crystallisation is another process that has to be considered. Normally, before crystallisation it is necessary that a nucleation point is present (besides a saturated mixture). A lot of material can act as a nucleus in the oil paint as well as on the paint surface. Let's suppose that there are nuclei for the crystallisation to start with. Then the question remains why the crystals grow so big in certain cases. This phenomenon can be explained by so-called Oswald ripening [396]. This is a spontaneous process because larger crystals are more energetically favoured than smaller crystals. At first it is easier to nucleate small crystals. However, large crystals have a greater volume to surface ratio, which means a lower energy state. Thus, many small crystals will attain a lower energy state, if transformed into large crystals. The nucleation of small crystals reduces the degree of supersaturation and that may be a reason why the larger crystals (sometimes) don't have a chance to form.

7.1.2 Case studies

In this chapter two cases will be studied where the formation of a whitish haze, consisting of different types of crystalline material, was observed on the surface of the object. The first case involves two unfinished canvases of the American painter Frederic Edwin Church that show a number of typical defects, including bloom formation. The second case concerns a number of paints found on different panels of two works of art by the American painter Frank Stella. The first question that has to be answered in these cases is the composition of the whitish material that is found on the surface. Once this has been established, a more detailed study on the chemical state of the object itself is needed to be able to draw up an inventory of the possible causes and mechanisms of the bloom formation. The model of the oil paint system as has been presented in Chapter 2 plays a central role in this analysis. Important questions that should be dealt with are the type of paint and pigment, the amount of mobile vs. stationary components and the degree of network formation. A range of analytical techniques has been used to

address these matters in more detail, including DTMS, Curie-point pyrolysis-TMAH-GC/MS, GC/MS with on-column injection after transethylation/trimethylsilylation derivatisation, FTIR, secondary ion mass spectrometry (SIMS), both on the surface and cross-sections, and scanning electron microscopy/ electron dispersive X-ray spectrometry (SEM/EDX). The information that is obtained subsequently is related to the theories that have been put forward in literature.

7.1.2.1 Case I

This study was performed on a white haze observed on two pieces of rolled and primed canvas found in the Olana archive upstate New York and originally belonging to the American painter Frederic Edwin Church (1826-1900) a representative of the Hudson River School of painters [397]. This group of artists is known for their light and translucent media that capture the evanescent colours of dawn and sunset, the dramatic clear light of midday and the warm haze of Indian summer afternoons [397]. The colour and properties of the ground were critically important in their work. The materials of the ground and the colour of the ground both have a significant impact on the stability and tonality of the works of art. During this period, artists' colourmen were experimenting with various recipes to economically produce primed canvases that could be stored and rolled. As a consequence of these experiments some problems were encountered with the grounds in 19th-century American paintings and those of the Hudson River School in particular. In the Olana archive an unused, rolled piece of primed linen with a cream colour is available for study. A sample of this material (OL.NA), which shows bloom formation, was investigated in detail. A second sample of an unfinished painting on canvas, 1984.122 (circa 1865), showing the same phenomenon but partially covered with an additional thin dark brown imprimatura on top was also available for studies. Besides the problem of bloom formation two other defects are observed on these canvases: ground staining, and the formation of so-called protrusions. Protrusions appear as a whitish globule within the prime layer that sometimes rises above the surface. Ground staining, the appearance of dark coloured patches, must have taken place reasonably fast as the phenomenon already was observed at the time of Church. Church related the ground staining to the canvas he had bought from Winsor & Newton. Church identified the cause of ground staining to be the incorporation of lead acetate in the ground layer [397]. Indeed, a strong colour effect has been found by Carlyle in lead white pigmented oil paints made with traditionally processed oils which contain lead acetate as drier [315]. Dwyer suggested another possible cause and proposed the darkening as a chemical alteration of lead-white. The upper particles of this pigment, which is normally opaque, had turned into unidentified transparent crystals. The mechanism of ground staining will not be further discussed in this chapter. The second phenomenon, the formation of voluminous whitish globules within the paint, that (sometimes) disrupt the paint surface, is thought to relate to the bloom formation

and will be addressed to some extent when investigating the relation between the composition of the bloom and the underlying paint it was found on.

7.1.2.2 Case II

The second study was performed on a number of samples taken from two modern works of art made by the American painter Frank Stella (1936-) and presently in the collection of the Art Institute of Chicago. Both works are from Stella's "Pillars and Cones" period, which spanned from 1982 into the mid-to-late 1980's [398]. The first work entitled "Gobba, Zoppa e Collotorto" (1986.93) was made in 1985, whereas the second work, "Cricche, Crocche e Manico d'Unico" (1992.285), was executed in 1986 [398]. The works are made of several smaller panels mounted on two larger back panels, which altogether form a three-dimensional structure. Crystalline growths were discovered on four panels: #4, #5, and #6 from "Cricche" and #7 from "Gobba". They were present as a white disfiguring film on the dark red and dark blue paints. The other paints were not affected unless they were painted over the red or blue. The crystals were abundantly present where the paint was more thinly painted, and were hardly visible in areas of high impasto. On panel #6 remnants of masking tape were seen, which were painted over with brushstrokes of red paint, which shows no crystals and has a lighter colour, compared to the same brushstrokes outside the masking tape area. The crystals were less apparent on panel #5 from "Cricche" when the dark blue paint lay directly over areas that were very thinly painted with a pink polyurethane paint. In addition to the bloom a number of worm-like patterns could be observed distributed over the surface of the magnesium support of "Cricche" panel #6 and on metal supports of several other panels from both works. These were identified as filiform corrosion. This indicates that these paints were very likely exposed to relative high humidity levels [395].

7.2 Materials and methods

7.2.1 Chemicals

Tetramethylammonium hydroxide pentahydrate (minimum 97%), sodium ethoxide 21 wt% solution in denatured ethanol (approximately 99%) were obtained from Sigma-Aldrich Chemie Bv., Zwijndrecht, The Netherlands. Bis-(trimethylsilyl)trifluoroacetamide (BSTFA), containing 1% trimethylchlorosilane, was purchased from Fluka, Zwijndrecht, The Netherlands.

7.2.2 Samples

7.2.2.1 Church

The two canvas samples OL.NA and OL.1984.122 were supplied for analysis by J. Zucker, Painting laboratory, Peebles Island Resource Center, New York State Office of Parks, Recreation, and Historic Preservation (NY, USA). Previous investigations of sample OL.NA with polarising microscopy have revealed that the linen is coated with a layer of calcium carbonate with a very thin cream-coloured layer of lead white on top [397]. Sample OL.1984.122 was constructed in the same manner and was partially covered with an additional darker, brownish layer of imprimatura. The different layers of interest were removed carefully from the piece of canvas using a microscope and a fine scalpel, resulting in three different samples. Two samples of a combination of ground and lead white-pigmented paint and a sample of the brown imprimatura layer present on top of OL.1984 122 were collected. This last separation was extremely difficult due to the thinness of the brown layer and pieces of the underlying layer still attached were present as well. Closer inspection of the surface of both samples showed ground staining, bloom and a number of whitish protrusions. On the imprimatura a thin white-grey surface coating is visible on top. In case of the cream-coloured layer the bloom and protrusions are hardly visible with the naked eye but under the microscope the phenomena can be clearly identified. The whitish material has a waxy appearance and seems not soluble in water, (m)ethanol or hexane. It can be displaced easily with a light mechanical force. Lead was confirmed as the primary metallic component in the bloom material using an analytical electron microscope interfaced with an energy dispersive X-ray detector [397].

7.2.2.2 Stella

Four types of blue paint (two from panel #4, one from panel #6 and #5 each), two red paints, one including crystals (panel #6), taken from “Cricche, Croache e Manico d’Unico” (1992.285) and a red paint with crystalline growth on top of it (panel #7) from “Gobba, Zoppa e Collotorto” (1986.93) were supplied by B. Rimer and I. Fiedler from the Art Institute of Chicago. The materials that Stella likely has used could be established through discussions with Frank Stella’s studio, via his private conservator. It also has been determined how these paint materials may have been treated or altered to enhance their working properties. The top skins of the panels, made of aluminium, magnesium, or fibreglass, were coated with clear polyurethane. Some of the edges of each panel were sealed with Bondo® polyester resin mixed with talcum. The paints on the surfaces were oil paints made by Blockx, a two-part polyurethane marine paint called Awlgrip® and a third paint made by Stella, consisting of dry pigments in Acryloid B-72® resin. Stella’s studio assistant and his conservator identified the paints that exhibited crystals as most likely to be the oil paint obtained from Blockx. They further informed that Blockx’

oil paints, although of high quality and preferred for their strong bright tints, tended to stay tacky for a long time. For this reason, the studio often added cobalt driers to these paints, sometimes in large amounts, to speed up the drying process [395, 398]. The crystalline growth appears under the microscope as finely branched networks and occasionally as small needle-like crystals. They seem to rest on top of the surface and do not rupture the paint surface. The waxy crystals could be easily detached from the surface by a slight mechanical force using a scalpel blade or brush. The paint samples were stored between welled glass-slides. After a few months it was observed that a halo of fine crystals had formed around the paint samples, indicating that the migration of material was continuing.

A number of the paint samples were previously analysed at the Canadian Conservation Institute (CCI), Ottawa, using FTIR, X-ray diffraction (XRD), and SEM/electron dispersive X-ray spectrometry (SEM/EDX) and at MVA, Inc., Glenview, Illinois, using infrared microscopy (IM) [395]. The paints, which exhibited crystal growths, were identified as cobalt blue and alizarin crimson oil paints. The crystals were identified to be fatty acids, especially palmitic (C16) and stearic (C18) acids. The results are depicted in Table 1.

Table 1. Summary of analytical data on paint Stella panels

Sample	FTIR/IM[a]	XRD	SEM/EDX[b]
Dark blue paint panel 5	Cobalt blue Drying oil	Cobalt blue ($CoAl_2O_4$)	Co, Al (Si, S, Fe)
Crystals on dark blue paint panel 5	Fatty acids – C16 fatty acids, possibly a small amount C18	N/A[c]	N/A
Pale blue paint panel 5	Polymer with urethane	Titanium white (TiO_2, rutile)	**Ti**, Al, Si (Cl, Fe)
Yellow paint panel 6	Barium sulphate Drying oil Soaps (possibly Al drier)	Cadmium yellow (CdS), barium sulphate ($BaSO_4$)	Cd, S, Ba (Zn, Al, Se)
Red paint panel 6	Alizarin crimson Drying Oil	N/A	N/A
Crystals on red paint panel 6	Fatty acids	N/A	N/A

[a] The red paint and the crystals were analysed using IM, all other samples were analysed using FTIR

[b] **major elements**, minor, elements, (trace elements)

[c] N/A = not available

7.2.3 TOF-SIMS

Time of Flight- Secondary Ion Mass Spectrometry (TOF-SIMS) measurements were performed on a TRIFT II instrument of PHI Electronics Inc. (USA) [399]. The surface of the sample was scanned with a 25keV primary ion beam from an Indium liquid metal ion gun, which has an input beam diameter of about 100 nm. The triple ESA ion focussing system guarantees a spatial resolution of at least 1 micron at a mass resolution of about 5000. Positive or negative ions emitted by the primary beam were mass analysed and detected on a position sensitive detector. The surface of the sample was charge compensated with electrons pulsed in between the primary ion beam pulses.

7.2.4 DTMS

The crystals or protrusions were carefully removed from the paint surface using a scalpel blade and suspended in a drop of methanol, which had been applied onto the analytical filament beforehand. Paint samples of typically 20 to 60 µg were homogenised in methanol using a mini-glass mortar. Aliquots of the obtained suspension or extracted materials were applied onto the analytical filament and dried *in vacuo*. The analyses were performed on a Jeol SX-102 double focussing mass spectrometer (B/E) using a direct insertion probe equipped with a Pt/Rh (9/1) filament (100-micron diameter). The probe filament was temperature programmed at a rate of 0.5 A/min to an end temperature of about 800 °C. Compounds were ionised at 16 eV under electron ionisation conditions in an ionisation chamber kept at 180 °C, mass analysed over the range *m/z* 20-1000, with 1 second cycle time. Data were processed using a JEOL MP-7000 data system.

7.2.5 Transethylation/Trimethylsilylation

For every mg of sample in a GC vial, 250 µl of a 0.01 M ethanolic sodium ethoxide solution was added. Typical amounts of material analysed are in the order of 250 µg to 2 mg. The vial was flushed with dry nitrogen, sealed and placed in an oven at 75-80 ˚C. After 90 minutes the vial was taken out of the oven and allowed to cool to room temperature (RT). To neutralise the sample, 30 µl of a saturated ethanolic ammoniumchloride solution is added. After 20 minutes the ethanol is evaporated under a gentle stream of dry nitrogen and the residue is dissolved in

hexane (250 µl per mg of sample). The resultant suspension is treated with 15 µl bis(trimethylsilyl)trifluoroacetamide (BSTFA), sealed and returned to the oven for a further 30 minutes. After cooling down (RT), and evaporation of the solvents the analytes are redissolved in dichloromethane, containing hexadecane as an internal standard (100 mg/l). The mixture is subsequently centrifuged and the upper layer is carefully separated from pigment particles and remains of the paint film and subsequently transferred into a new GC vial.

With the aid of an AS 800 on-column autoinjector (Fisons Instruments) 1 µl of derivatised sample was directly injected into a SGE BPX5 column (25 m, 0.32 mm i.d., 0.25 µm film thickness), mounted in a Carlo-Erba gas chromatograph (series 8565 HRGC MEGA 2). The gas chromatograph was directly coupled to the ion source of a JEOL DX-303 double focusing (E/B) mass spectrometer via a home built interface, which was kept at 180 °C. Helium was used as carrier gas at a flow rate of approximately 2 ml/min as regulated with a CP-CF 818 pressure/flow control box (Fisons Instruments). The initial temperature of the gas chromatograph was 50 °C, which was maintained for 2 minutes. The oven temperature was programmed at a ramp of 6 °C to an end temperature of 320 °C (50(2)-6-320). Ions were generated by electron impact ionisation (70 eV) in the ionisation chamber (180 °C), accelerated to 3 keV, mass separated and post-accelerated to 10 keV before detection. The mass spectrometer was scanned from m/z 40-700 with a cycle time of 1 s. A Jeol MP-7000 data system was used for data acquisition and processing.

7.3 Results Case I

7.3.1 Cream-coloured grounds of Frederic Edwin Church

7.3.1.1 SIMS analysis of the surface and in cross-section

The analytical technique that lends itself well for the investigation of both inorganic and organic components in/on solid surfaces is SIMS [400]. SIMS makes it possible to investigate the presence of a number of typical compounds found in oil paintings, for example, the (in)organic materials used for priming and the components of the oil paint: inorganic and organic pigments, triacylglycerols and their hydrolysis products, including free fatty acids, and metal soaps as the reaction products of (basic) inorganic pigments and free FAs. In this study, the technique is only used as a qualitative technique. The actual response of the different materials upon SIMS analysis is not known yet and it is therefore not possible to quantify the different compounds.

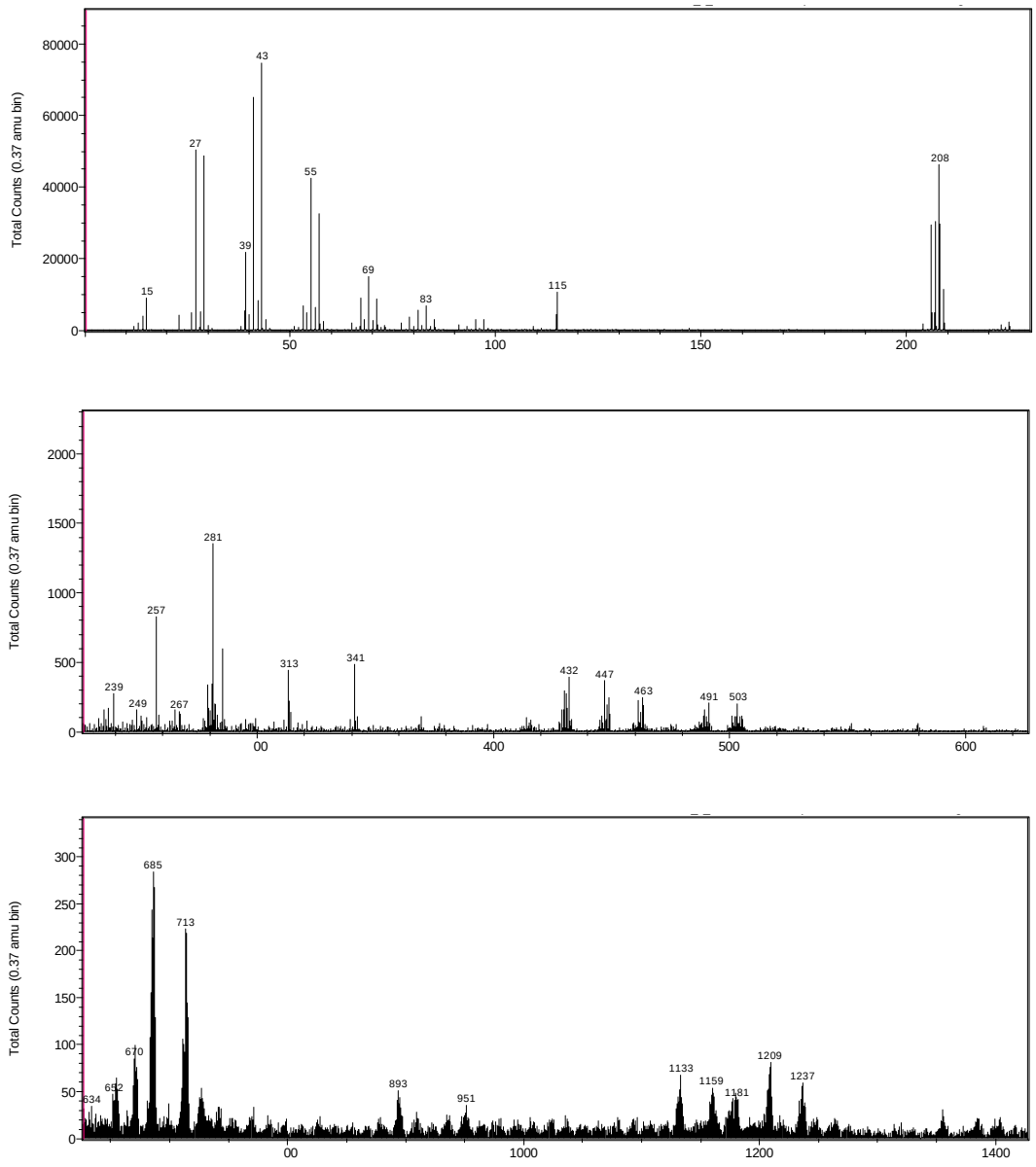


Figure 1. SIMS spectrum of the surface of sample OL.1984.122 (a) mass range m/z 0-230; (b) mass range m/z 230-62, and (c) mass range m/z 625-1425.

The cream-coloured surfaces of both primed canvas samples including the bloom on top were examined using positive and negative ionisation SIMS. The SIMS spectrum of sample OL.1984 122, recorded in the positive mode, is depicted in Figure 1a-c, and was shown to be similar to the spectrum obtained for OL.NA. Ions of high intensity are observed indicative of lead (m/z 208), and clusters of lead oxides (Pb_2O, Pb_3O_2, and Pb_4O_3) and lead hydroxides (PbOH, Pb_2O_2H, Pb_3O_3H, and Pb_4O_4H) (Fig. 1; Table 2). These lead (hydr)oxides most likely originate from lead white due to thermal processes induced by the primary ion beam. A similar reduction of lead also has been observed when irradiating lead

white with a Nd:YaG laser [401]. Fragment ions indicative for both palmitic and stearic acid soaps (C16 and C18 FA-Pb^+, respectively) are observed in Fig. 1b at m/z 463 and 491, with a typical monolead isotopic distribution. The basic forms of these last two compounds, C16/C18 FA-$PbOPb^+$, are also observed 224 mass units higher, at m/z 685 and 713, respectively. Apart from these ions, ions are observed at m/z 257 and 285, which are ascribed to hydrogen cationised $(M+H)^+$ C16 and C18 fatty acids. These ions can be derived from free FAs or esterified FAs. SIMS studies on pure compounds (FAs and TAGs) have shown that in case of the latter compounds a more intense acylium $[FA-H_2O]^+$ is present as well [402]. It should be noted that formation of protonated free FAs is not a very efficient process under SIMS conditions. The presence of such ions, together with a minor presence of acylium ions, therefore is good marker for free FAs. The ions at m/z 313, 341, 369 and 397 are inferred to be derived from acylglycerols of C16, C18, C20 and C22 fatty acids, respectively ($[RCO+74]^+$; see also Chapter 3). The latter two ions are only observable in trace amounts. In theory, it cannot be excluded that these ions are partially derived from hydrogen cationised C20 to C26 fatty acids, although their relative concentration in the paint is very low (see GCMS data below). The intensity pattern of these ions is indicative for their origin, especially because ions indicative for diacylglycerols with two C16, a C16 and C18, and two C18 FAs (m/z 551, 579, and 607, respectively) are also present in the SIMS data. Both sets of ions are evidence for the presence of low amounts of glycerol-esterified fatty acids on the surface. Above mass 1000 high mass ions of unknown identity are present in the spectrum. These ions form PbO clusters as well, as can be deduced from the mass increment of 224 for most of these ions. It should be noted that it is more difficult to obtain exact mass data for the ions in this mass range due to the counting statistics, which are very poor due to the low ion intensity.

Table 2. Positive and negative ions observed in the TOF-SIMS spectra of samples OL.NA and OL.1984 122

m/z	Intensity	Assignment (positive mode)
115	Medium	In^+
208	Strong	Pb^+
225	Medium	$PbO{\cdot}H^+$
239	Weak	C16 acylium$^+$ ($C15CO^+$)
257	Medium	C16 $FA{\cdot}H^+$
267	Weak	C18 acylium$^+$ ($C17CO^+$)
281	Medium	Unknown
285	Medium	C18 $FA{\cdot}H^+$
313	Medium	$C16CO+74^+$ / $C20{\cdot}H^+$
341	Medium	$C18CO+74^+$ / $C22{\cdot}H^+$
369	Weak	$C20CO+74^+$ / $C24{\cdot}H^+$
397	Weak	$C22CO+74^+$ / $C26{\cdot}H^+$
416	Weak	Pb_2^+
430	Medium	$PbOPb^+$

447	Medium	$PbOPbO \cdot H^+$
463	Medium	$C16\text{-}Pb^+$
491	Weak	$C18\text{-}Pb^+$
551	Weak	$C16\text{-}C16\ DAG^+$
579	Weak	$C16\text{-}C18\ DAG^+$
607	Weak	$C18\text{-}C18\ DAG^+$
654	Weak	$PbOPbOPb^+$
671	Weak	$PbOPbOPbO \cdot H^+$
687	Medium	$C16\text{-}PbOPb^+$
713	Weak	$C18\text{-}PbOPb^+$
878	Weak	$PbOPbOPbOPb^+$
894	Weak	$PbOPbOPbOPbO^+$
1132	Very weak	$PbOPbOPbOPbOPb^+$
1160	Very weak	$PbOPbOPbOPbOPbO^+$
m/z	Intensity	Assignment (negative mode)
208	Weak	Pb^-
224	Weak	PbO^-
255	Strong	$C16\text{-}H^-$
283	Strong	$C18\text{-}H^-$

Cross-sections made of the paints of OL.NA and of OL.1984 122 were subjected to imaging SIMS to map the distribution of the different materials in relation to the bloom formation [403]. At the same time a better insight into the layer build-up of the samples was acquired. Figure 2 compares the positive ion maps of different classes of compounds with the positive ion image of the total ion current (TIC^+) of OL.NA (Fig 2a). The paint sample is lying upside down, with the paint surface downwards. Calcium, which is the marker for chalk ($CaCO_3$) used in the ground, is confined to the lower part of the cross-section (Fig. 2b; highest concentrations are shown in white, decreasing *via* grey to black). Lead (Fig. 2c), on the other hand, shows highest concentrations in the top layer, but is also present in the ground. Fig. 2d-g shows that ions indicative of the different classes of materials identified in the surface layer are present in localised areas in the cross-section. Lead soaps (m/z 489-491; Fig. 2d) and lead (hydr)oxides (m/z 430 en m/z 447; Fig. 2e,f) are present in high concentrations in the top layer of the cream-coloured paint, whereas ions derived from ester bound FAs (m/z 267; Fig. 2g and m/z 341; Fig. 2h) are mainly found in the calcium rich ground. Similar distributions were found in both cross sections.

The OL.1984 122 sample was investigated in more detail using microscopy and SEM-EDX in our laboratory. The ground layer that looked like a single layer in the earlier microscopic examination [397] was shown to be a multi-layered system that consisted of four superimposed layers. The imprimatura layer is placed on top of these layers. EDX analysis shows that the lower layer consists mainly of chalk. The two subsequent layers contain a mixture of lead and calcium, whereas only lead is observed in the upper layer. This layer build-up is confirmed by the SIMS data.

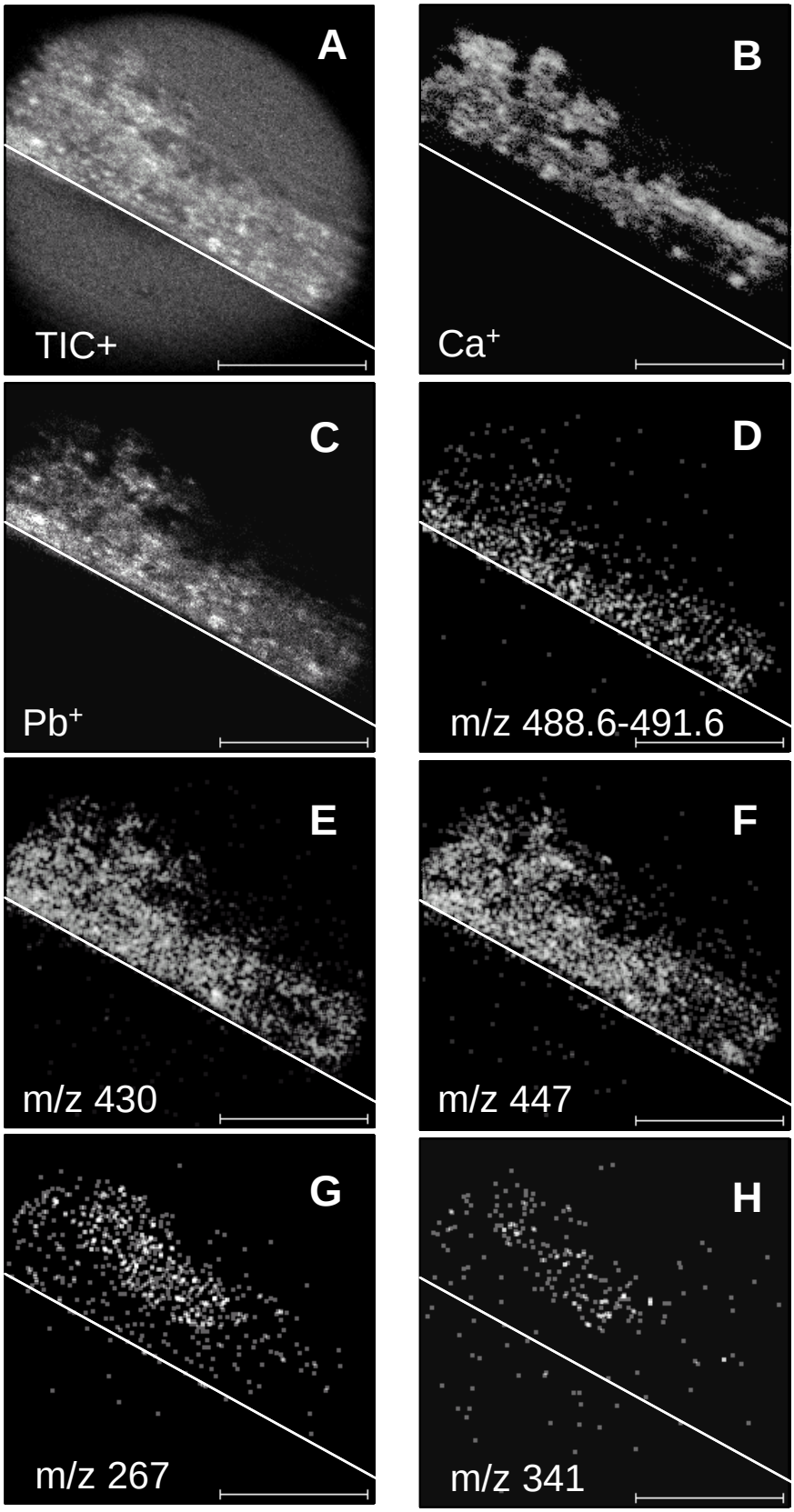


Figure 2. Positive ion maps of the cross-section of OL.NA: (a) TIC; (b) calcium; (c) lead; (d) lead soaps; (e) lead oxides; (f) lead hydroxides; (g) C18 FA acylium ions; and (h) C18 MAG fragment ions

The surface of the reverse of the canvas OL.1984 122 was analysed as well to investigate the possible migration of oil derived compounds to the back. Hardly any lead oxide or lead hydroxide clusters are detected, which was expected. Ions of high intensity are observed indicative for the presence of C16 and C18 substituted mono- and diacylglycerols (m/z 313, 341, and 551, 579, 607, respectively) (results not shown).

The results of the surface analysis obtained with negative ionisation (not shown) are less informative. High intensity ions indicative for palmitic and stearic FAs, $[FA-H]^-$, are seen at m/z 255 and 283 for the two cream-coloured samples. Both esterified and free FAs are known to produce these ions when analysed with SIMS [400]. Whether lead soaps will give $[M-H]^-$ ions upon SIMS analysis is not clear yet. In addition low intensity peaks are observed 14 mass units higher at m/z 269 and 297, respectively. It is not clear how these ions are formed. Low intensity ions at m/z 208 and 224 are observed, which can be ascribed to Pb^- and PbO^- [404]. In the high mass range low intensity peaks are observed at m/z 974, 1002, and 1030 in a typical ratio which suggest they are derived from saturated triacylglycerols. At least two peaks are observable that are two and four mass units lower, which suggest that unsaturated analogues are present as well. A similar cluster is observed 256 amu lower at m/z 718, 746, and 774. Their composition is presently not known. An ion at m/z 315 (unknown identity) is present with a reasonable intensity. The intensity of light ions (m/z 13, 16, 17, 25) is very high. Throughout the whole mass range weak clusters of ions are observed consisting of three peaks differing two mass units each. These clusters differ 14 mass units and are indicative for pyrolytic processes occurring during the analysis. A series of unknown ions of decreasing intensity are observed starting at m/z 521, with an increment of 14 mass units towards m/z 603. It is clear from these findings that the information obtained in the negative ionisation mode doesn't contribute significantly to the identification of the material on the surface.

7.3.1.2 Degree of hydrolysis of the paint

A two-step chemical chemical work-up [405] was applied on the cream-coloured paint samples with the objective to determine the degree of hydrolysis of the glycerol ester bound fatty acids. With this method, all esterified FAs, including those from the triacylglycerols and waxes, are transethylated in the first step. In a second reaction all free and metal-bound FAs are trimethylsilylated, together with hydroxy groups present. In this way information can be obtained on the degree of hydrolysis of the oil paint present. Figure 3 depicts the resulting gas chromatogram (GC/MS TIC trace) obtained for the cream-coloured layer sampled from OL.NA. The analysis of the paint from OL.1984 122 gave a similar result. The identified compounds are given in Table 3.

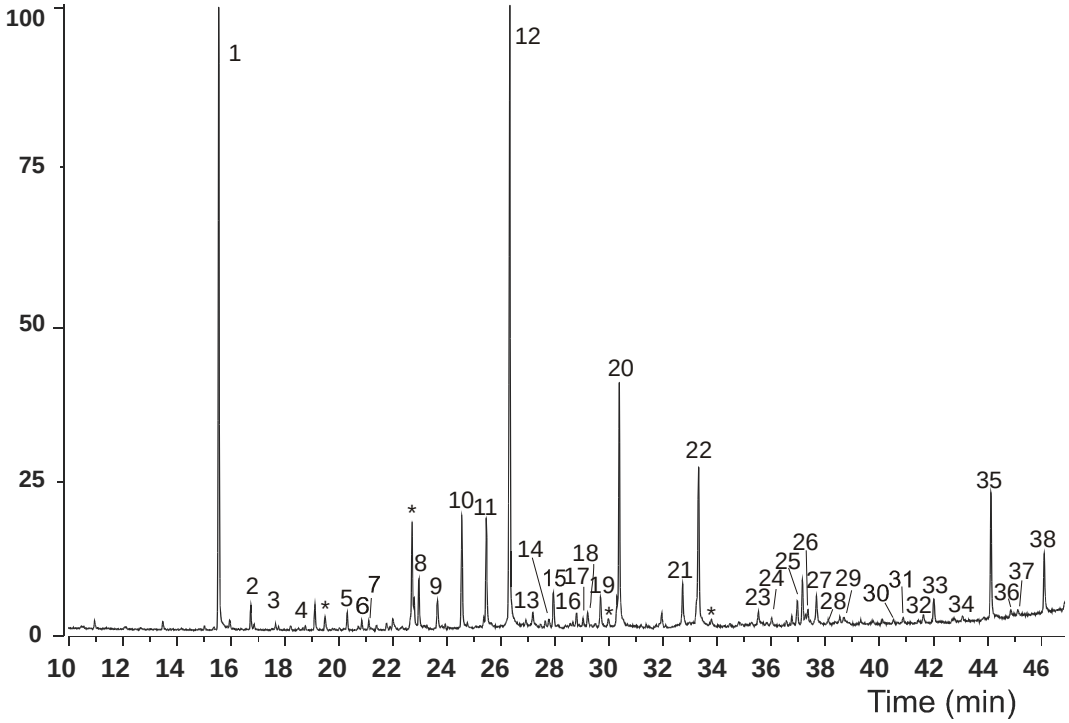


Figure 3. Total ion chromatogram of cream-coloured paint of OL.NA after transethylation and trimethyl silylation derivatisation in combination with GC/MS using an on-column injector. Temperature program: 50(2)-6-320. Peak numbers correspond to Table 3. Peaks labelled * are identified as polysiloxanes.

Table 3. List of compounds identified in the cream-coloured layer of sample OL.NA after transethylation/trimethylsilylation derivatisation and GC/MS analysis using on-column injection. Identification is based on the 70 eV mass spectrum.

Label	Retention Time (min)	Compound name	Molecular Weight
40.	15.5	1,2,3-Tris(trimethylsilyloxy)propane	308
41.	16.7	Butanedioic acid, di-TMS ester	262
42.	17.6	Hexanoic acid, 2-TMS ether, TMS ester	280
43.	18.7	Pentanedioic acid, di-TMS ester	276
44.	20.3	Butanedioic acid, 2-TMS ether, di-TMS ester	350
45.	20.8	Hexanedioic acid, di-TMS ester	290
46.	21.1	Heptanoic acid, 7-TMS ether, TMS ester	290
47.	22.8	Heptanedioic acid, di-TMS ester	304
48.	23.6	Octanedioic acid, ethyl TMS ester	274
49.	24.6	Octanedioic acid, di-TMS ester	318
50.	25.5	Nonanedioic acid, ethyl TMS ester	288

51.	26.3	Nonanedioic acid, di-TMS ester	332
52.	27.2	Decanedioic acid, ethyl TMS ester	302
53.	27.6	Octanedioic acid, 2-TMS ether, di-TMS ester	406
54.	27.9	Decanedioic acid, di-TMS ester	346
55.	28.8	Nonanedioic acid, 2-TMS ether, di-TMS ester	420
56.	29.0	Nonanedioic acid, 3-TMS ether, di-TMS ester	420
57.	29.2	Nonanedioic acid, 4-TMS ether, di-TMS ester Nonanedioic acid, 5-TMS ether, di-TMS ester	420
58.	29.6	Hexadecanoic acid, ethyl ester	284
59.	30.4	Hexadecanoic acid, TMS ester	328
60.	32.7	Octadecanoic acid, ethyl ester	312
61.	33.3	Octadecanoic acid, TMS ester	356
62.	35.5	Icosanoic acid, ethyl ester	340
63.	36.0	Icosanoic acid, TMS ester	384
64.	36.7-37.0	Mixture of unidentified C18 oxidation products	488/532
65.	37.3	Octadecanoic acid, 9,10-bis[(TMS)oxy], ethyl ester	488
66.	37.6	Octadecanoic acid, 9,10-bis[(TMS)oxy], TMS ester	532
67.	38.1	Docosanoic acid, ethyl ester	368
68.	38.5	Docosanoic acid, TMS ester	412
69.	40.4	Tetraicosanoic acid, Ethyl ester	396
70.	40.9	Tetraicosanoic acid, TMS ester	426
71.	41.6	Nonaicosane	408
72.	42.0	Heptaicosanol	454
73.	43.1	Hexaicosanoic acid, TMS ester	468
74.	44.1	Nonaicosanol	468
75.	44.9	Octaicosanoic acid, ethyl ester	452
76.	45.1	Octaicosanoic acid, TMS ester	496
77.	46.1	Untriacontanol	510
78.	46.9	Triacontanoic acid, ethyl ester	480

The most intense peak is derived from glycerol (compound 1) that has been liberated from the oil network upon hydrolysis or transethylation. Besides palmitic- and stearic acid in a ratio typical for linseed oils (P/S=1.48), breakdown products formed upon oxidation of unsaturated C18 FAs are observed (see Table 2). The most prominent one being azelaic acid, a C9 dicarboxylic acid, indicative of a relatively high degree of oxidation. Relatively low amounts of C8 and C10 dicarboxylic acids are present as well. The ratio of C8, C9, and C10 diacids is general considered to be an indication that the oil has not been heat-treated prior to its usage. Ethylated and trimethylsilylated derivatives are present for most of the compounds (see in the chromatogram and Table 2). The trimethylsilylated compounds predominate. This indicates that most of the ester bonds were hydrolysed prior to analysis, as can be expected for an “oil paint” sample of at least

100 years old. The average degree of hydrolysis of the samples analysed was found to be around 88%.

Apart from the compounds already mentioned, short chain diacids down to C4 were observed. A range of oxidised C18 FAs, hydroxy fatty acids and diacids, present in low abundance, could also be identified. These are typical compounds formed upon ageing of oil paint. At higher retention times traces of a series of even-carbon numbered long chain FAs (C20-30) and odd-numbered alcohols are detected. The TMS derivatives of C29 and C31 alcohols are the most abundant homologs in this series. These compounds arise from wax esters and are either present as ethylated or TMS derivatives (in case of the esters) or TMS derivative only (alcohols). It is concluded from the data that the waxes were also hydrolysed due to aging but less severe compared to the paint because their ethyl derivatives predominate over TMS derivatives.

7.3.1.3 Characterisation of free and bound fractions by DTMS

The DTMS data of the cream-coloured layer of the primed canvases OL.NA and OL.1984 122 shows that both samples have a very similar thermal desorption and pyrolysis profile with multiple peaks. A typical example is depicted in the insert of Figure 4a for OL.NA. This is an unusual desorption profile for an oil paint because many compound classes are present as relatively volatile matter. Free palmitic- and stearic acid (C16 and C18 FAs, respectively) are present and desorb at a relatively low temperature (scans 10-30, results not shown), together with lower amounts of C20 and C22 FAs. Peaks at m/z 256, 284, 312, and 340 are indicative for these compounds. In addition two peaks of relatively high intensity with an unknown origin are seen at m/z 243 $[M-15]^+$ and 258 $[M]^{+\cdot}$ in the MS data of the cream-coloured layer of OL.1984 122. Compared to the low temperature domain relatively higher amounts of masses indicative for long-chain C20, and C22 fatty acids are seen at scans 30-45 (Fig. 4a). New ions ascribed to long chain FAs are seen at mass m/z 368 (C24), 382 (C25), and 396 (C26). At higher temperatures (scans 60-70), m/z 44, ascribed to the evolution of CO_2 from both the network and lead white, is observed (results not shown). A typical m/z pattern frequently observed for the oil network is seen in the mass spectrum of scans 72-85, which is depicted in Figure 4b. A relatively high peak at m/z 91, which was identified as indicative for an aromatic pyrolysis product of oil paint in previous DTMS investigations (see Chapter 4 of this thesis), is one of the more informative ions in a broad envelope of ions from other low molecular weight pyrolysis breakdown products. Furthermore, m/z 256 and 284, released thermally from the network and a clear lead isotope pattern at m/z 206, 207, and 208 are observed. At the end of the measurement, at high temperatures (scans 85-110, results not shown) the thermal breakdown of lead carbonate is observed as indicated by a high m/z 44 and m/z 206-208.

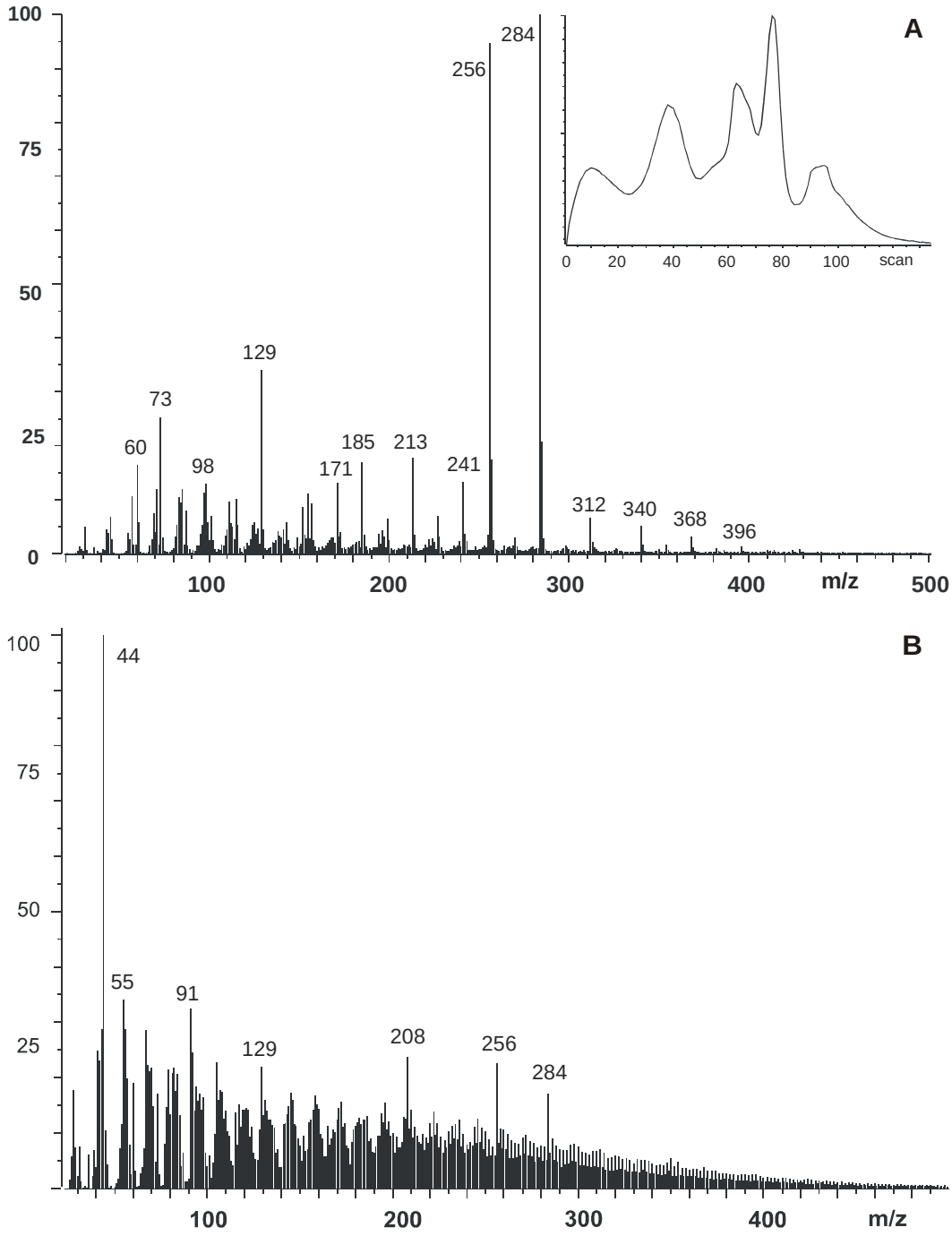


Figure 4 DTMS summation spectrum of OL.NA (a) scans 30-45, insert: TIC, and (b) scans 72-85.

The appearance of long chain FAs in the DTMS spectrum was not expected since the canvas has not been lined, a process that can introduce wax into a painting. An extraction of the canvas fibres of sample OL.1984 122 with either ethanol or dichloromethane (DCM) was performed to investigate the origin of this

wax. The DTMS data of both extracts shows the presence of an envelope of ions over a large temperature range indicative for fatty acids and waxes. Figure 5 depicts the summed mass spectrum of scans 25 to 110 of the ethanol extract. The most abundant wax esters are observed at mass m/z 620, 648, 676, and 704, corresponding to a total number of carbon atoms of 42 to 48 for the wax esters. This profile is typical for beeswax, which consists exclusively of ester of palmitic acid (C16 FA) and long chain alcohols. At higher masses however, with an increment of 28 (CH_2) mass units low amounts of other wax esters are visible up to m/z 872. These high molecular weight waxes are unusual and most likely originate from plants. Surprisingly, the ratio of C16 and C18 FAs as present in a normal linseed oil seems not to have changed. Often an increased C16 FA peak can be observed in DTMS spectra of beeswax containing paint material. This implies the wax in the canvas is not hydrolysed. However, especially for the dichloromethane extract increased amounts of apolar chains of C16 and C18 FAs fragment ions (m/z 257 and 285) are observed (results not shown), together with those of C20 to C28 FAs (m/z 313, 341,369, 397 and 425). In the same temperature range at which the waxes are desorbed most dominantly a peak at m/z 280 is observed, which is assigned to a C20 alcohol moiety. A special feature of the summed mass spectrum of scans 30-44 (not shown) is m/z 337, which is quite abundant. The origin of this ion is unknown and merits further study.

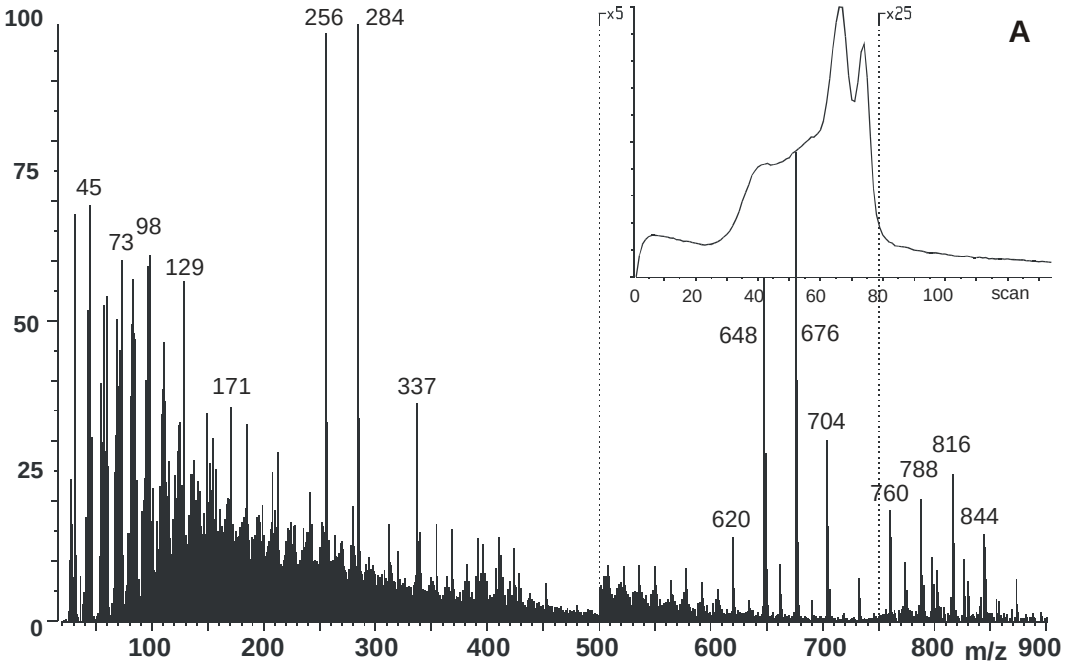


Figure 5. DTMS summation spectrum of scans 25-110 of an ethanol extract of canvas fibres of OL.1984 122. Insert: TIC.

DTMS analysis of the bloom on the cream-coloured paint of sample OL.1984 122 indicates that it is composed of relatively volatile components (see insert Figure 6a) as can be deduced from the TIC profile. Most material

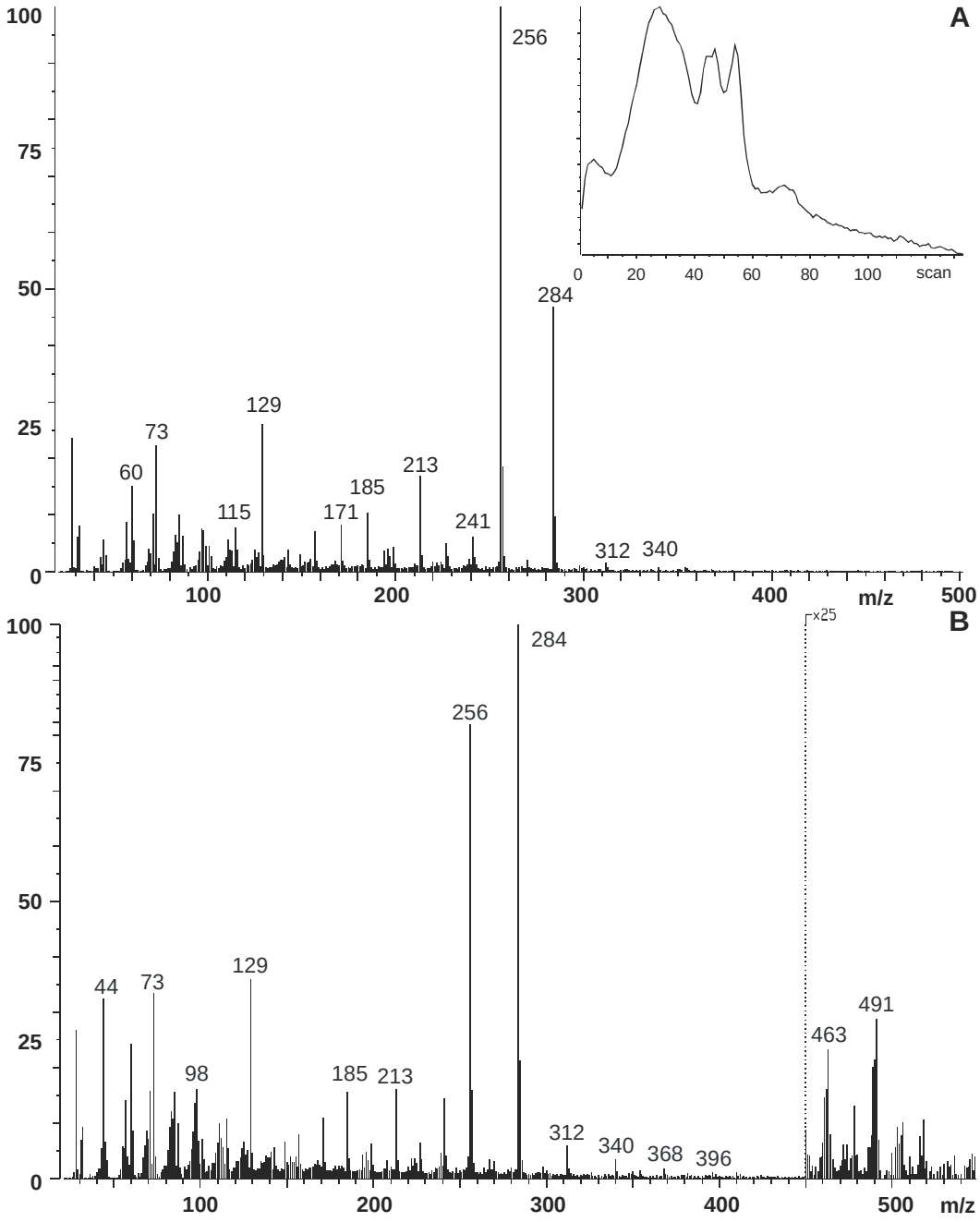


Figure 6 DTMS summation spectrum of bloom on surface OL.1984 122 (a) scans 1-25, insert: TIC, and (b) scans 33-50.

evaporates at low temperature and there is no indication for pyrolysis of oil network constituents. The DTMS spectrum of scans 1-25 (Figure 6a) shows that mainly free C16 (m/z 256) and C18 FAs (m/z 284) are desorbed at low temperature, next to smaller amounts of C20 and C22 FAs. Closer inspection of scans 20-30 (not shown) reveals that low intensity peaks are present at m/z 522, 550, 578, 606 and m/z 648, 676 and 704. This indicates the presence of trace amounts of diacylglycerols and long chain wax esters, respectively. At slightly

higher temperatures (scans 33-50, Figure 6b) two isotopic distributions are seen at m/z 463 and 491, indicative for lead soaps of C16 and C18 FAs, respectively. This is seldom observed and indicates that reasonable amounts of saturated lead soaps are present on the surface[1]. Their acylium ions at m/z 239 and 267 are also observable although with low intensity. Peaks indicative for lead are only seen from scan 60 onward and with a low intensity. The same holds for m/z 44, ascribed to CO_2, which is encountered in the DTMS spectra of aged oils and oils that contain lead white.

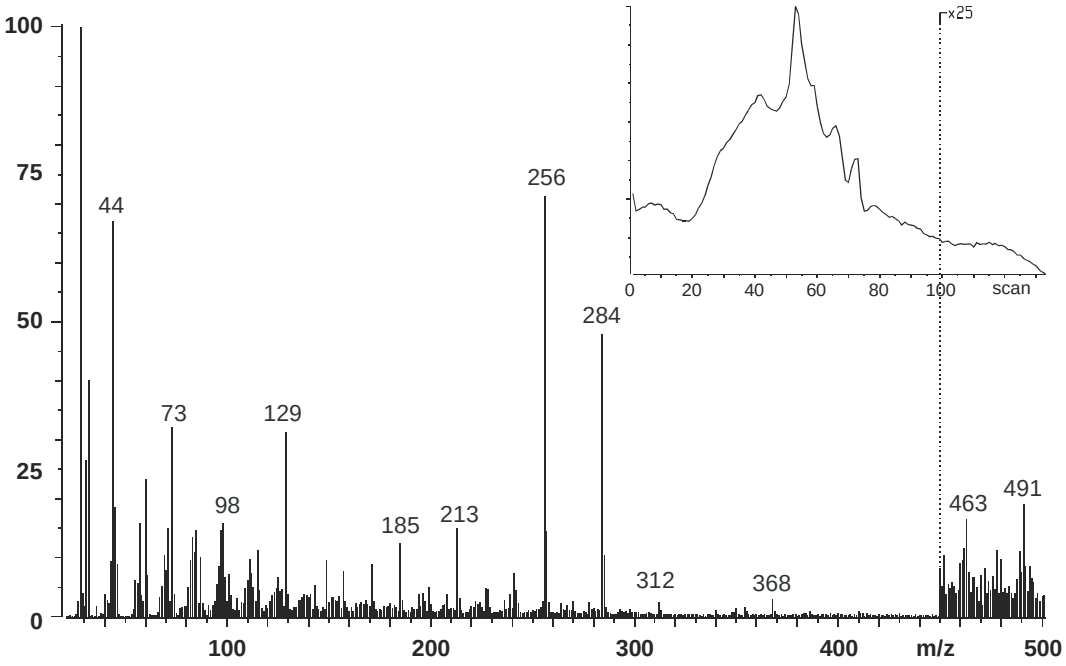


Figure 7. DTMS summation spectrum of scans 50-60 of a protrusion in paint OL.NA. Insert: TIC.

In addition to the bloom, a sample was taken from a white circular mass, with a transparent rim around a more opaque core, which erupted (or protruded) through the paint surface of OL.NA. Analysis by DTMS gives a multiple-peak desorption profile as is depicted in the insert of Figure 7. At low temperature free FAs are observed (not shown), whereas at higher temperatures (scans 50-60) peaks m/z 463 and 491, attributed to lead soap formation, can be seen. In the region where the oil networks start to pyrolyse normally, no distinct signal is observed in the TIC profile. At even higher scan numbers (80>), traces of lead are detected. These findings indicate that the free fatty acids and their soaps are present in this

[1] The presence of metal carboxylates was confirmed by FTIR analysis of the bloom [406], which absorbs at wavelengths of 2926 cm^{-1} (C-H stretch vibration), 2844 cm^{-1} (C-H symmetric stretch vibration), 1723 cm^{-1} (saturated CO stretch), 1418 cm^{-1}, (symmetric CO_2 stretch, 1252 cm^{-1} (C-O stretch) and 1521 cm^{-1} (asymmetric CO_2 stretch). This data correlates with fatty acids present as lead soaps [205, 270].

protrusion and suggest that there has been a reaction between the basic lead white and the free organic acids within the paint (or at/near the surface)[1].

7.3.2 The brown imprimatura layer on OL.1984 122

Part of the cream-coloured layer of OL.1984 122 was covered with a brown imprimatura layer. Despite the presence of this layer the same phenomena were observed on the surface and apparently the thin layer didn't form a barrier for the migration of fatty acids and their lead soaps.

The surface analysis of the brown paint layer with SIMS resulted in a qualitatively similar mass spectrum compared to the cream-coloured paint. This is to be ascribed in part to the fact that remnants of the underlying ground/paint still were present. There are, however, differences in the relative abundances and the overall signal intensity, which is lower for the imprimatura. The most noticeable difference is the relatively high abundance of the unknown ion at m/z 281 and the less abundant ions ascribed to acylglycerols. In the negative mode, two new peaks are observed in the low mass region of the mass spectrum specifically (not shown), which were not seen before. These peaks, m/z 64 (SO_2) and 80 (SO_3), suggest the presence of sulphates in/on the surface of the brown layer.

The results of the SIMS analysis of the cross-section of the canvas with the brown imprimatura layer (results not shown) indicates that free FAs are concentrated at the top layer and not in the bulk of the ground layers. The same holds for the lead soaps of C16 and C18 FAs, which are confined to the upper layer.

The degree of hydrolysis of the brown paint system determined by the derivatisation-GCMS method and the P/S ratio was determined to be 87% and 1.55, respectively, which agrees well with the values obtained for the cream-colored paint system. The ratio of C9 diacid to C16 FAs is slightly lower compared to the cream paint (2.11 vs. 2.78). The chromatogram (not depicted) that was obtained for the brown paint layer is almost identical to the one measured for the cream-colored paint.

The composition of both the bloom and the imprimatura layer also were investigated with DTMS and the composition was found to be similar to the cream-

[1] The results of FTIR analysis of the protrusion by van der Weerd [406] confirm these data. A strong carbonate signal could be observed at 1422 cm^{-1}. Additional peaks at 3539 and 683-1 cm^{-1} indicate that lead white is present. The lead carboxylate absorption was clearly seen 1518 cm^{-1}.

colored layer build-up. The shape of the desorption profile (see insert Fig 8) is identical to Figure 4, although relatively less compounds are evaporated at low temperatures, indicating that lower relative amounts of volatile compounds are present. Figure 8 depicts the mass spectrum of scan 55-70 and shows (fragment) ions typical for the presence of free fatty acids, long chain alcohols, bees wax, DAG fragments and lead white. In addition, two unknown monolead-containing compounds are seen at m/z 348-350 and 516-518.

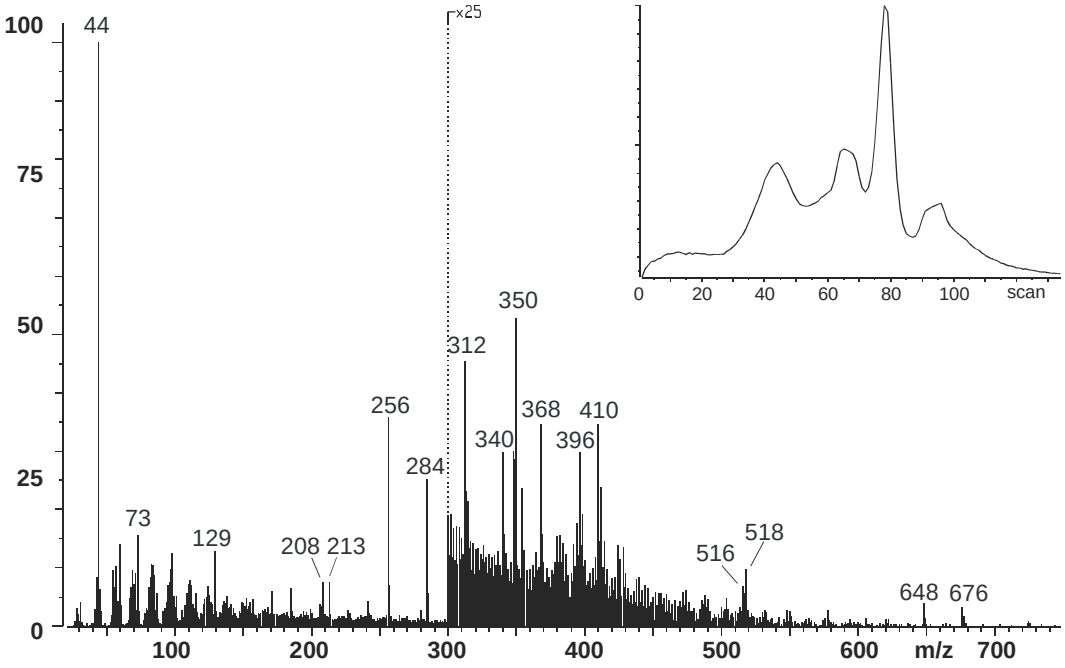


Figure 8. DTMS summation spectrum of scans 55-70 of brown imprimatura layer of OL.1984 122. Insert: TIC.

Analysis of the whitish bloom scratched off the surface of the brown paint layer gave a relatively simple desorption profile (not shown). Besides free fatty acids detected at low temperature a second peak is observed ranging from scans 40-75. This coincides with the region where the oil network is pyrolysed. Apparently some paint was scraped off as well while sampling the crystals. Only trace amounts of lead soaps could be identified. At higher temperatures (scans 75-95, DTMS spectrum not shown) peaks m/z 64 and 208, indicative for sulphur and lead containing inorganic components are observed. The presence of sulphur was also inferred from the SIMS data. Upon re-inspection of the low-mass region of the DTMS spectrum of the cream-coloured ground (Fig. 4) traces of sulphur were visible there as well.

7.3.3 Discussion

The same phenomena are observed for the two primed canvases, independent of the type of paint. This indicates it is the combination of lead white and ground layer composition that is playing an important role. The cream-coloured layers are shown to have been made of linseed oil and lead white, on top of a chalk layer, whereas the brown imprimatura layer is made with a combination of linseed oil, lead white and an unknown brown pigment. Within the cream-coloured layer of both canvases indications were found for wax, and the bloom of both layers was shown to contain some traces of wax esters as well. These are thought to have migrated to the surface from beneath the ground as it was shown that the reverse of the canvas contained reasonable amounts of bees wax and a waxy material, most likely derived from plants. The main differences between the two cream-coloured paints and the brown imprimatura are the relative abundances of the compounds identified with surface analysis by SIMS. The cream-coloured canvas sample of OL.1984 122 has higher relative amounts of $FA \cdot H^+$, $FA\text{-}H^-$ and diacylglycerol ions in its SIMS mass spectra compared to the brown paint layer. Besides, the brown imprimatura layer differs from the two other samples in the relatively lower amounts of the diacids. The SIMS mass spectrum of the cream-coloured paint of sample OL.NA shows lead containing ions as the most prominent whereas ions indicative for glycerol esters of FAs are almost absent.

The two-step derivatisation experiments have shown that the relative amount of free fatty acids is relatively high within the cream-coloured layers (>85%). In a well-defined paint system the presence of free FAs is not supposed to lead to the exudation of the free fatty acids. The paint system itself normally traps these acidic compounds through the formation of non-volatile metal soaps by reaction with the pigment surface or these compounds are “stored” within the paint and gradually released by evaporation from the paint surface of the paint (given that the hydrolysis rate is not too fast). The exact mechanism of the bloom formation has been subject of discussion for a long time already and different hypotheses have been postulated. It has been suggested in literature that only a specific number of pigments seems to be responsible for the formation of bloom. Lead white is not supposed to contribute to the formation of bloom, as it is a reactive pigment that can normally trap free carboxylic acids and even should prevent the formation of bloom. Apparently, the relative amount of free carboxylic acid anions in the prepared paintable surface available to Church is too large to be compensated by the cations of the pigment particles. We also propose that the surface of many of the lead white particles is saturated with fatty acid soaps. In extremo this could lead to lead soap masses as observed in protrusions in which lead white particles seem to have been consumed completely.

It is clear from the literature [397] that the bloom formation has occurred at a relative young age and was independent of the type of pigmentation of the upper layer. The fact that the commercial canvases were prepared in such a way that they could be rolled suggests that the priming may have contained materials that kept it relatively soft for a prolonged time. The high oil to pigment ratio of the upper lead white layer observed with microscopy suggest that there is a problem. This is not in accordance with the general idea that a lead white paint only needs a low amount of oil because it is a lean paint. Normally, some additional oil is deliberately added to the final priming layer so it can be absorbed by the ground. This practice should prevent that the ground takes up too much oil when painted over. We suspect that the thick calcium carbonate layer unintentionally could have been made less absorbent for oil and suggest that the waxy components observed play a role in the lower absorbency. Obviously, a reduced capacity to absorb oil will lead later on to a surplus of free fatty acids within the top layer when hydrolytic processes are starting up. In the presence of reactive basic lead white particles this will not only lead to the formation of lead soaps, but also will lead to the formation of bloom when the amount of free FAs formed is too large to be reacted away by the pigment particles in the paint compartment.

7.4 Results Case II: Bloom on paintings by Frank Stella

7.4.1 Panel #5 "Cricche, Crocche e Manico d'Unico"

DTMS

The first samples of bloom analysed were taken from panel #5 from "Cricche, Crocche, e Manico d'Unico". The panel was covered with pink, orange and light blue epoxy paints. Examination of the paint surface revealed a fine layer of white crystals on dark cobalt blue paint. The other paints on this panel only exhibited crystals when they were painted over this blue paint. The DTMS TIC obtained from the crystals is depicted in the insert of Figure 9a. The main constituents are evaporating in the beginning of the analysis from scan 2 to 30 and are identified in the mass spectrum depicted in Fig. 9a as volatile C16 and C18 free fatty acids (m/z 256 and 284), of which the first are present in relatively large quantities. Fragment ions typical for free fatty acids are seen at lower masses (for an explanation of the observed masses see Chapter 4, Figure 2). At a slightly higher temperature (scans 35-45; Figure 9b) next to free fatty acids, fragments ions $[RCO+74]^+$ and $[TAG-RCOO]^+$ are observed (m/z 313 and 341, and 551, 579, and 607), consisting of C16 and C18 saturated fatty acids only.

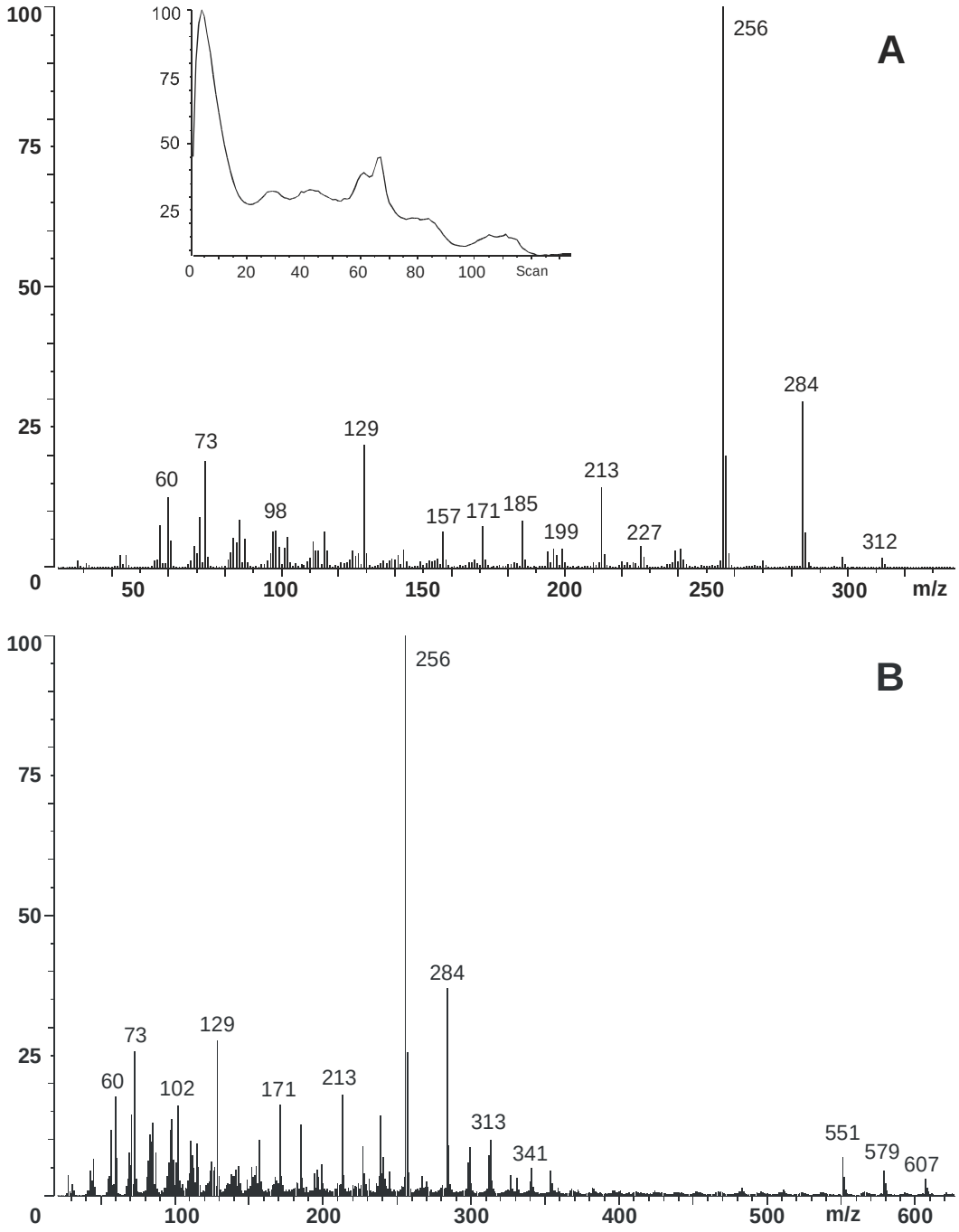


Figure 9 DTMS summation spectrum of crystals on surface of cobalt blue paint panel #6 “Cricche, Crocche e Manico d’Unico”, (a) scans 0-30, insert: TIC, and (b) scans 37-44

DTMS analysis of the dark blue paint (Figure 10a) shows a desorption/pyrolysis profile consisting of a relatively small fraction of volatile components and two

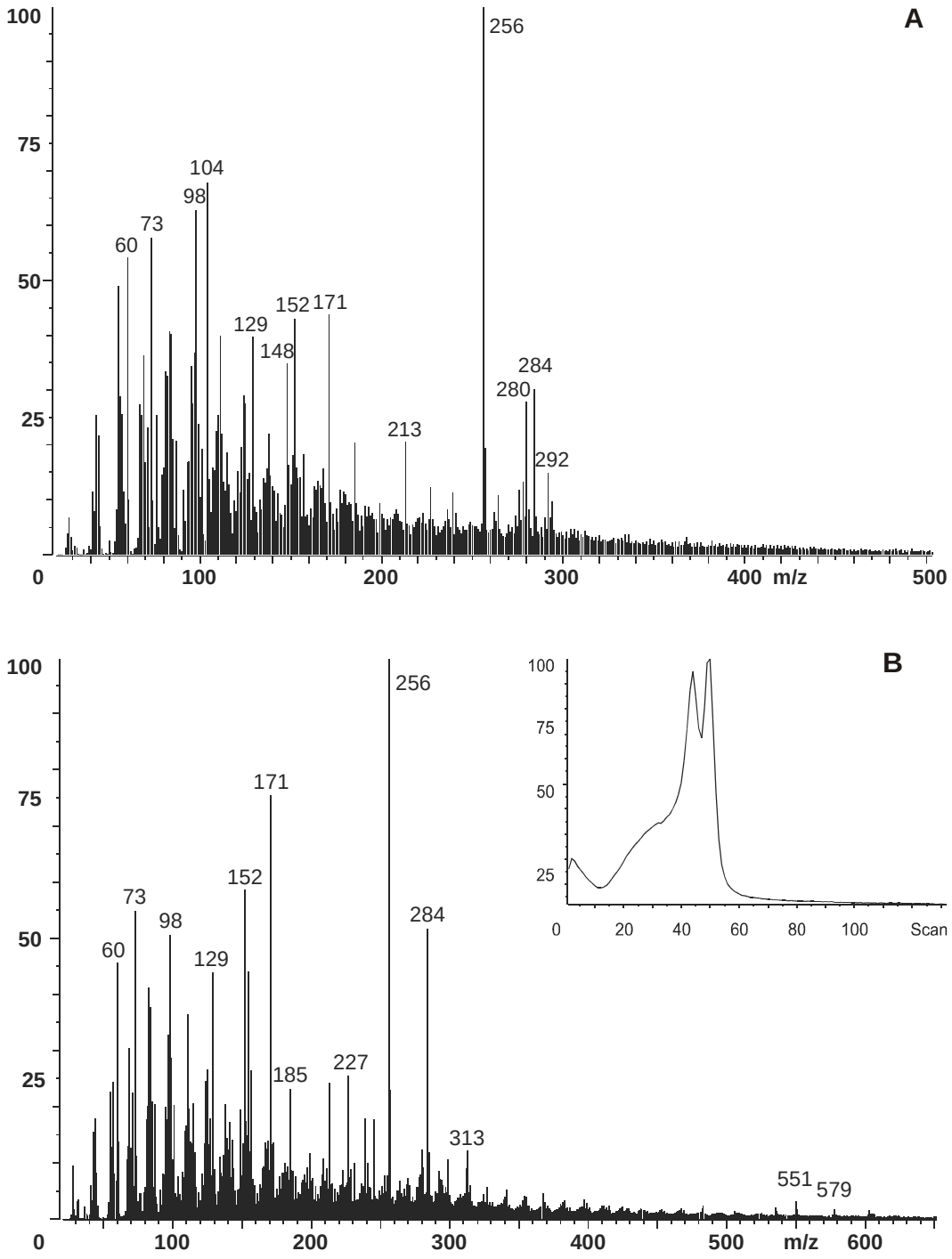


Figure 10. DTMS analysis of dark blue paint panel #5“ Cricche, Crocche e Manico d’Unico”, (a) scans 40-46, insert: TIC, and (b) scans 15-35.

peaks at a higher temperature. In the mass spectrum of scan 40 to 46 the presence of a modern orthophthalic alkyd resin is recognised by the presence of ions at m/z 104 and 148 (see Fig. 10a), which are characteristic for phthalic anhydride, the pyrolysis product of this type of paints [339, 407, 408]. This type of paint

previously has been found on “Hyena Stomp” (1962), a painting also made by F. Stella and at present in the collection of the Tate Gallery (TG ac. No. T00730) [408]. Another paint material, which is present in higher relative amounts, looks like a normal drying oil paint and is characterised by typical (fragments) ions such as m/z 98, 152, 155, 256, 284, 313, and 551 (see mass spectrum of scan 15 to 35, Fig. 10b).

Fatty acid composition by Cu-Py-TMAH-GC/MS

Curie point pyrolysis-GC/MS analysis of the cobalt blue paint (#5) could not positively confirm the identity as a modern synthetic paint. All peaks observed in the gas chromatogram depicted in Figure 11 have been encountered before when analysing traditional oil paints (See Table 3 for the identification). The peaks labelled with *, however, are derived from (tere)phtalates. These compounds are typical for alkyd resin paints, but also can be picked up relatively easily during sampling or sample preparation. It is clear that this component is only present in low quantities. A pure alkyd resin would have given more intense peaks for the dimethyl orthophthalate. The P/S ratio of 2.33 is in the range of walnut oil, or mixtures of linseed oil with walnut and/or poppy seed. However, other oils like

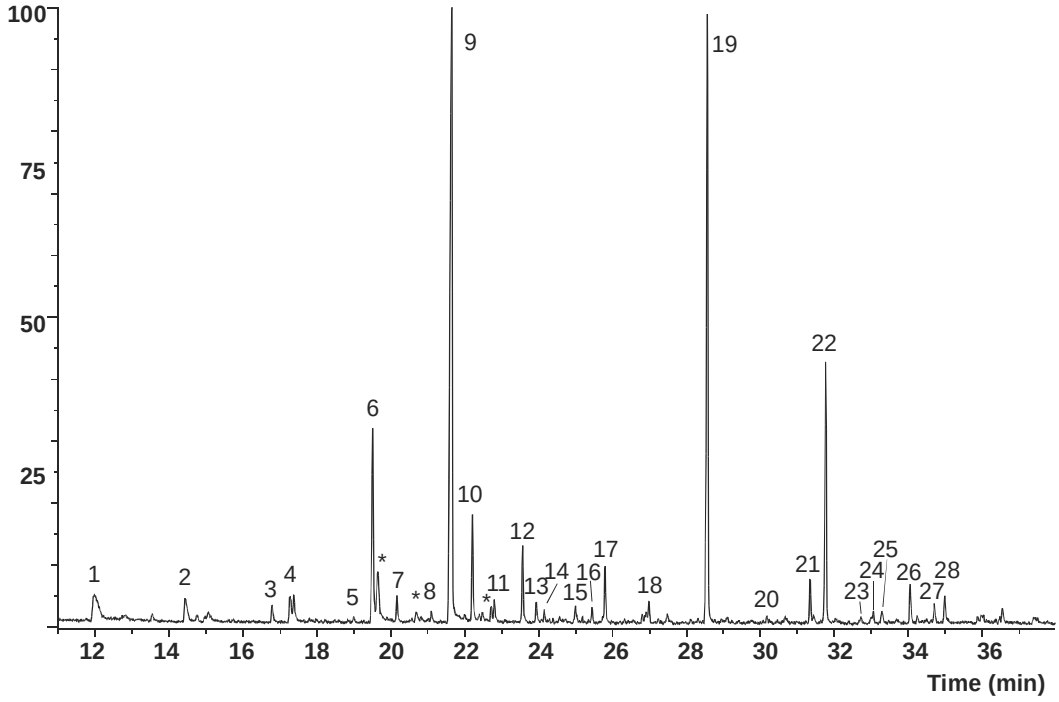


Figure 11. TIC of cobalt blue paint panel #6 “ Cricche, Crocche e Manico d’Unico”, obtained by Cu-Py-TMAH-GC/MS analysis. Temperature program: 50(2)-6-320.

safflower, soybean or tall oil, may have been used as well in the production of the alkyd/oil paint mixture. The relative amount of the C9 diacids is in the same order of magnitude as the C16 fatty acids and not unusual for an oil paint. No data are available on the degree of hydrolysis of the glycerol ester bonds of the fatty acids.

Table 3. Identified compounds in the Cu-Py-TMAH-GC/MS analysis of paints from work of arts by F. Stella. Identification is based on the 70 eV mass spectrum.

Label	Retention (min.)	Compound name	MW
1.	12.0	Hexanoic acid, methyl ester	130
2.	14.5	Heptanoic acid, methyl ester	144
3.	16.8	Octanoic acid, methyl ester	158
4.	17.3	Heptanedioic acid, dimethyl ester	188
5.	19.0	Nonanoic acid, methyl ester	172
6.	19.5	Octanedioic acid, dimethyl ester	202
7.	20.2	-Methyl octanedioic acid, dimethyl ester	216
8.	21.1	Decanoic acid, methyl ester	186
9.	21.6	Nonanedioic acid, dimethyl ester	216
10.	22.2	-Methyl nonanedioic acid, dimethyl ester	230
11.	22.8	,. -Dimethyl nonanedioic acid, dimethyl ester	244
12.	23.6	Decanedioic acid, dimethyl ester	230
13.	23.9	-Methoxy nonanedioic acid, dimethyl ester	246
14.	24.1	-Methyl decanedioic acid, dimethyl ester	244
15.	25.0	Tetradecanoic acid, methyl ester	242
16.	25.4	Undecanedioic acid, methyl ester	244
17.	25.8	-Methoxy decanedioic acid, dimethyl ester	260
18.	26.8	Pentadecanoic acid, methyl ester	256
19.	28.6	Hexadecanoic acid, methyl ester	270
20.	30.2	Heptadecanoic acid, methyl ester	284
21.	31.4	Octadecenoic acid, methyl ester	296
22.	31.8	Octadecanoic acid, methyl ester	298
23.	32.7	Octadecadienoic acid, methyl ester	294
24.	33.1	11-Methoxy-9-octadecenoic acid, methyl ester	326

25.	33.3	9-Methoxy-10-octadecenoic acid, methyl ester, 10-Methoxy-8-octadecenoic acid, methyl ester	326
26.	34.1	9,10-Epoxy-octadecanoic acid, methyl ester	312
27.	34.7	Icosanoic acid, methyl ester	326
28.	35.0	9,10-Dimethoxy-octadecanoic acid, methyl ester	358

7.4.2 Panel #6 "Cricche, Crocche e Manico d'Unico"

A red paint was sampled from a panel consisting of etched magnesium with a clear and lime-green epoxy paint on top. These paint layers are followed by three oil paints: red, yellow, and orange. Beneath the paint layers remnants of masking tape were still present. The only areas of red paint in panel #6 where crystals did not show up, is where the paint lies over the masking tape that most likely had been used for the etching process of the magnesium. The crystals could be easily removed from the paint and were analysed. Figure 12 presents the results of the DTMS analysis of the crystals. The summed mass spectrum of scans clearly shows the presence of free C16 and C18 FAs.

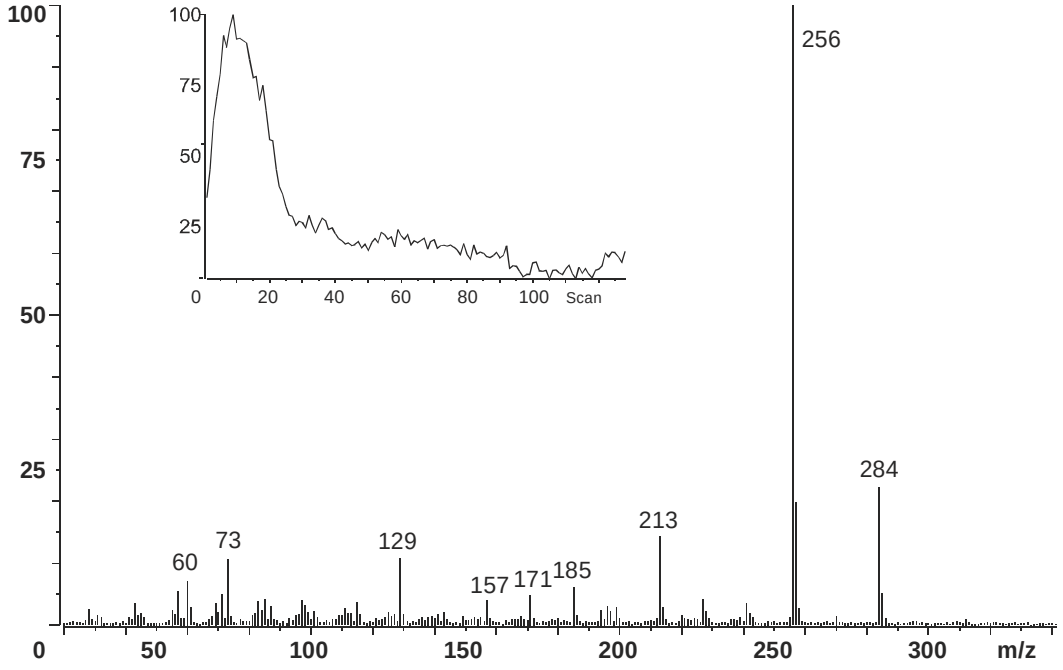


Figure 12. DTMS summation spectrum of scans 0-3- of crystals on surface of red paint panel #6 " Cricche, Crocche e Manico d'Unico". Insert: TIC.

The red paint shows a desorption/profile that indicates that mostly high molecular weight networks are present. Three different regions can be identified in the DTMS TIC depicted in Figure 13. In the mass spectrum of scans 1-70 typical compounds are seen that can be related to oil paint. From scans 70 towards 90 pyrolysis of the less mobile compounds takes place, indicated by the evolution of ions m/z 91 and 105, in combination with the unresolved envelope of low molecular weight (fragment) ions (result not shown). At the highest temperature (scans 95-125), m/z 44, 64, and 76, are formed. This is a clear indication for the presence of sulphur containing materials.

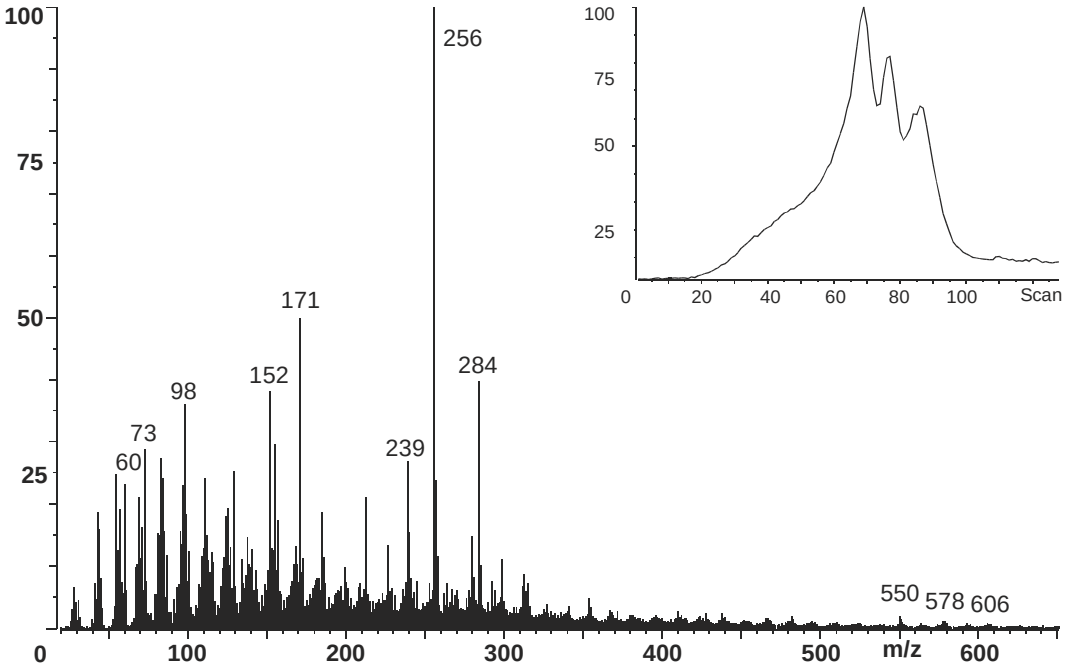


Figure 13. DTMS summation spectrum of scans 1-70 of red paint panel #6 " Cricche, Crocche e Manico d'Unico". Insert: TIC.

The red paint was subsequently analysed using Cu-Py-TMAH-GC/MS. The results are depicted in Figure 14 and Table 3. The P/S ratio has been determined and was in the same range as the previous paint (P/S=2,57). In the time range from Rt=34 to 38 min. peaks could be detected which are not derived from the oil network. The peak marked # at t_R=35.00 min. could be identified as 1-phenanthrenecarboxylic acid ($C_{21}H_{30}O_2$). A series of unidentified compounds with mass increments of 16 amu, is seen at t_R=37.76, 37.86 and 38.23, respectively. The high intensity molecular ions at m/z 340, 356 and 372, respectively, suggest that aromatic rings are present. Fragment ions at M-15, M-59, M-75 and M-115 are seen in all three mass spectra. No direct indications are found for the presence of the organic alizarin despite the fact that identification of alizarin with this technique was proven before [409].

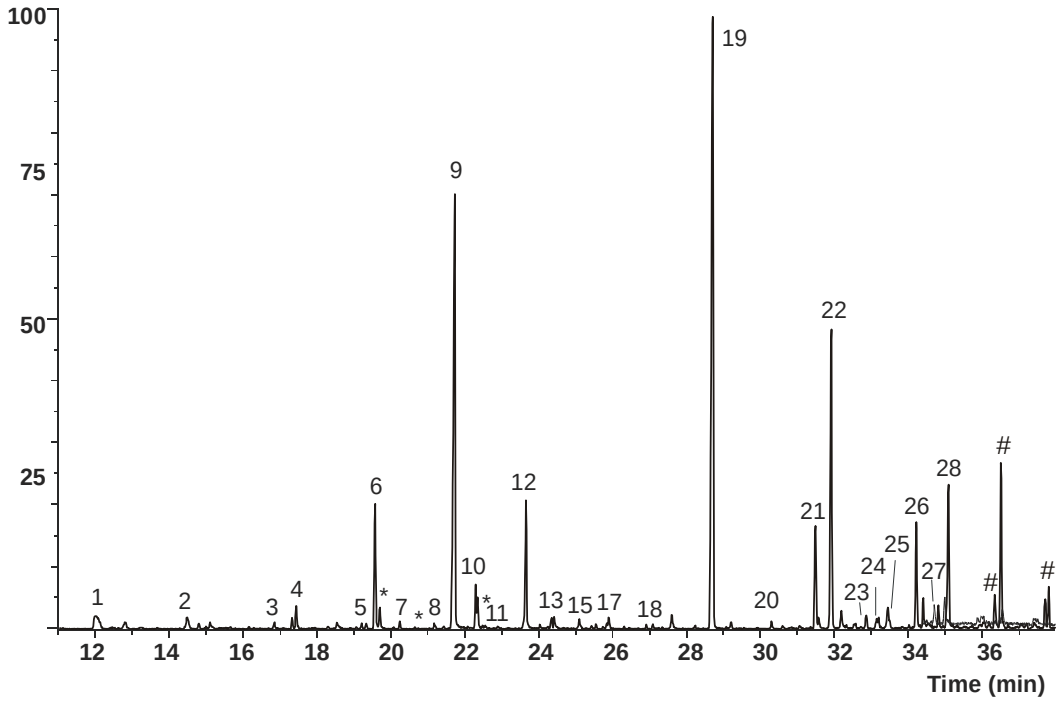


Figure 14. TIC of cobalt blue paint on panel #6 of " Cricche, Crocche e Manico d'Unico", obtained by Cu-Py-TMAH-GC/MS analysis. Peaks labelled * are identified as dimethyl esters of phthalic acids,. Temperature program: 50(2)-6-320.

The degree of hydrolysis of the red paint with bloom formation, and a red paint free from white crystals was determined using the two-step derivatisation method, as has been described in Chapter 6. The value obtained for the degree of hydrolysis of the relatively young red paint is already 40%. This is high for a young paint film but only 10% higher than value obtained for the red paint from the same panel that didn't develop bloom (results not shown). One should be cautious however, since it might be possible that a reasonable amount of free fatty acids already has left the paint by evaporation prior to analysis. This phenomenon was observed after the few months that the paint samples were stored for further analysis. White hazes of fine crystals had formed two to three halos around the paint particles. Apparently, the internal chemistry of the paint is severely disturbed and far from the ideal composition. Without the presence of appropriate pigment particles or other positively charged binders that can trap the freed FAs, it is likely that hydrolysed FAs will be mobile. Due to the high percentage of oil used in alizarin paints, in general 64-75%, and the non-drying properties of alizarin an excess of these mobile FAs is to be expected [410, 411].

It was observed that brushstrokes of red paint on top of the remnants of the masking tape did not show blooming whereas directly next to the tape, on top of the same brushstroke white crystals could be observed. At the same time another interesting observation was made. The red paint on top of the masking tape had kept its bright red colour, whereas the red paint with bloom formation had turned

darker. Recall that alizarin can be used as a pH-indicator for the transition from a very weak acidic environment to a strong alkaline environment, while it changes colour from red to a more violet colour. We infer that changes in the internal chemistry of the alizarin paint are a response to a higher pH that caused an increase in the degree of hydrolysis of the fatty acids. To test this hypothesis two spectral absorption curves were recorded of the two types of red paint using an imaging reflection VIS microspectrometer. It is known from literature that the maximum wavelength of absorption increases when the alizarin is brought at higher pH and that the percentage transmittance is increased [410]. Although the two measured curves only differed slightly, the pH effect was observable as a shift to higher wavelength.

SEM/EDX analysis of the two red samples was performed at Shell Research and Technology Centre, Amsterdam (SRTCA) to determine whether magnesium from the substrate could have migrated into one of the red paints, and whether this could have played a (catalytic) role in the process of bloom formation. This thought presented itself since the remnants of the masking tape could have acted as a barrier against a hydrolytic process that is somehow stimulated by corrosion products from the support. However in both samples of red paint investigated, no signals were observed that could be ascribed to magnesium. In most of the spots investigated only the elements carbon, oxygen, aluminium and sulphur and sometimes traces of calcium and zinc were observed. A few white spots were observed that contained low amounts of barium or zinc. Most likely the aluminium is derived from the alizarin red lake, which is often prepared with either aluminium and/or calcium. Other materials have been used as substrate as well, including barium sulphate and zinc oxide [410]. However, the excessive use of (aluminium) driers is not to be excluded, especially since Stella's private conservator has suggested this. The presence of zinc and calcium can be caused by small amounts of modern driers as these metals often are mixed in to assist the primary driers. Barium sulphate is often used as filler and this may account for its presence.

7.4.3 Panel #4 "Cricche, Crocche e Manico d'Unico"

The last paint sample analysed from "Cricche, Crocche, e Manico d'Unico" was taken from panel #4. A fine whitish haze of crystals was observed on a cooler blue paint. A second warmer blue paint on the same panel did not suffer from bloom formation and was sampled as well to compare the composition of both materials. DTMS analysis of the bloom resulted in a desorption profile and mass spectra (not shown) similar to the blue paint from panel #5. Only free C16 and C18 FAs are

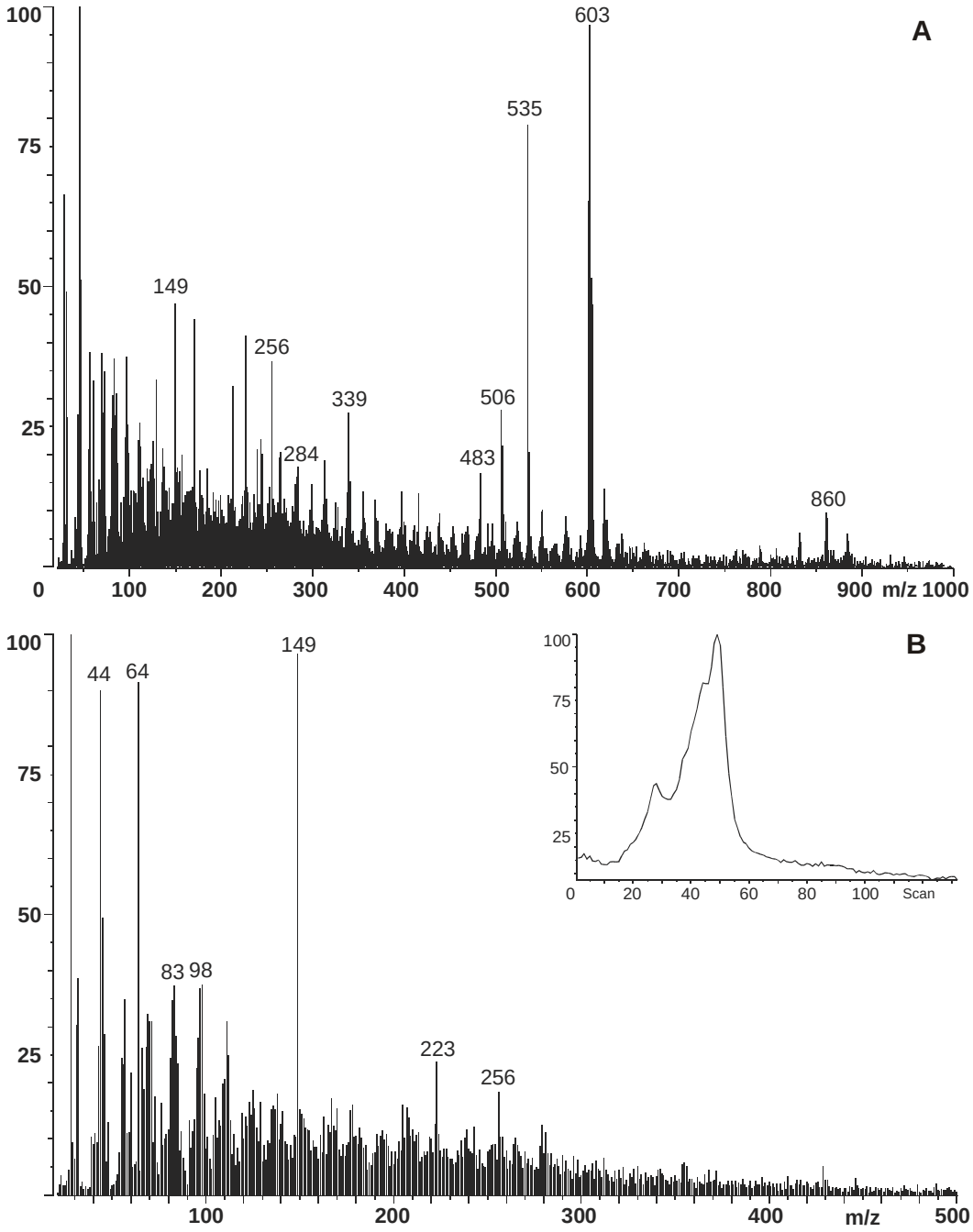


Figure 15 DTMS summation spectrum of warmer blue paint on panel #4 " Cricche, Crocche e Manico d'Unico"; (a) scans 17-30, insert: TIC, and (b) scans 40-50.

observed. The paint itself is identified as modern alkyd paint, both with DTMS and Cu-Py-TMAH-GC/MS. In the DTMS analysis CS_2 (m/z 76) is observed at high temperatures. It is formed upon pyrolysis of a sulphur containing char remaining on the analytical filament. The TIC of the gas chromatogram (not depicted) showed a P/S ratio in the same order of magnitude as the other paints from this work of art, namely 2.45. The relative amount of C9 diacids and the degree of

oxidation of this particular sample were significantly lower compared to the other paints.

The DTMS TIC of the analysis of the warmer blue paint is depicted in Figure 15a. The profile obtained is typical for a number of paints analysed. Fragment ions indicative of phthalates and ortho-phthalates can be observed at m/z 149 and 148, respectively, throughout the whole analytical run. In the beginning of the DTMS run, two clusters of ions are seen at m/z 603 and 535 (scans 17-30, Fig 15a). The origin of the ions is unknown but they are most likely related to the organic binder. Surprisingly, hardly any free fatty acids are observed within scans 1 to 30. These compounds are only visible after 40 scans at higher temperatures, together with the two ions indicative for orthophthalate alkyd paints (m/z 104 and 148). This suggests that the FAs are still present as esters and are not hydrolysed to a large extent. In Figure 15 b, the summed mass spectrum of scans 65-94 is depicted. A high intensity ion is seen at m/z 64 (SO_2), probably derived from sulphates, and m/z 149, in combination with m/z 223 (phthalates) desorbing from the walls of the ion source.

A P/S ratio of 2.65 was obtained with Cu-Py-TMAH-GC/MS. This is in the same range as for the other paints. The same holds for the relative amount of C9 diacids.

7.4.4 Panel #7 "Gobba zoppa e collotorto"

The other work of art by Franks Stella partially covered with bloom is panel #7 of "Gobba zoppa e collotorto". Red paint had crystalline material on its surface, which was shown to be mainly composed of free C16 and C18 fatty acids upon DTMS analysis. Investigation of the red paint itself gave a profile with two peaks close to each other (see Figure 16). In the beginning of the analytical trace free C16 and C18 fatty acids are desorbed, followed by more polar and higher molecular weight material constituents of oil paint (Fig. 16; scans 14-32). The typical mass spectrum observed after pyrolysis of the cross-linked oil networks is obtained upon summation of scan 50 to 60 (not shown). M/z 91 and 105 are present in high abundance, together with the broad envelope of low molecular weight compounds and fragment ions as has been seen before (see Chapter 4). At the end of the analytical run peaks are seen that point towards the presence of sulphates in the sample (m/z 64, ascribed to SO_2 from a released XSO4). In addition, the CS_2 ion at m/z 76, formed upon pyrolysis of a sulphur containing char residue, is seen.

A Cu-Py-TMAH-GC/MS analysis of the red paint showed typical products of an aged drying oil with an average distribution. The P/S ratio of 3.17 was slightly higher than determined for the other oil paints used by Stella.

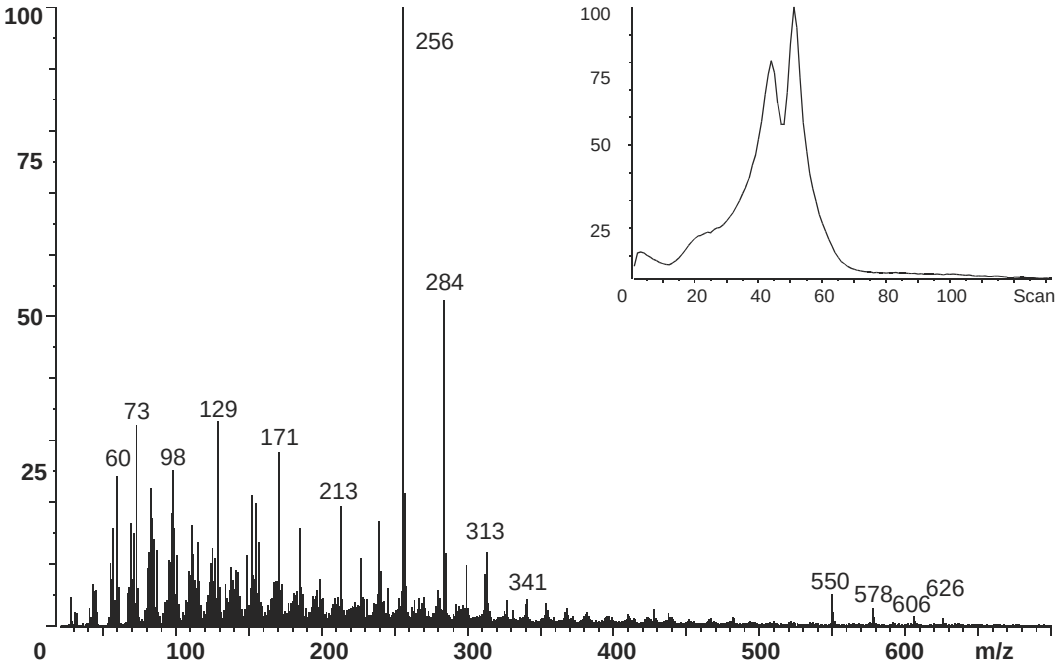


Figure 16. DTMS summation spectrum of scans 14-32 of red paint panel #7 " Gobba, zoppa e collotorto". Insert: TIC.

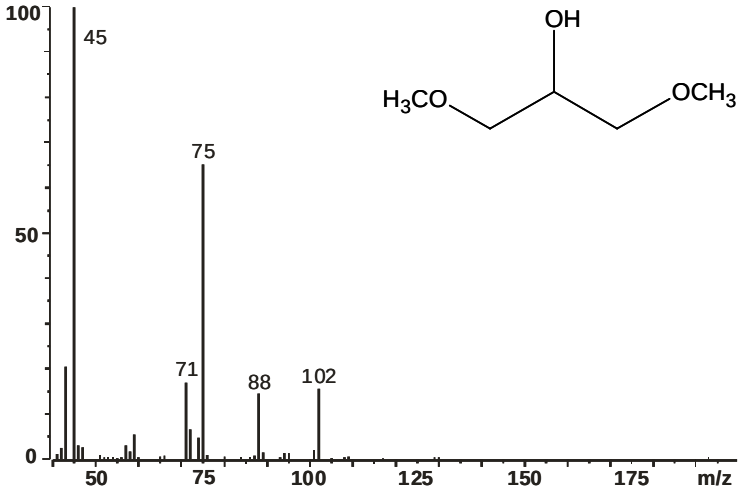
7.4.5 Discussion/Conclusion

All the waxy, whitish, crystalline growths found on the two works of art by Stella were shown to consist of free C16 and C18 fatty acids. The ratio of these free FAs, as is obtained with DTMS, is relatively high, especially when compared to the numerous traditional linseed oil paints that were investigated within the MOLART project. It is clear from the P/S ratios obtained with Cu-Py-TMAH-GC/MS, ranging from 2.5 to 3.2, that the oil medium is the supplier of the bloom. Although it is thought that most of these paints are made with Blockx oil paint composed of poppy seed oil, the P/S ratios point toward walnut oil or a mixture of poppy seed oil with walnut an/or linseed oil. This suggests that the P/S ratio may have been altered upon ageing. The relatively volatile C16 FAs, as suggested by Schilling [15], can have evaporated more effectively from the surface of the paint, leading to an increase of the percentage of C18 FAs. The fact that relatively large amounts of free fatty acids had formed around the stored paint sample supports this idea.

The fact that deviating P/S ratios are found may also be caused by the addition of different amounts of alkyd paints. These have been identified in a number of paints. Unfortunately, it was impossible to study all paints in detail to clarify the specific mechanism underlying the bloom formation. It is clear that the

paints investigated are composed of organic pigments that do not have (sufficient) capacity to trap the fatty acids that have been hydrolysed. The specific pigments that exhibited bloom formation need high amounts of oils (70% for the alizarin and up to 140% for the cobalt blue). In the absence of an alkaline reserve even a moderate degree of hydrolysis can lead to an excess of FAs within the paint. Whether this is caused by increased rates of hydrolysis in these specific cases is not known and merits further study. This will require more fundamental studies on the synergy of the different factors known to be involved in the hydrolytic processes.

Only the red paint of Panel #6 was investigated in more detail, partially because of the intriguing phenomena that could be observed. Alizarin crimson, a high oil absorbing pigment, was the only colorant identified in the affected red paint by FTIR. No other compounds are present that might have reacted with the free carboxylic acids to trap them or to reinforce the paint film. On the basis of the observations the idea is put forward that a high internal alkalinity of the red paint led to an increase in the rate of hydrolysis of the fatty acids. Apparently, the big difference between the bright and darker red is caused by the underlying remnants of tape. The observations suggest that the increase in alkalinity and thus the hydrolysis process is catalysed from the underlying paint layers or the magnesium support. This may be caused by the filiform corrosion of the magnesium and /or the high humidity that is required for this process [395]. The paint on top of the remnants of tape is protected against the negative effects of the corrosion. The fact that thick layers are less prone to bloom formation would also support this idea. Besides, thick layers can be seen as large reservoirs for liberated fatty acids, something that also holds for the tape. I.e. it just takes longer for them to reach a critical concentration of free FAs. The formation of free FAs is a process not clearly understood. Humidity, the internal pH of the paint system, type of pigment(s), and heat for instance, will contribute to the rate of the hydrolysis. Once the free FAs have been formed they are retained within the paint or transported to the surface. The reason why the fatty acids are moving to the surface of the paint is even less understood. Several theories have been put forward in the introduction and all of the suggestions are reasonable possibilities. These two matters are questions that cannot be answered at present due to the complexity of a paint system. Simple model systems will have to be developed that address the trapping efficiency of pigments or other paint materials. Mixtures of free fatty acids added to paint systems can also be used to imitate the conditions that are expected to lead to blooming. Such studies should give a better insight into the problems and possible solutions.



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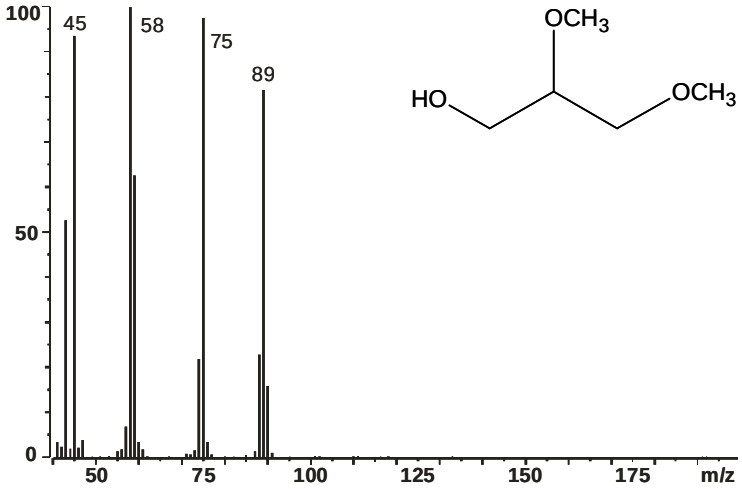

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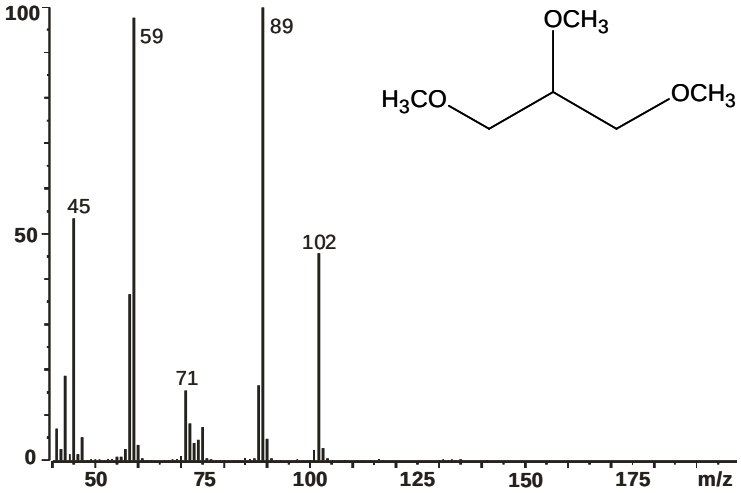
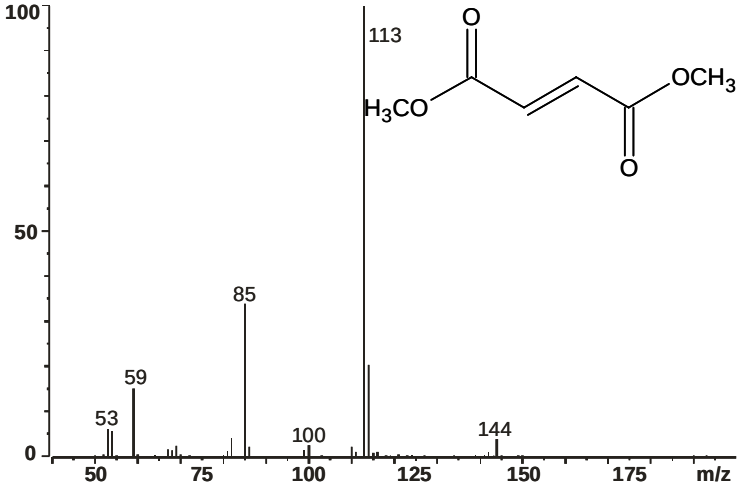
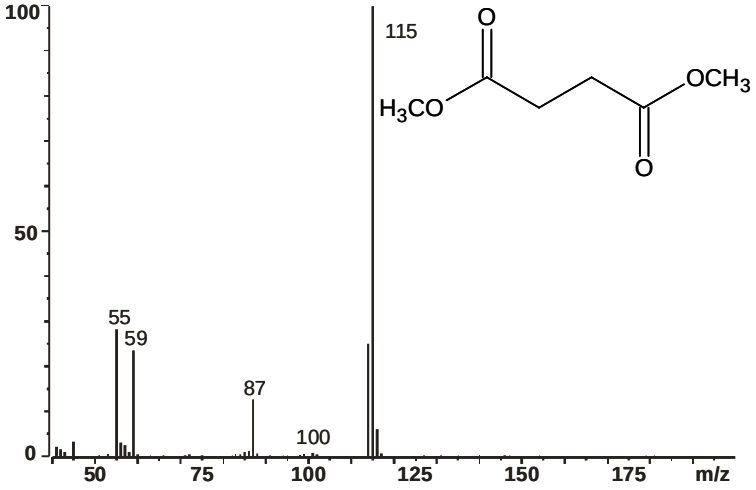


MW 134 1,2,3-trimethoxypropane



MW 144 butenedioic acid, dimethyl ester



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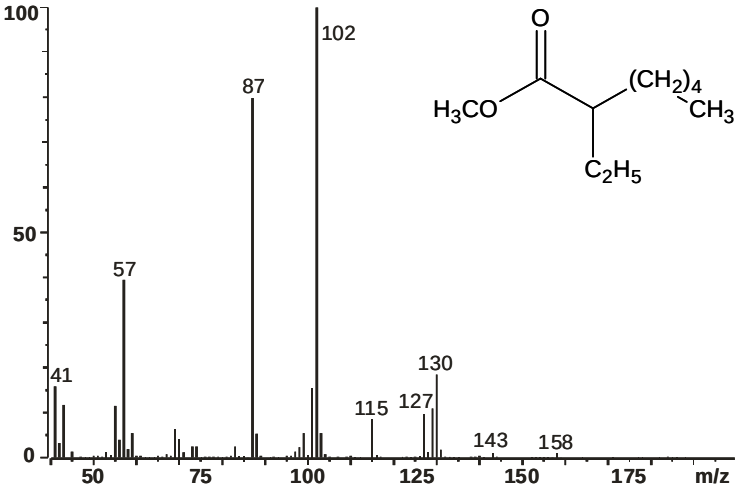

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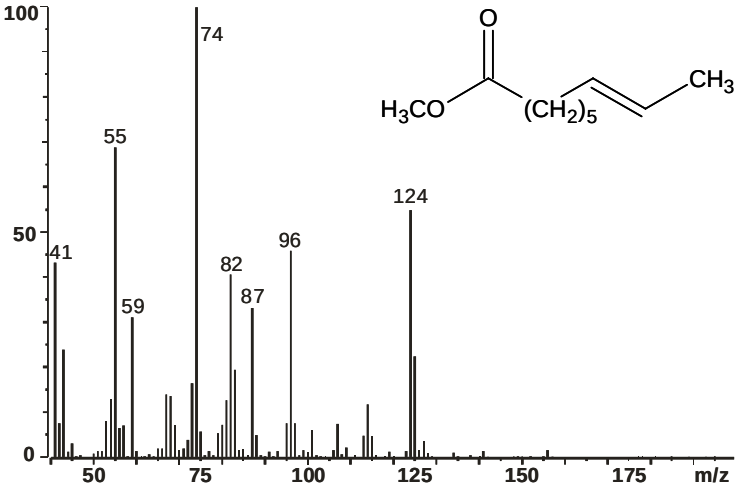
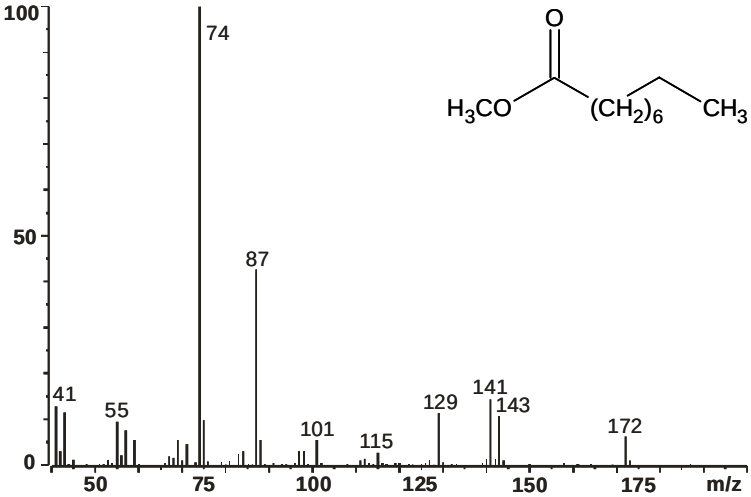
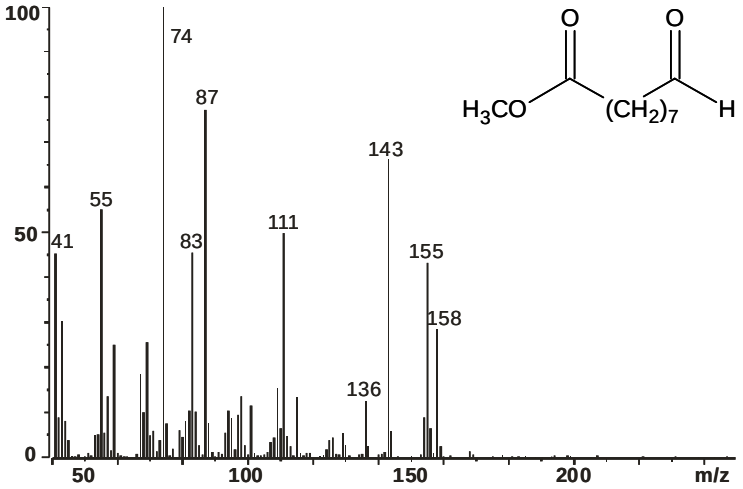


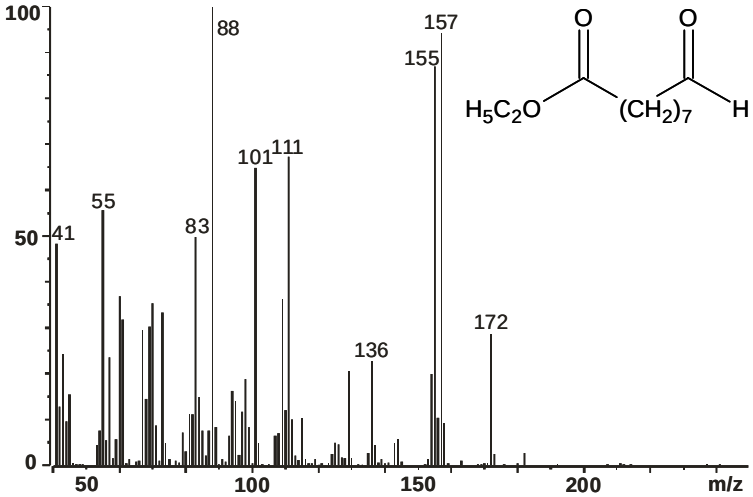
MW 170 non-7-enoic acid, methyl ester



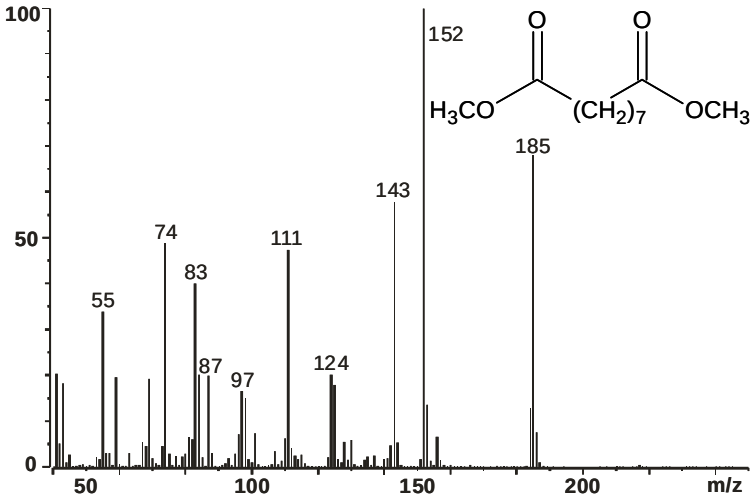
MW 172 nonanoic acid, methyl ester



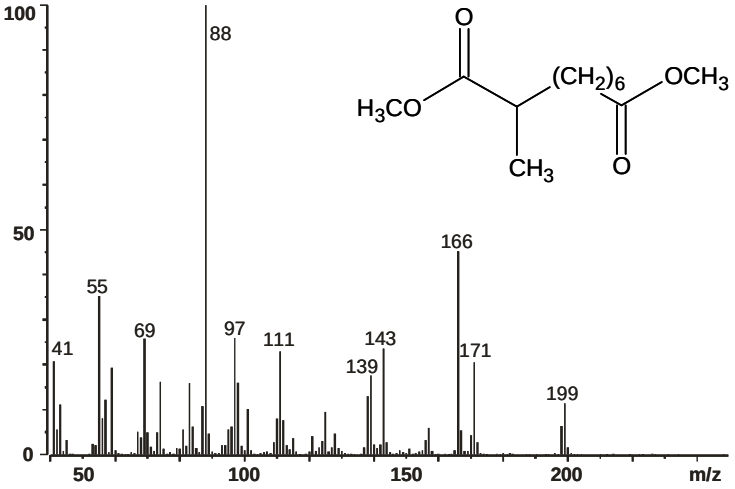
MW 186 9-oxononanoic acid, methyl ester



MW 200 9-oxononanoic acid, ethyl ester



MW 216 nonanedioic acid, dimethyl ester



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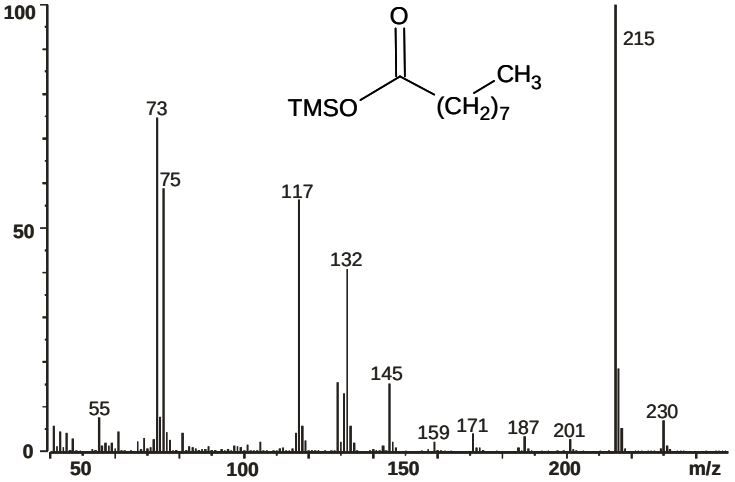
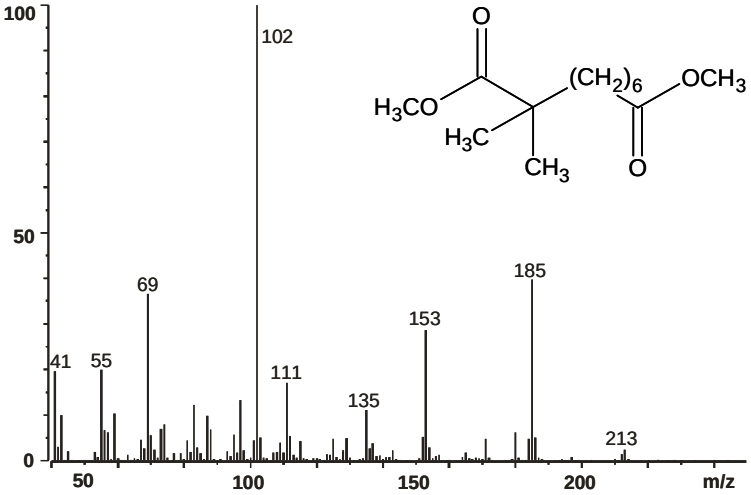
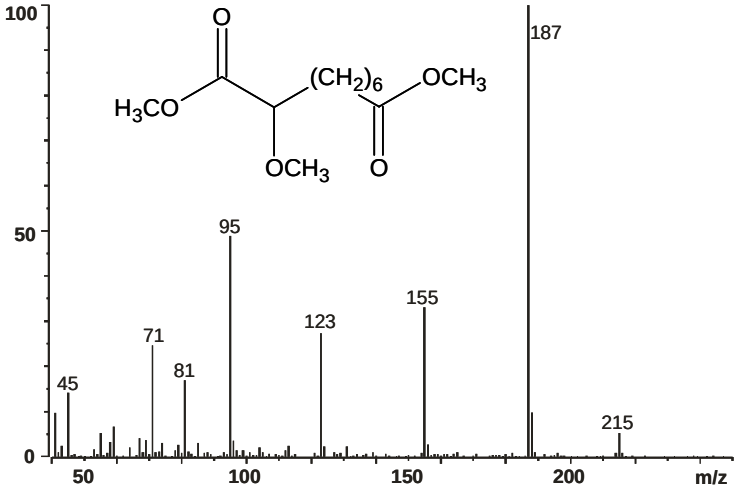


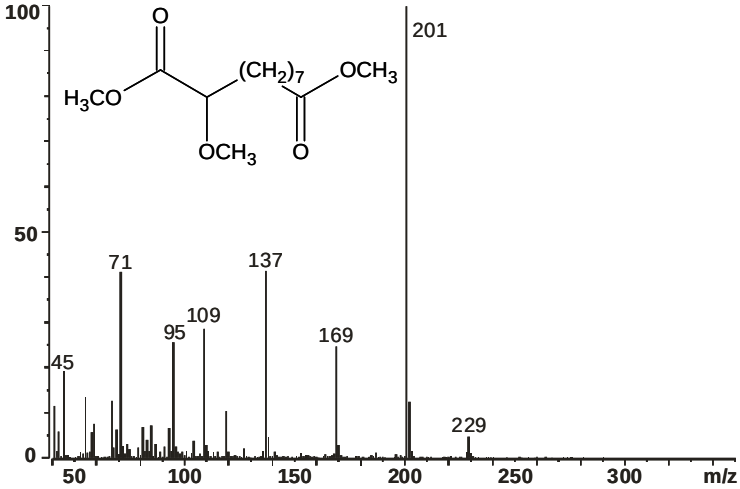
MW 230 nonanoic acid, trimethylsilyl ester



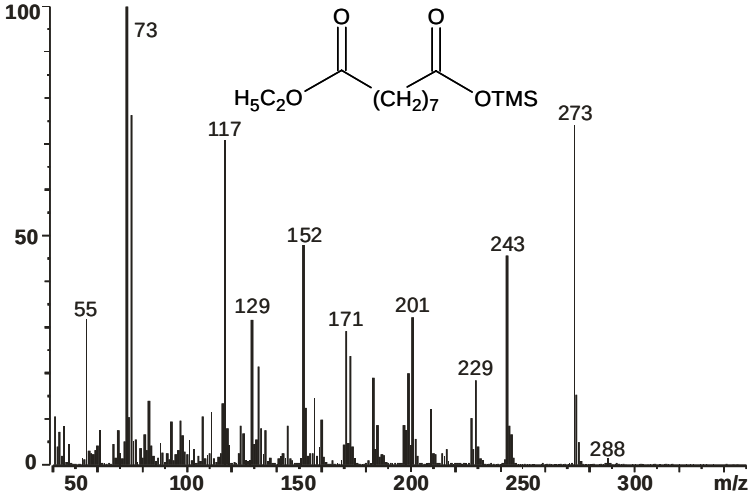
MW 244 2,2-dimethylnonanedioic acid, dimethyl ester



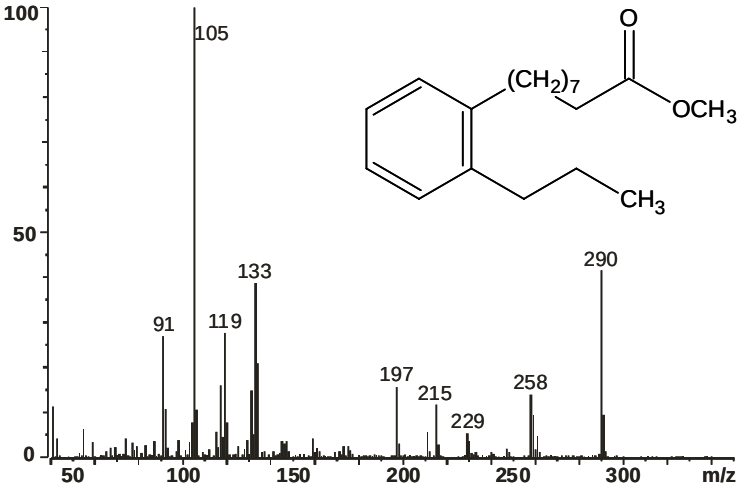
MW 246 2-methoxynonanedioic acid, dimethyl ester



MW 260 2-methoxydecanedioic acid, dimethyl ester



MW 288 nonanedioic acid, ethyl trimethylsilyl ester



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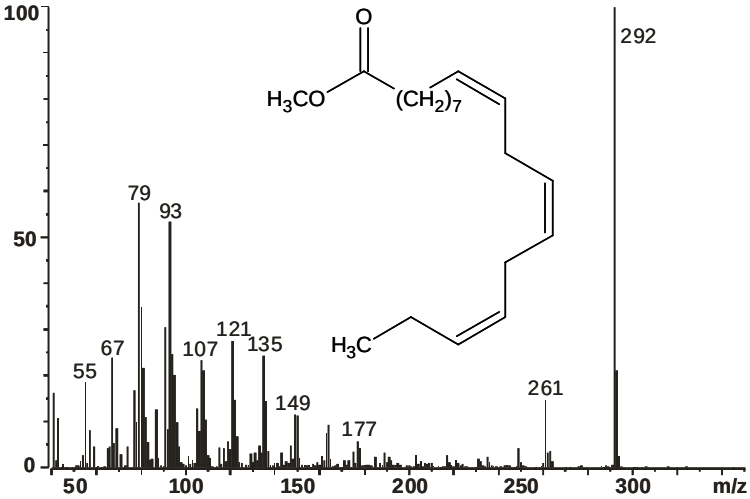
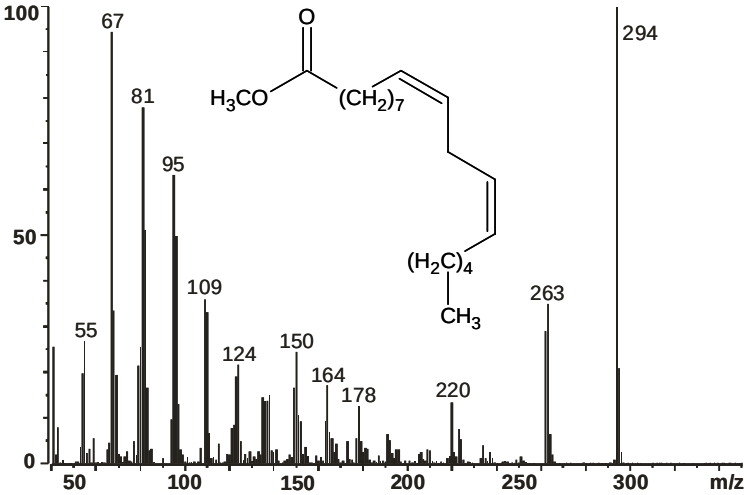


MW 292 octadeca-9,12,15-trienoic acid, methyl ester



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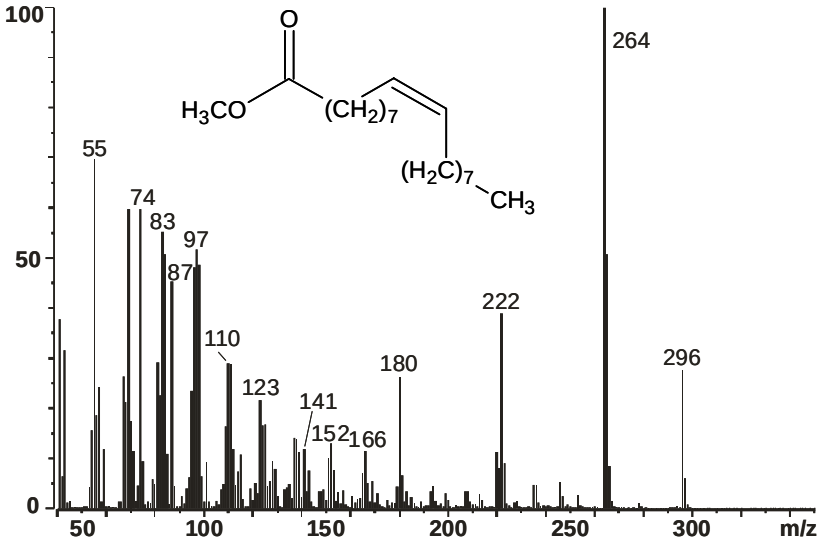
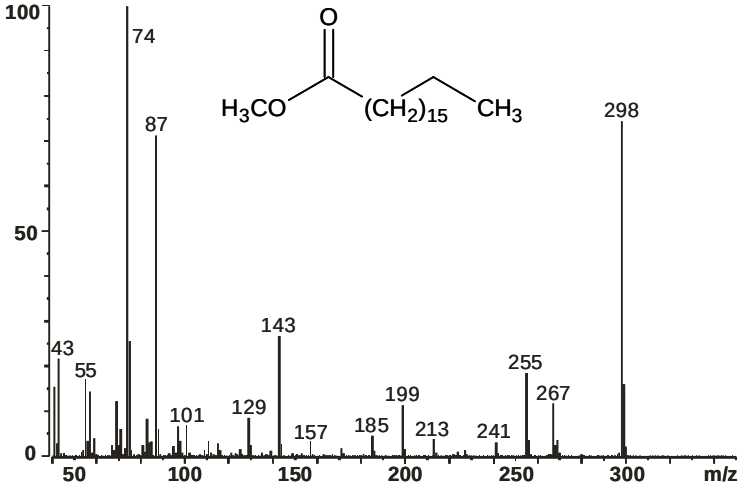
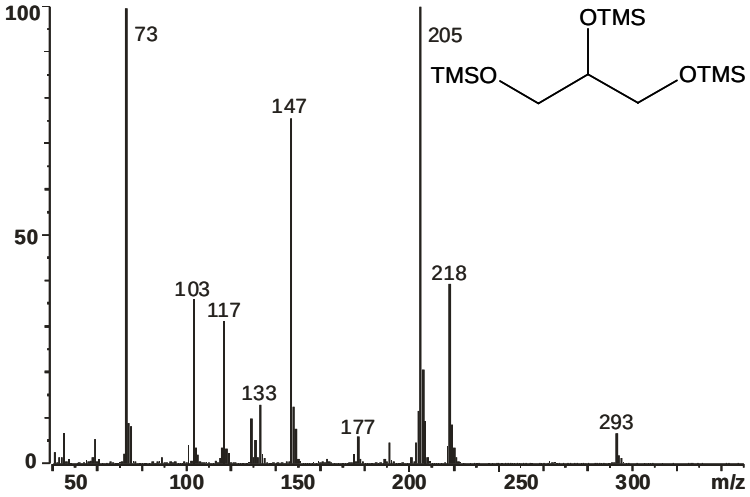


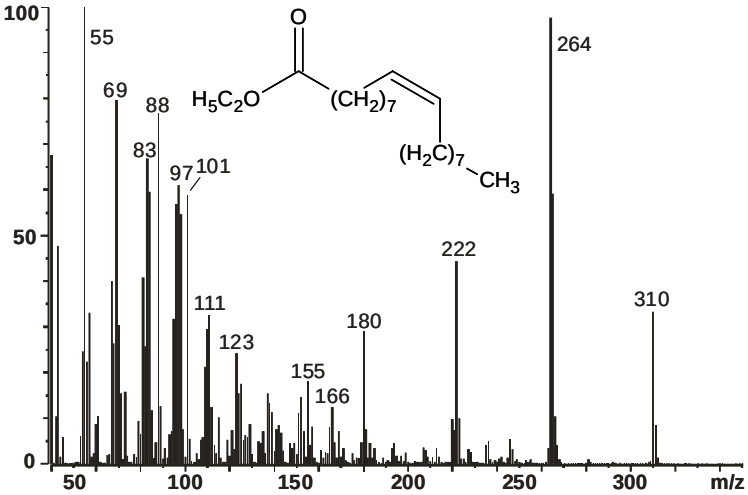
MW 296 octadec-9-enoic acid, methyl ester



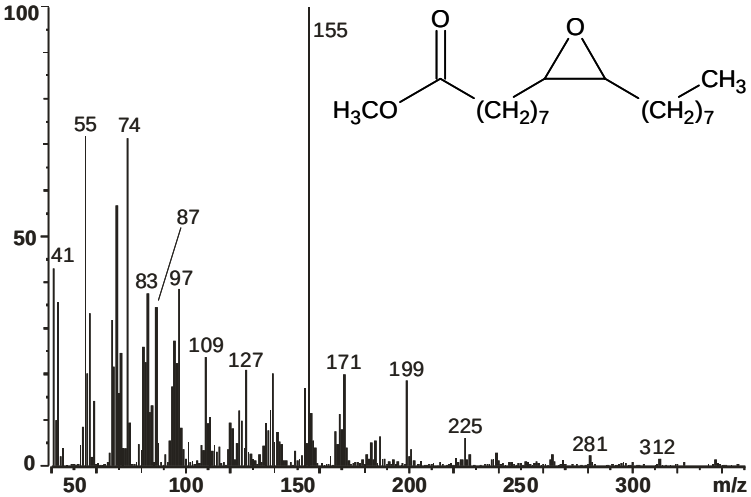
MW 298 octadecanoic acid, methyl ester



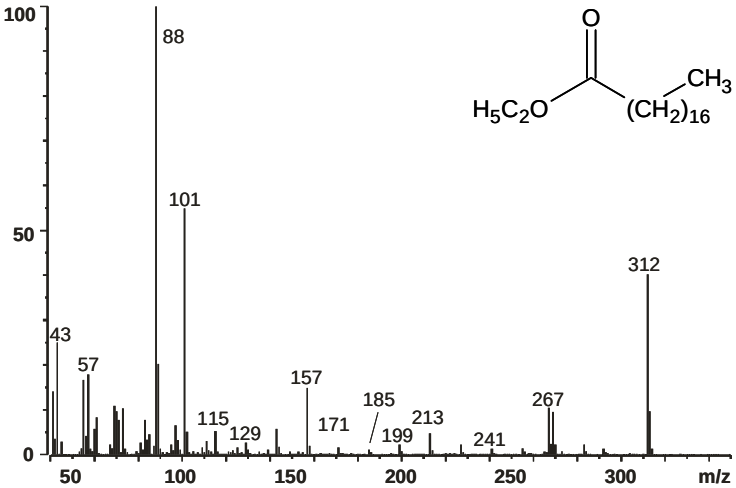
MW 308 1,2,3-tris(trimethylsilyloxy)propane



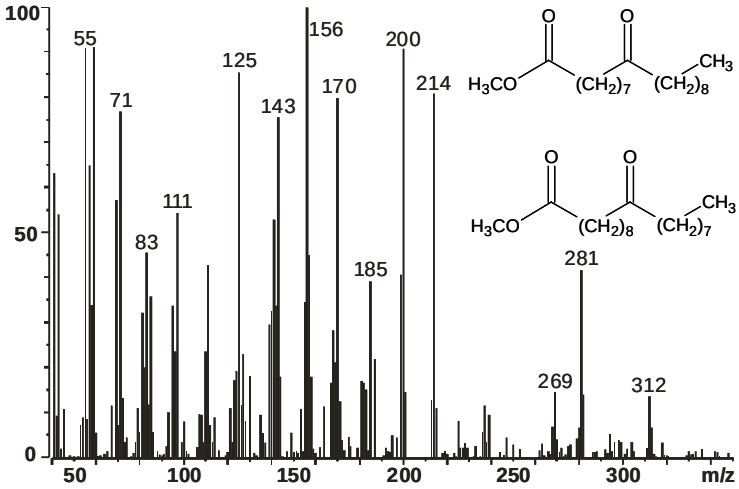
MW 310 octadec-9-enoic acid, ethyl ester



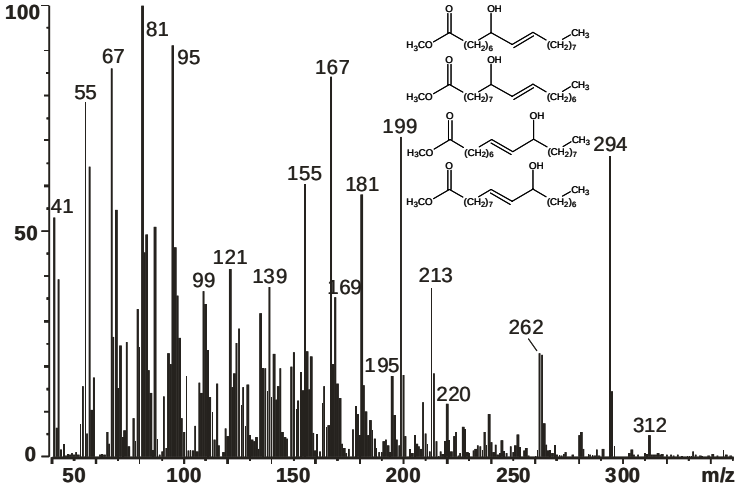
MW 312 9,10-epoxy-octadecanoic acid, methyl ester



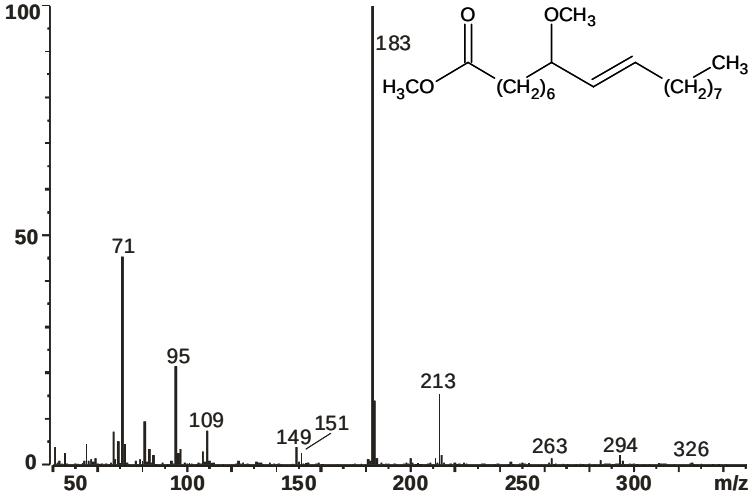
MW 312 octadecanoic acid, ethyl ester



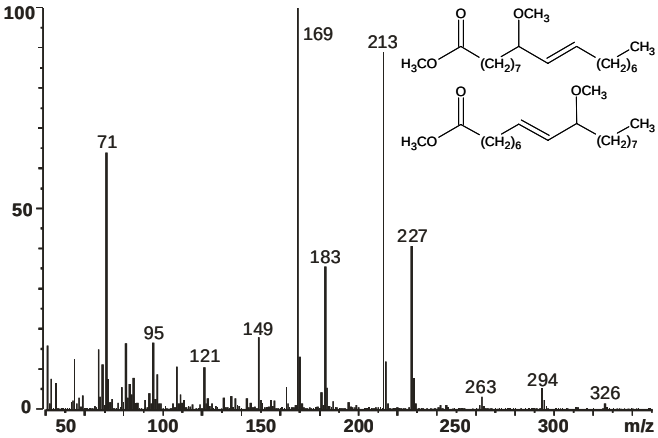
MW 312 9-oxooctadecanoic acid, methyl ester and 10-oxooctadecanoic acid, methyl ester



MW 312 11-hydroxyoctadec-9-enoic acid/ 9-hydroxyoctadec-11-enoic acid/ 10-hydroxyoctadec-8-enoic acid/ 8-hydroxyoctadec-10-enoic acid, all methyl esters



MW 326 8-methoxyoctadec-9-enoic acid, methyl ester



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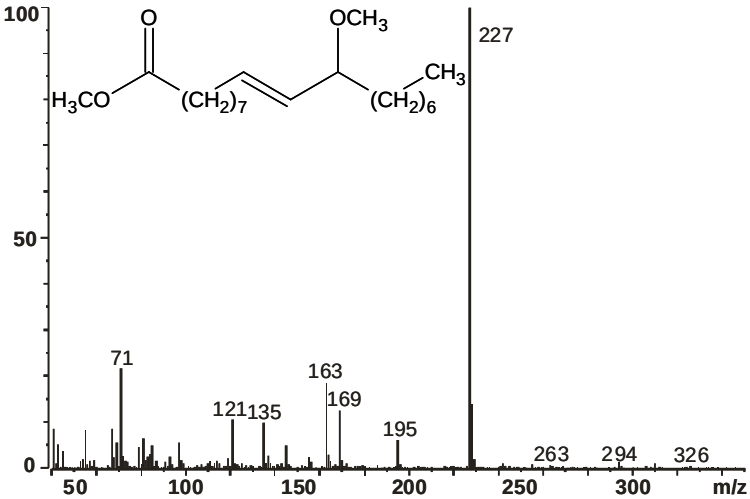
10-methoxyoctadec-8-enoic acid, methyl ester



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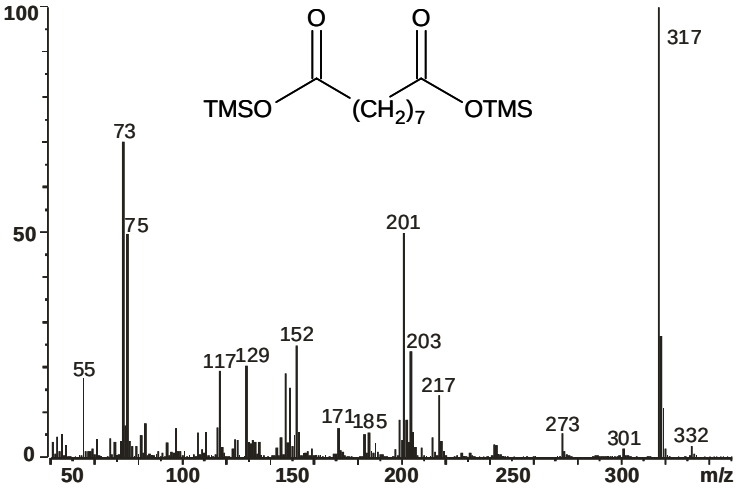
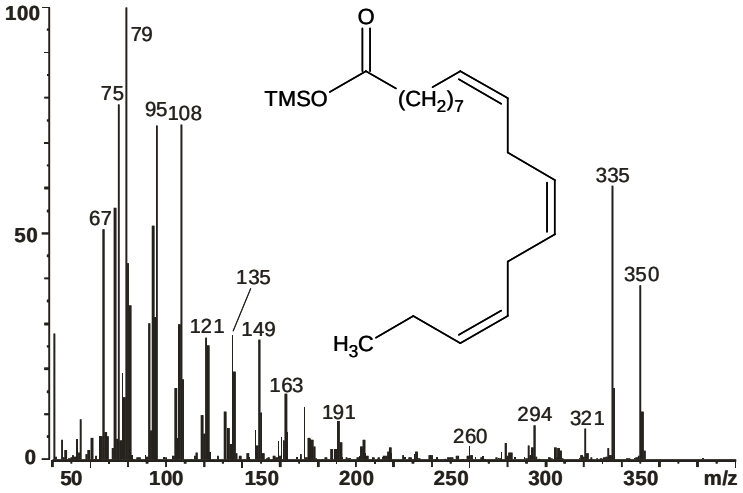


MW 332 nonanedioic acid, bis(trimethylsilyl) ester



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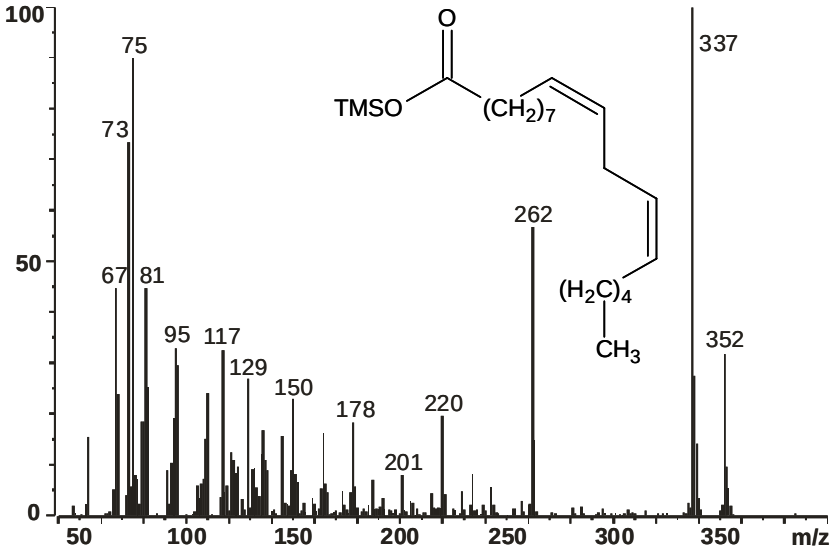

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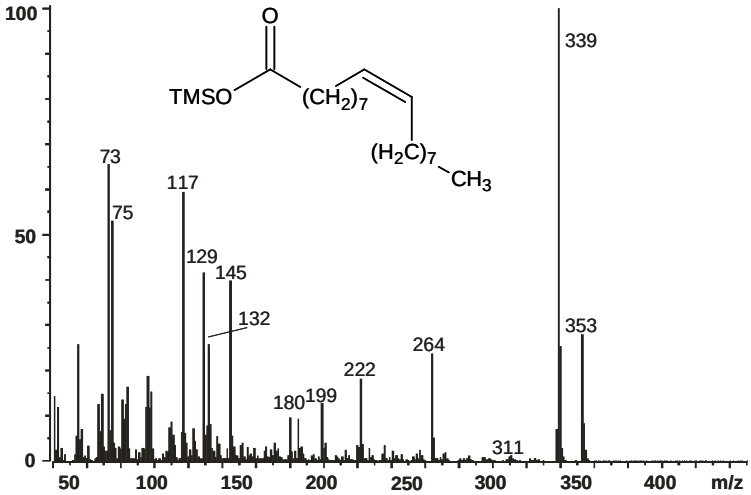

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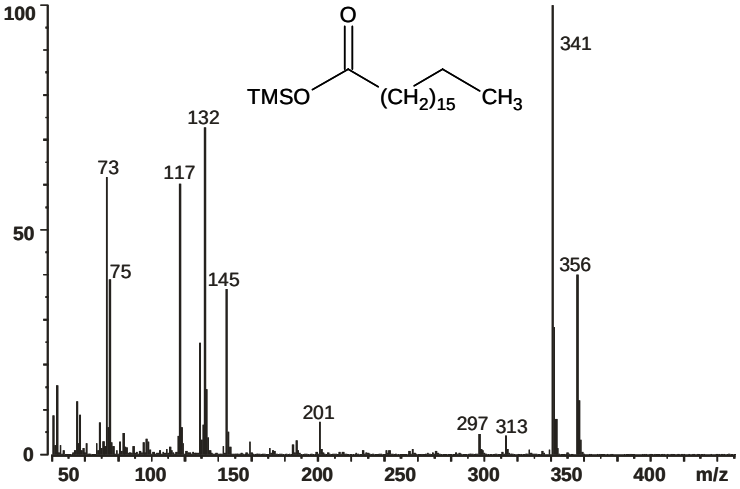
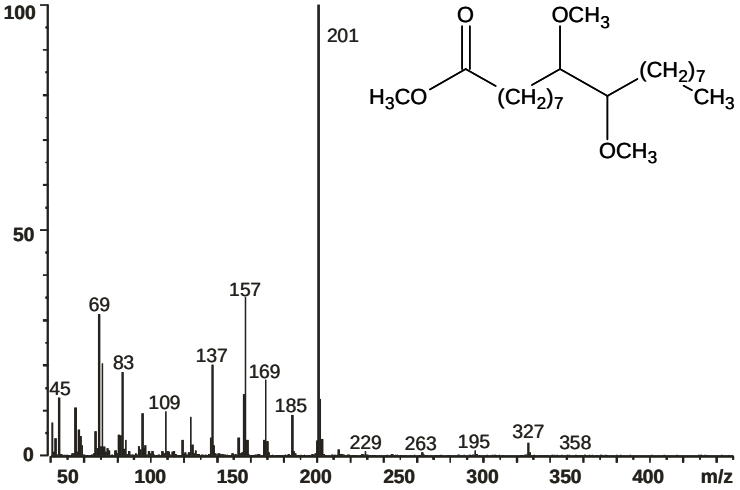
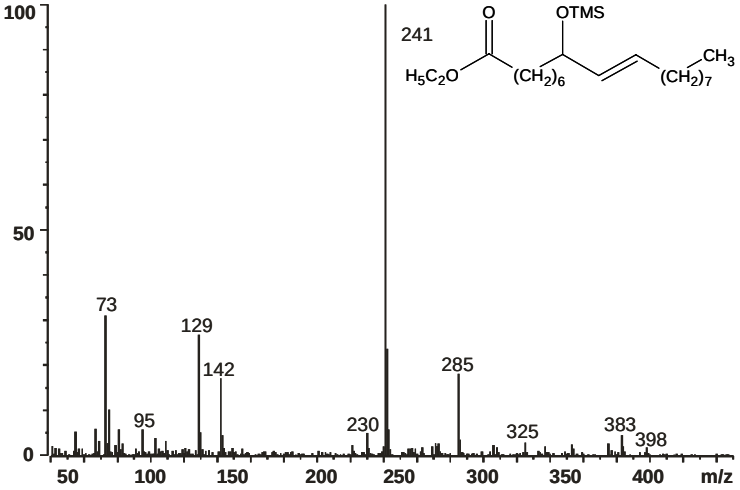


MW 356 octadecanoic acid, trimethylsilyl ester



MW 358 9,10-dimethoxyoctadecanoic acid, methyl ester



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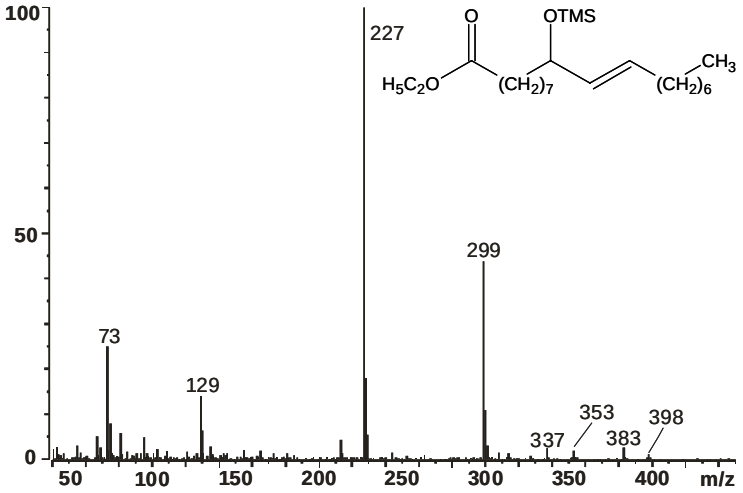

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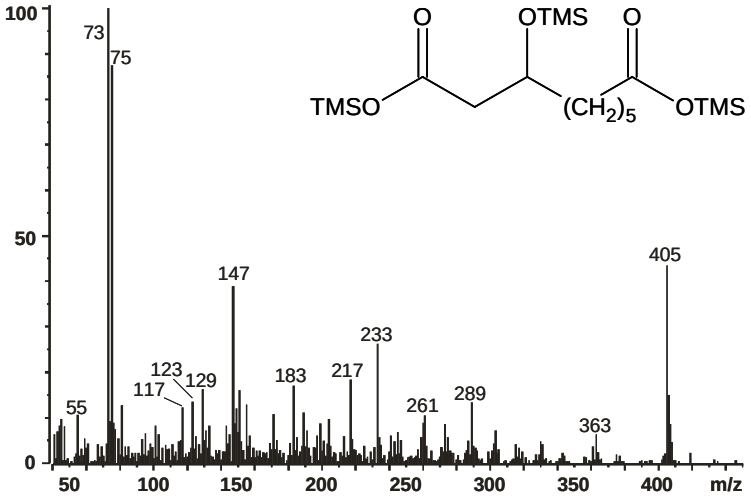

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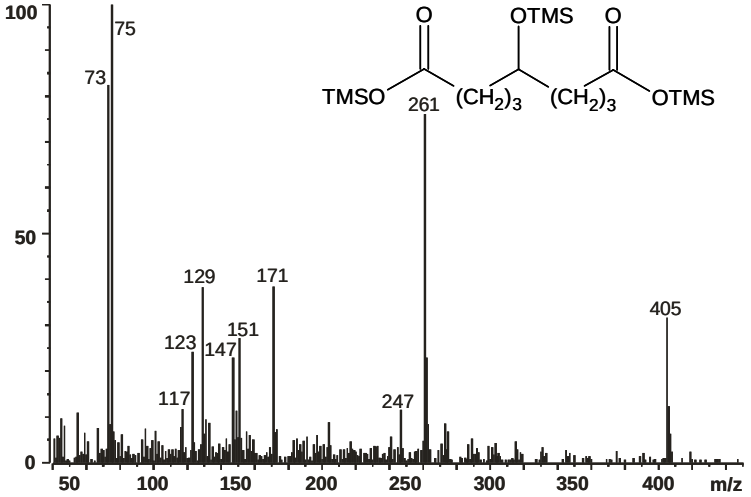

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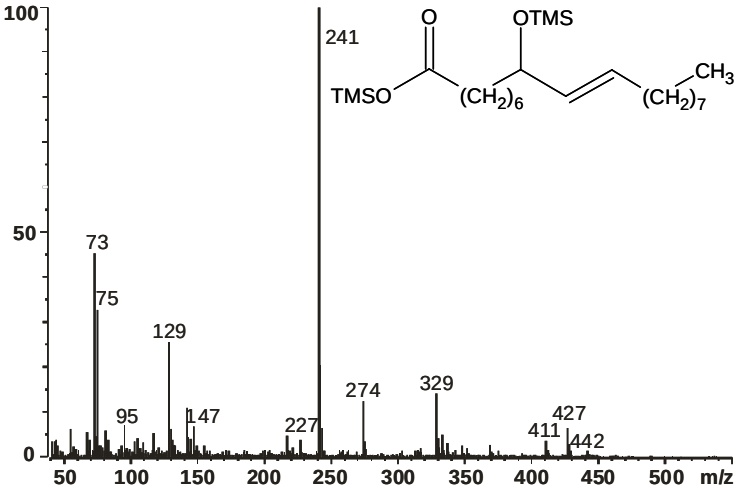

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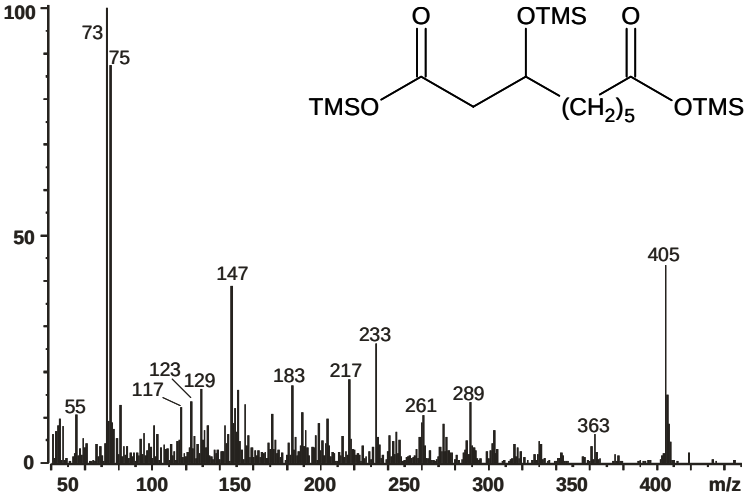

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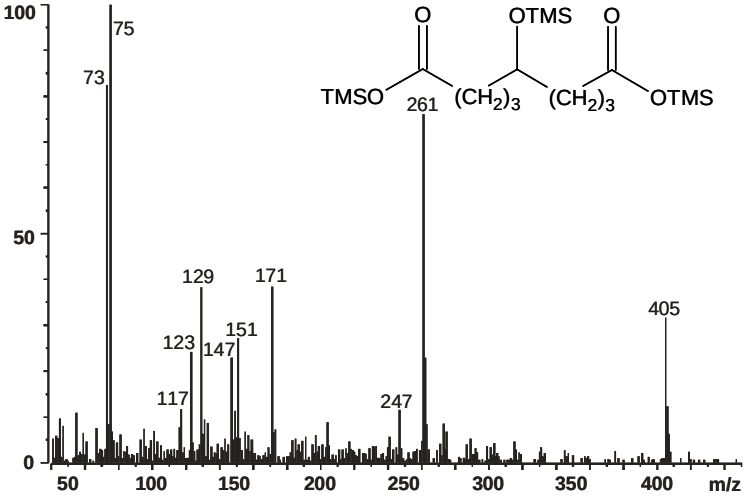

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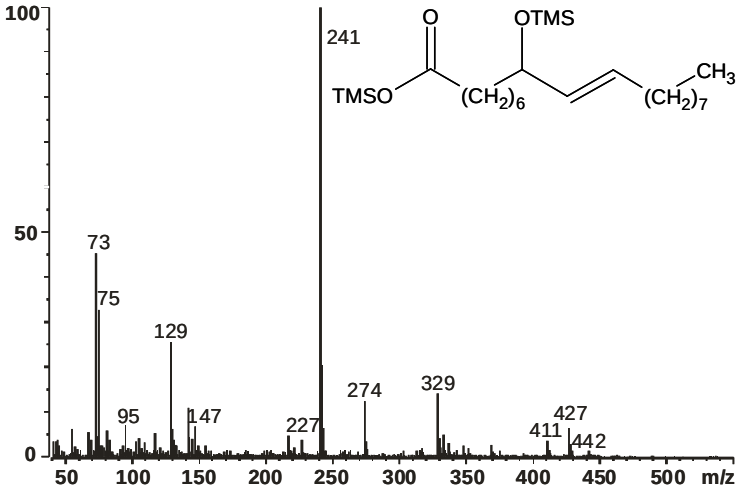

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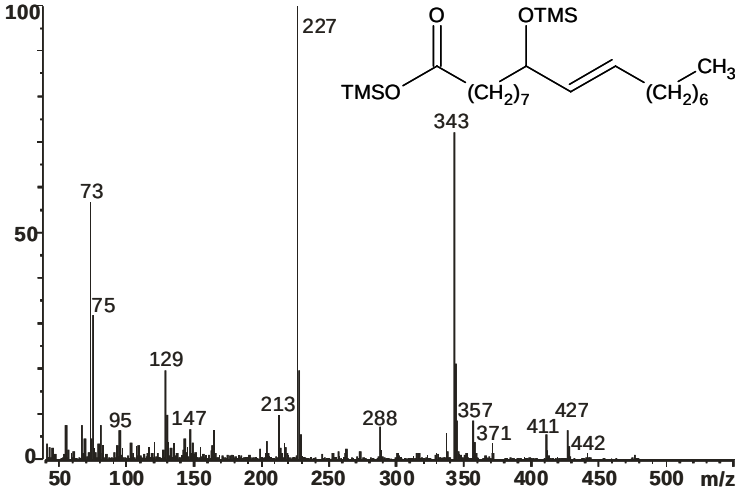

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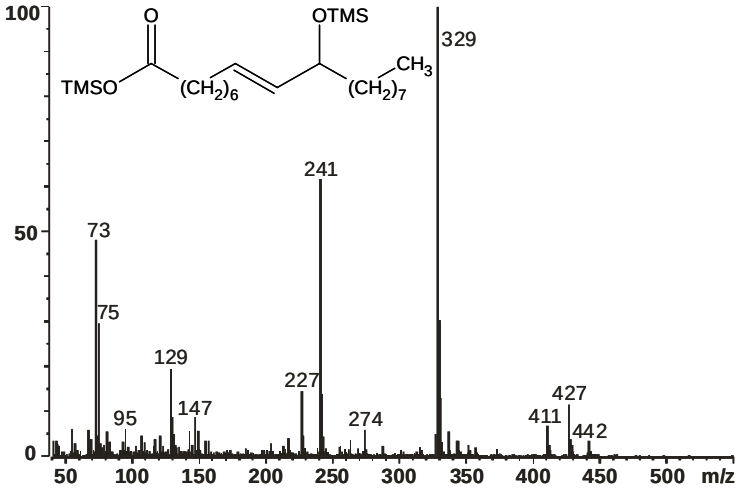

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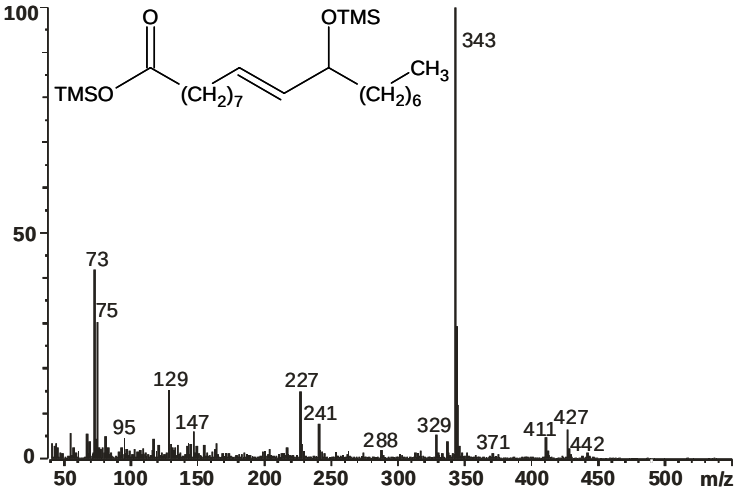

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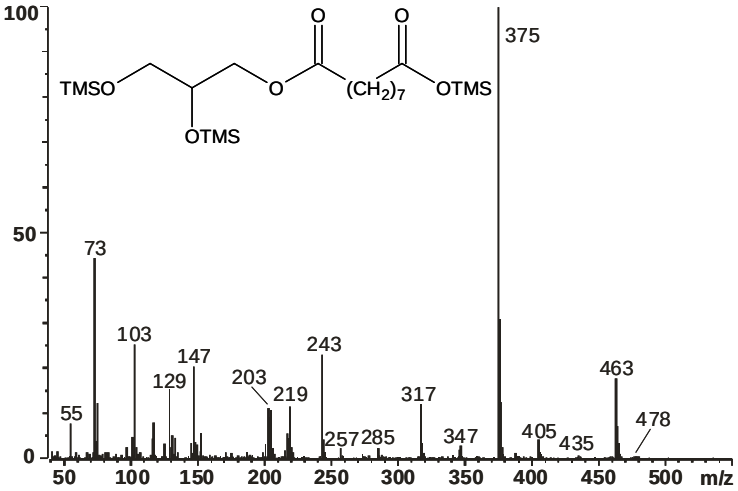

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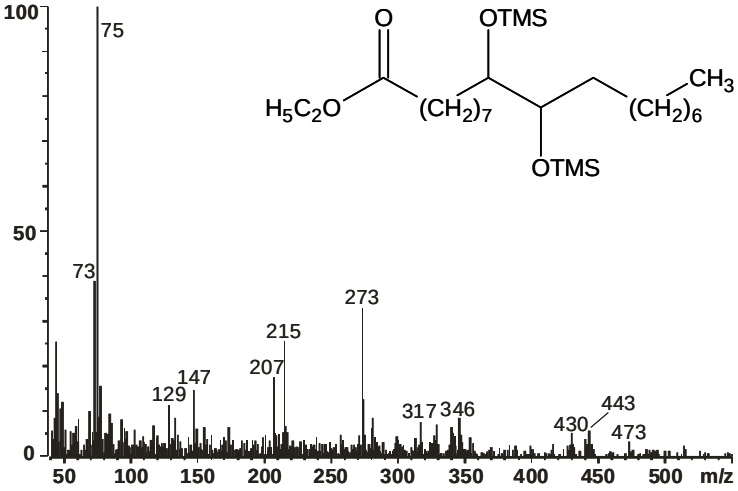

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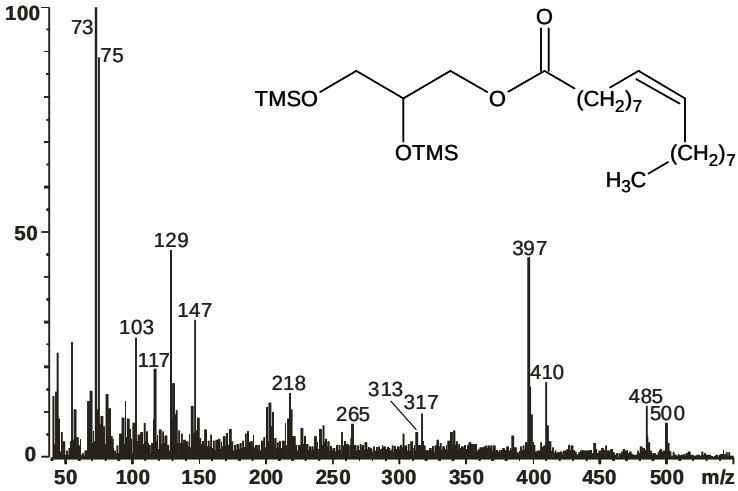

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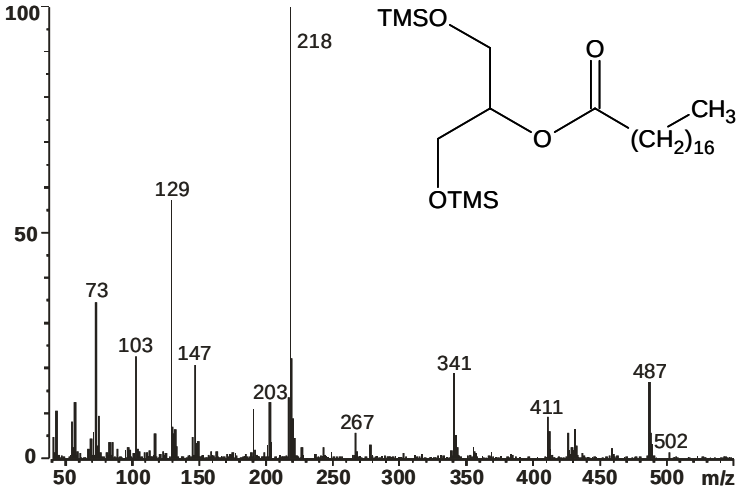

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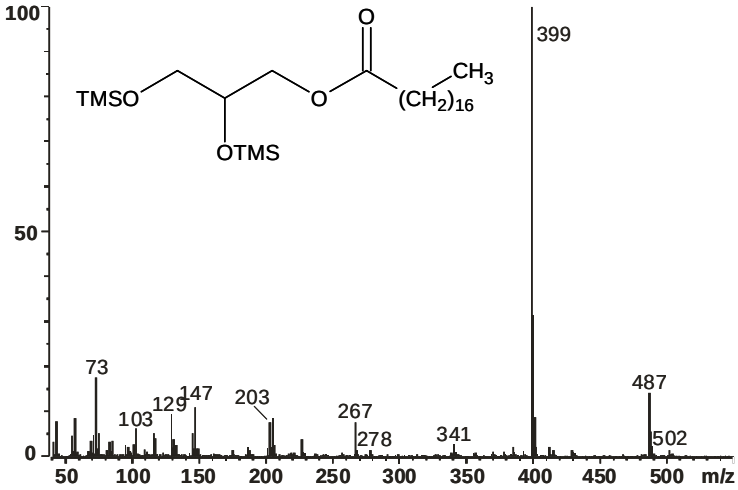

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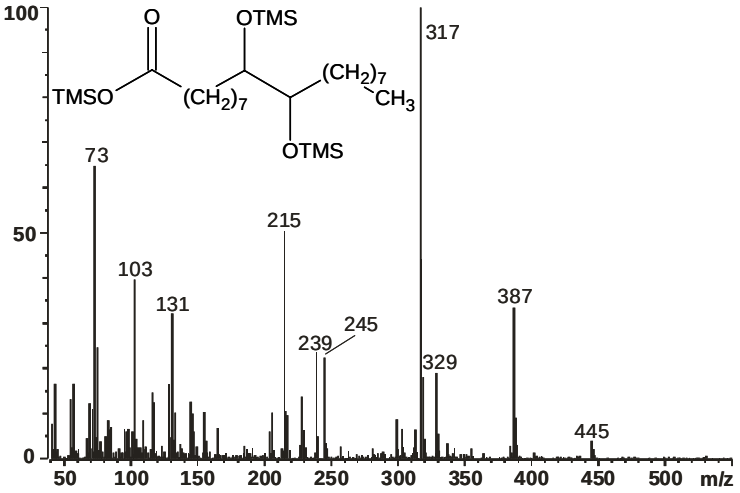

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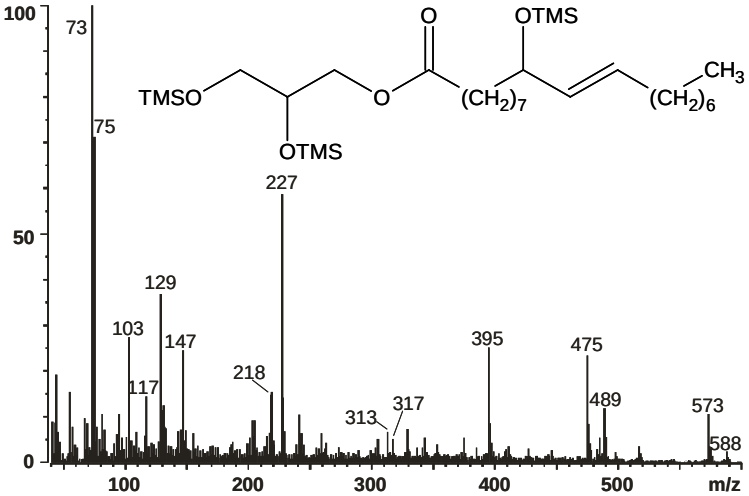

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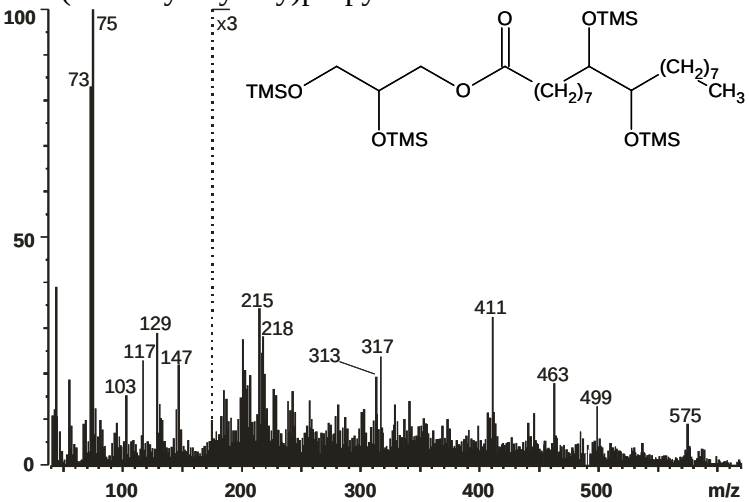

MW 678 9,10-bis(trimethylsilyloxy)octadecanoic acid, 2,3-bis(trimethylsilyloxy)propyl ester

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Glossary

amu	atomic mass unit
APCI	atmospheric pressure chemical ionisation
CID	collision-induced dissociation
Cu-Py	Curie point pyrolysis
DAG	Diacylglycerol
DCM	dichloromethane
DHB	2,5-dihydroxybenzoic acid
DTMS	direct temperature resolved mass spectrometry
EDX	electron dispersive X-ray spectrometry
EI	electron ionisation
ESI	electrospray ionisatie
EV	electronvolt
FA	fatty acid
FT	Fourier transfom
GC	gas chromatography
HPLC	high performance liquid chromatography
HPSEC	high performance size exclusion chromatography
ICR	ion cyclotron resonance
IM	infrared microscopy
In	initiator
IR	infrared spectroscopy
IV	iodine value
L	linoleic acid
Ln	linolenic acid
M	molecular ion
M^N	metal with oxidation state N
MAG	monoacylglycerol
MALDI	matrix assisted laser desorption/ionisation
MS	mass spectrometry
MS/MS	tandem mass spectrometry
m/z	mass over charge ratio
NMR	nuclear magnetic resonance
O	oleic acid
P	palmitic acid
PDA	photo diode array
PV	peroxide value
RH	compound with removable hydrogen
ROOH	hydroperoxide
S	stearic acid
SEC	size exclusion chromatography
SEM	scanning electron microscopy

SIMS	secondary ion mass spectrometry
TAG	triacylglycerol
THF	tetrahydrofuran
TIC	total ion current
TMAH	tetramethylammonium hydroxide
TMS	trimethylsilyl
TOF	time of flight
t_R	retention time
XRD	X-ray diffraction

Summary

For centuries, painting has been man's visual expression of his imagination and observation. Already in medieval times the oils of linseed, poppy seed, and walnut were used as a pigment binding medium to achieve this. These so-called drying oils were known to form dry films when exposed to air. Although oil paint has been used for centuries, there are still many questions about the properties, use and curing and ageing of the oil paint system, despite all our present physical and chemical knowledge and the availability of a wide range of analytical instruments and methods.

Once the oil painting has been created it is not a stable system and in time this system changes as a result of processes that take place on a molecular level. Some of these processes are necessary for the paint to harden, e.g. oxidation, but at the same time these processes also have a negative effect on the durability of both the organic and inorganic materials used to create the work of art. These changes are driven by external factors such as the temperature, humidity, light intensity and the presence of pollutants. Unfortunately, the processes inevitably will lead to the decay of the work of art, a process that is now understood to be irreversible. The fate of every oil paint will be different due to the large number of variables that determine its quality and drying properties and due to the methods of paint modification and application by the painter. However, by studying the changes on a molecular level in more detail, a detailed insight into the chemical condition of paintings is obtained. This will help in taking measures to reduce the rate of decay and it may help the conservator in deciding how to treat the work of art with a minimal effect on its delicate internal chemistry to prevent further damage.

This thesis contributes to the determination and understanding of the internal chemistry of oil paint systems at the different stages in their lifetime. Chapter 1 gives a short introduction into the historical development of (oil) paints, followed by the most relevant analytical research on (aged) oil paints that has been published and a short overview of the chapters to come. Subsequently, in Chapter 2 an extensive literature survey is given about the properties and chemistry of oil paints. This includes (traditional) manufacturing methods of the freshly pressed oils and their effects on the oil composition, the (chemical) drying of oil and oil paints, and the possible interactions with organic and inorganic pigments that are introduced into the oil. All this has been done with a special focus on the resulting chemical composition and the effects of ageing. The developmental stages of oil paint are summarised in a theoretical model that is introduced at the end of the chapter. The main idea put forward and explored further in this thesis is the transition from a cross-linked network of triacylglycerols to a metal bound

ionomeric network resulting from hydrolysis of the glycerol ester bonds and the interaction with pigments.

The work described in this thesis focuses on analytical chemical investigations of oil paint systems and the information that can be obtained from these studies in relation to a model of an ageing oil paint. Different analytical methods are used for the investigation of degradation products and cross-linked materials formed upon processing of a number of traditionally prepared linseed oils. The techniques included high performance size exclusion chromatography (HPSEC), Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography - atmospheric pressure chemical ionisation - mass spectrometry (HPLC-APCI-MS), direct temperature resolved mass spectrometry (DTMS), matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS. The results obtained are presented, compared and evaluated in Chapter 3. The various 19th century methods for preparation of the oil were found to have a great influence on the composition of the oil used for the paint manufacture. Different levels of both *cis-trans* isomerisation and oxidation could be observed. Oils that had been treated in the presence of lead show incorporation of higher numbers of oxygen atoms and in addition, divergent oxidation patterns were observed. Cross-linked products up to hexamers were preferentially observed in the heated oils.

The combination of desorption/pyrolysis and mass spectrometry for the fingerprinting of linseed oil and paints derived from it, is described in Chapter 4. This approach makes it possible to derive information on the chemical and physical properties of the dried oil paint. Direct temperature resolved mass spectrometry (DTMS) of relevant reference materials, e.g. free fatty acids, metal soaps, and triacylglycerols, as well as cured and aged paint is presented in the form of total ion currents and 16 eV ionisation mass spectra. The applicability, advantages and disadvantages of the technique are discussed in detail. It is shown that the DTMS technique not only provides information on the relatively volatile non-cross-linked fractions within a paint sample but also on the presence of non-volatile cross-linked material and/or metal salts.

Apart from cross-linked systems, low molecular weight breakdown products are formed within the oil paint due to oxidative cleavage of the triacylglycerols and the hydrolysis of their ester bonds. This fraction of the paint system has been characterised in more detail. A comparison of the analytical results obtained by on-column injection gas chromatographic (GC(/MS)) analysis after wet chemical work-up and by on-line Curie point pyrolysis GC/MS using *in-situ* (trans)methylation with tetramethyl ammonium hydroxide (TMAH) is presented in Chapter 5. A variety of paint samples of different age, history and compositions have been analysed and new compounds formed upon oxidation of the unsaturated fatty acids are presented that have not been reported before. The second part of Chapter 5 describes a study on the formation of -methylated fatty acids. These compounds are by-products formed upon the use of TMAH as a (trans)methylation reagent in combination with Curie-point pyrolysis GC/MS. Their formation could be related to the presence of methanol during the drying

step, before the thermal dissociation of the fatty acid TMAH derivatives. A hypothetical explanation is presented to account for the differences observed between the results when using aqueous or methanolic TMAH solutions.

The hydrolysis of ester bonds of the cross-linked oil triacylglycerols within the paint is addressed in Chapter 6. A two-step analytical derivatisation method has been developed suitable for the determination of the degree of hydrolysis of oil paint samples. In the first step, esterified fatty acids have been converted to their ethyl esters whereas in the second step both free fatty acids and metal soaps are trimethylsilylated. Subsequent analysis by GC/MS using on-column injection showed the ratio of the two derivatives, which gives information about the % of acylglycerols remaining in the paint. In this way, a general relationship is established between the age of the oil paint and the progression of the hydrolytic processes on the basis of the analytical results obtained on an extended range of (model) oil paint samples. The decrease in the relative amount of esterified fatty acids as the oil paint ages supports the idea of an aged oil paint as an ionomeric structure.

Chapter 7 reports two case studies in which the variety of analytical methods is applied to characterise the so-called bloom on the paint surface. The possible origin and theoretical considerations in relation to this phenomenon are discussed in more detail. The first case of bloom formation is studied on primed pieces of canvas in the possession of the 19th century American artist F. Church. The bloom phenomenon was investigated using several mass spectrometric techniques, including secondary ion mass spectrometry (SIMS), GC/MS with on-column injection, and DTMS. The bloom of the paint surface was shown to be composed of long-chain saturated C16 and C18 fatty acids and their lead salts. The degree of hydrolysis of the unusually oil-rich upper paint layer, was shown to be 85%. A very substantial part of these fatty acids is not trapped in ionomeric structures, which is the main cause why fatty acid crystals appear at the surface.

The second case involves a study on the exudation of whitish organic crystals observed on a number of paints of the painted works of art by the American painter Frank Stella. A number of analytical techniques are used to identify the paint materials, including DTMS, GC/MS, and scanning electron microscopy (SEM). The crystals were identified as saturated C16 and C18 fatty acids. The composition of the different paints is complex and includes a mixture of alkyd paint with another oil paint. One alizarin-lake coloured paint with exuded fatty acids shows a colour change that is related to an increase in internal alkalinity. The degree of hydrolysis of this relatively young paint is already 40%. The absence of particles with the capability to trap (negatively) charged FAs leads to an excess of mobile FAs that end up on the paint surface.

Gas chromatography, coupled with mass spectrometry is the main technique used in this thesis for the separation and identification of the low molecular weight compounds present within oil paints. Different derivatisation methods are used throughout the thesis and large number of compounds has been presented. An atlas of the 70 eV ionisation spectra of the most important compounds and their derivatives is provided as an appendix at the end of the thesis.

Samenvatting

Door de eeuwen heen zijn schilderijen gebruikt als manier om expressie te geven aan de verbeelding en het waarnemen van de mens. In de Middeleeuwen gebruikte men hiervoor al een mengsel van pigmenten met bindmiddelen als lijnzaadolie, papaverolie of walnotenolie. Het was bekend dat deze zogenaamde drogende oliën onder invloed van de lucht een elastische film vormen. Hoewel olieverf al eeuwen gebruikt wordt zijn er nog vele vraagtekens met betrekking tot de eigenschappen, het gebruik, het drogen en verouderen van het olieverfsysteem, ondanks al onze kennis op het gebied van fysische en chemische verschijnselen en de beschikbaarheid van een breed assortiment aan analytische apparatuur en methoden.

Vanaf het moment dat een schilderij gemaakt is, kan het beschouwd worden als een in de tijd veranderend systeem ten gevolge van processen die op moleculair niveau plaatsvinden. Een aantal van deze processen, zoals bijvoorbeeld oxidatie, zijn noodzakelijk om de verf te laten drogen. Aan de andere kant hebben dezelfde processen een negatieve invloed op de duurzaamheid van het organische en anorganische materiaal waaruit het schilderij is opgebouwd. De veranderingen die in de verf optreden worden vooral beïnvloed door factoren als temperatuur, luchtvochtigheid, licht intensiteit en de aanwezigheid van verontreinigingen in de lucht. Op den duur zullen die processen leiden tot het verval van het kunstwerk, hetgeen we met de huidige kennis als onomkeerbaar kunnen zien. Het samenspel van de afbraakprocessen hangt af van een groot aantal variabelen, die uiteindelijk bepalen wat de eigenschappen en de kwaliteit van de verf zullen zijn. Ook de methoden van verfbereiding en de manier waarop de verf door de schilder opgebracht worden zijn hierop van invloed. Door het grondig bestuderen van de veranderingen op moleculair niveau kan men echter een beter inzicht krijgen in de chemische toestand van de verf. Hierdoor zijn we beter in staat een inschatting te maken van hoe het schilderij behandeld moet worden bij een restauratie opdat geen of minimale schade aan het schilderij wordt toegebracht en een verdere afbraak vertraagd wordt.

Deze dissertatie wil een bijdrage leveren aan de analyse en begrip van de interne chemie van het olieverfsysteem gedurende de ontwikkeling in de tijd. In het eerste hoofdstuk wordt een korte introductie gegeven van de historische ontwikkeling van (olie)verf, gevolgd door een samenvatting van de meest belangrijke publicaties die verschenen zijn op het gebied van het analytische onderzoek olieverfschilderijen. Daarna volgt een beknopte weergave van de overige hoofdstukken. Vervolgens wordt in Hoofdstuk 2 een uitgebreid literatuuroverzicht gegeven van de eigenschappen en de relevante chemie van olieverf. Besproken worden de traditionele methoden van bereiding van de verse olie, de effecten daarvan op de samenstelling van de olie, het harden van de olie(verf) en de eventuele interacties van organische en anorganische pigmenten, die aan de olie worden toegevoegd.

Hierbij is vooral speciale aandacht besteed aan de resulterende chemische samenstelling en de effecten daarvan op het verouderingsproces. De verschillende ontwikkelingsstadia worden samengevat in een theoretisch model dat aan het eind van het hoofdstuk gepresenteerd wordt. De hoofdgedachte, de overgang van een netwerk van triacylglycerolen naar een metaal gebonden ionomeer netwerk, door de hydrolyse van de glycerol esterbindingen en de interactie met pigmenten, zal hierbij worden geponeerd en nader uitgewerkt.

Het werk dat in deze dissertatie wordt beschreven heeft vooral betrekking op het analytisch chemisch onderzoek aan olieverfsystemen en de informatie die kan worden verkregen met deze studies in relatie tot de veroudering van olieverf. Verschillende analytische methoden worden beschreven, die gebruikt zijn om de afbraakproducten en de netwerken die gevormd worden tijdens het bereiden van een aantal traditionele lijnzaadoliën te onderzoeken. De technieken waar hierbij gebruik van gemaakt is zijn hoge prestatie op molekuulgrootte scheidende chromatografie (HPSEC), Fourier transformatie infrarood spectroscopie (FTIR), hoge prestatie vloeistof chromatografie - atmosferische druk chemische ionisatie - massaspectrometrie (HPLC-APCI-MS), direct temperatuur opgeloste massaspectrometrie (DTMS), matrix geassisteerde laser desorptie/ionisatie vluchttijd massaspectrometrie (MALDI-TOF-MS) en electrospray-ionisatie Fourier-transformatie-ionen-cyclotron-resonantie massaspectrometrie (ESI-FTICR-MS). In Hoofdstuk 3 worden de verkregen resultaten vergeleken en geëvalueerd. De verscheidene 19de eeuwse methoden van oliebereiding blijken een grote invloed te hebben op de samenstelling van de olie gebruikt voor de verfbereiding. *Cis-trans* isomerisatie en oxidatie worden in verschillende mate waargenomen. Oliën, die behandeld zijn in aanwezigheid van lood vertonen bovendien een toegenomen inbouw van zuurstof en bovendien worden afwijkende oxidatie patronen waargenomen. Het zijn vooral de verhitte oliën die een toename in molecuulgrootte vertonen: er worden oligomeren tot aan hexameren gevormd.

De combinatie van desorptie/pyrolyse en massaspectrometrie als een “fingerprinting”techniek voor de analyse van lijnzaadolie en daarop gebaseerde verven wordt beschreven in Hoofdstuk 4. Deze benadering maakt het mogelijk informatie te verkrijgen over de chemische en fysische eigenschappen van de gedroogde verf. Direct temperatuur opgeloste massaspectrometrie (DTMS) van relevante referentiematerialen zoals vrije vetzuren, metaalzepen, en triacylglycerolen, alsmede gedroogde en verouderde verf, wordt gepresenteerd aan de hand van totale ionen stroomverdelingen en 16 eV ionisatie massaspectra. De toepasbaarheid, en de voor- en nadelen van de techniek worden hierbij in detail bediscussieerd. Er wordt aangetoond dat DTMS niet alleen informatie geeft over de relatief vluchtige niet-vernette componenten in een olieverfmonster maar ook de aanwezigheid van vernet materiaal en/of metaal zepen kan laten zien.

Naast de vernette systemen worden ook laag-moleculaire afbraakproducten in de verf gevormd ten gevolge van de oxidatieve splitsing van de triacylglycerolen en de hydrolyse van hun esterbindingen. Deze fractie van het verfsysteem is in nader detail gekarakteriseerd. De analytische resultaten verkregen met gaschromatografie en “on-column” injectie (GC/MS) na nat-chemische opwerking en met on-line Curie punt pyrolyse (Cu-Py) GC/MS met behulp van *in-situ*

(trans)methylering met tetramethyl ammonium hydroxide TMAH worden gepresenteerd in Hoofdstuk 5. Een verscheidenheid aan verfmonsters met verschillende leeftijd, geschiedenis en samenstelling zijn geanalyseerd en nieuwe, niet eerder aangetoonde verbindingen, gevormd door oxidatie van de onverzadigde vetzuren worden gepresenteerd. Het tweede gedeelte van Hoofdstuk 5 beschrijft een studie naar de vorming van -gemethyleerde vetzuren. Deze verbindingen zijn bijproducten gevormd door het gebruik van TMAH als transmethylerings reagens, in combinatie met Curie-punt pyrolyse GC/MS. De vorming van deze verbindingen kon in verband worden gebracht met de aanwezigheid van methanol tijdens de droogstap voordat thermische dissociatie plaatsvond van het TMAH-derivaat van het vetzuur. Een hypothetische verklaring wordt gegeven om het verschil te verklaren tussen de resultaten verkregen met een methanolische en eenwaterige TMAH oplossing.

De hydrolyse van de vernette triacylglycerolen in olieverfsystemen wordt aan de orde gesteld in Hoofdstuk 6. Een twee-stapsderivatisering is ontwikkeld, die geschikt is om de graad van hydrolyse te bepalen van olieverfmonsters. In de eerste stap worden veresterde vetzuren omgezet in hun ethylderivaten, waarna in een tweede stap zowel vrije vetzuren als metaalzepen worden getrimethylsilyleerd. Aansluitende analyse met behulp van een GC/MS en on-column injectie liet de verhouding van de twee derivaten zien, hetgeen aangeeft welk percentage van de acylglycerolen nog in de olieverf aanwezig is. Op deze manier kon op basis van de analyse van een uitgebreide collectie van (model)verfsystemen een algemene relatie verkregen worden tussen de leeftijd van de olieverf en de voortgang van de hydrolytische processen. De afname in de relatieve hoeveelheden van veresterde vetzuren tijdens het verouderingsproces ondersteunt het idee dat een verouderde verf als een ionomere structuur gezien kan worden.

Hoofdstuk 7 behandelt twee praktijkgevallen waarbij een witte kristallijne uitslag op het oppervlak van olieverf met een verscheidenheid aan analytische methoden is gekarakteriseerd. De mogelijke herkomst en de relevante theoretische overwegingen in relatie tot dit verschijnsel worden hierbij uitgediept. Het eerste geval dat onderzocht wordt betreft gegrondeerde doeken dat in het bezit zijn geweest van de 19de eeuwse Amerikaanse schilder Frederic E. Church. Het verschijnsel werd onderzocht met behulp van verscheidene massaspectrometrische technieken, inclusief secondaire ionen massaspectrometrie (SIMS), GC/MS met on-column injectie, en DTMS. De uitslag op de verf blijkt te bestaan uit verzadigde C16 and C18 vetzuren en hun loodzepen. De graad van hydrolyse van de bovenste, ongewoon bindmiddelrijke verflaag is vastgesteld en blijkt 85% te zijn. Een aanzienlijk deel van deze vetzuren wordt niet ingevangen in ionomere structuren, hetgeen een belangrijke reden is waardoor deze vetzuren als kristallen aan het oppervlak (gaan) verschijnen.

Het tweede geval betreft de vorming van witte, organische kristallen op het oppervlak van een aantal verven op moderne werken van de 20^{e} eeuwse Amerikaanse schilder Frank Stella. Een aantal analytische technieken, inclusief DTMS, GC/MS, en raster electronenmicroscopie (SEM), is gebruikt om de aard van de kristallen en de verf te bepalen. De kristallen blijken uit verzadigde C16 en C18 vetzuren te bestaan. De samenstelling van de verschillende verven is complex

en een mengsel van een alkydverf en een andere olieverf werden onder andere gevonden. Eén met alizarine gepigmenteerde verf waarop vetzuren uitkristalliseerden, liet bovendien een kleurverandering zien, die gerelateerd is aan de interne zuurgraad van het verfsysteem. De graad van hydrolyse van deze relatief jonge verf was al 40%. Het ontbreken van (pigment)deeltjes die de (negatief) geladen vetzuren kunnen invangen leidt al spoedig tot een overmaat aan vrije vetzuren die uiteindelijk op het verfoppervlak terechtkomen.

Gas chromatografie, gekoppeld met massaspectrometrie is de meest gebruikte techniek voor de scheiding en de identificatie van de laag-moleculaire componenten die in de verf aanwezig zijn. Hierbij worden meerdere derivatiseringstechnieken gebruikt, waarvan een groot aantal wordt gepresenteerd in deze dissertatie. Een atlas van 70 eV ionisatiespectra van de meest belangrijke en nieuwe verbindingen, en hun derivaten, is als bijlage aan het eind van de dissertatie toegevoegd.

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