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Study of Aging in Oil Paintings by 1D and 2D NMR Spectroscopy

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Nuclear magnetic resonance spectroscopy is proposed as an efficient analytical tool in the study of painted artworks. The binding medium from two original oil paintings, dated from the early 20th and the late 17th century, was studied via high-resolution 1D and 2D NMR, establishing the advanced state of hydrolysis and oxidation of the oil paint. Studies of the solvent-extractable component from model samples of various drying oils, raw oil paints, and aged oil paints allowed the definition of several markers based on the integral ratios of various chemical species present in the ^{1}H and ^{13}C NMR spectra. These markers are sensitive to hydrolytic and oxidative processes that reflect the extent of aging in oil paintings. The rapidity, simplicity, and nondestructive nature of the proposed analytical NMR methodology represents a great advantage, since the usually minute sample quantities available from original artwork can be subsequently analyzed further by other analytical techniques, if necessary.

The study and characterization of materials in painted works of art represents a challenge for the analyst, who is called to identity the chemical constituents of heterogeneous matrixes, composed of both inorganic and organic substances, to gain knowledge on the paint materials and techniques employed or assess the state of preservation of a painting.[1] In particular, organic materials used in paintings, such as for example terpenoid resins or drying oils, employed as varnishes and binders, respectively, are natural products (plant or tree extracts) and thus multicomponent mixtures whose composition is chemically rich and moreover subject to continuous changes over time because of ongoing degradation processes including oxidation, polymerization, and hydrolysis.[2,3] Obviously, powerful analytical techniques and methodologies are required to characterize such complex materials, which are typically available in very low quantities due to the strict limitations imposed on sampling works of art. In this respect, gas chromatography/mass spectrometry (GC/MS) in combination with various off-line[4−6] or on-line[7] derivatization procedures, direct temperature resolved mass spectrometry,[8] and FT-IR spectrometry[9] has been used extensively to study the composition and degradation of organic paint binders.[1] Static secondary ion mass spectrometry[10] and FT-IR microscopic imaging[11] have been recently used for the examination of paint cross sections, providing an insight to the stratigraphy of the paint by identifying the different pigments and the binding medium.

Despite its established and prominent role in lipids research,[12−14] NMR spectroscopy has been restricted in terms of applications to studies of the chemical drying of oils[15,16] and alkyd resins,[17−19] while the direct analysis of painted artwork has not received any attention. Recently, the introduction of gradient 2D NMR techniques has enabled the thorough characterization of complex organic mixtures, while in parallel, detection limits have been reduced significantly. Thus, we were prompted to examine the analytical capabilities of NMR spectroscopy in the study of original painted works of art and in this paper present results from the analysis of binding media in oil paints.

A paint consists of pigments, in the form of a fine powder, dispersed in a suitable matrix, which normally is called the binding medium, and can be of proteinaceous, oil, or synthetic polymer

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origin.[1,2] Linseed, poppyseed, and walnut oils have traditionally found most use in western European painting practice as the binding media of oil paints. Cross-polymerization of the triglyceride unsaturated fatty acids during drying solidifies the paint, thereby trapping and stabilizing the pigments. A varnish coating is applied on the paint, functioning as a transparent, protective layer that enhances color and gloss.[2] Storage and cleaning history, past restoration attempts, and the original materials used by the artist are all important factors that need to be understood for a proper analysis of an oil painting. The "mobile phase" of an oil paint sample is easily separated by solvent extraction from the insoluble, highly cross-linked "stationary phase"and the solid pigment grains.[6] It consists of tri-, di-, and monoglycerides of fatty acids, free fatty acids, and lipid oxidation products such as diacids and hydroxy acids.[6,7,20−22] The composition of the solvent-extractable fraction can provide information regarding the levels of hydrolysis[6] and oxidation that have occurred during aging of an oil painting and can be of great interest to art conservators, who often use solvents during cleaning and restoration of painted artworks.[20,23]

In this report, we present the study of the "mobile phase" from two oil paintings by high-resolution NMR spectroscopy, using an analytical protocol that contains no chemical workup and utilizes simply sonication in a deuterated NMR solvent for the direct extraction of organic material from the painting sample. The composition of the solvent-extractable fraction is directly determined by NMR spectroscopy and will be used to provide insight into the extent of hydrolysis and oxidation processes during aging of the painted works of art. A systematic study of drying oils, the aging of drying oil model films, artists' oil paints, and aged oil paints using the same analytical protocol was carried out before the analysis of the two original paintings, to afford a better understanding of the aging procedure of oil paintings. Gradient 2D NMR spectroscopy was used to assign the peaks observed in the ^{1}H and ^{13}C 1D NMR spectra of the paint extracts. The main advantage of NMR spectroscopy over GC/MS analytical methods involving derivatization procedures that have been used to study solvent extracts of paints[4−6] is its nondestructive character. Complications that may arise from incomplete derivatization reactions or side products during chemical workup are avoided, and the whole of the paint sample can be reclaimed and made available for further analyses upon completion of the NMR experiments, if required. The ability to apply a series of different analytical methodologies is critical, since the amount of chemical information obtainable from limited quantities of original material from works of art is thus maximized.

EXPERIMENTAL SECTION

Deuterated acetone-d_6 and methanol-d_4 were obtained from Aldrich Chemical Co., Inc. Films of ∼0.1-mm thickness were prepared by coating glass slides with linseed oil (LO), boiled linseed oil (b-LO), and poppy seed oil (PO). These model films were kept under room temperature conditions and monitored by NMR spectroscopy over a period of five years. Model oil paint samples in the form of thin coatings were investigated including titanium white (TW), lamp black (LB), cadmium red (CR), and lead white (LW). TW and LB were commercially available paints (Rowney Georgian) while CR and LW were made in the laboratory by mixing the corresponding pigment in powder form with LO. These samples were naturally aged in the laboratory over a period of five years.

Two paintings from a private collection were investigated (Figure S-6 in Supporting Information): the first one, Portrait of Young Man (oil on canvas) by S. Vandoros, a modern Greek painter, was dated to the early 20th century;[24] the second, The Duke (oil on canvas), by an unknown artist, was dated to the late 17th century.[24] Samples from the two original paintings were collected by carefully removing a small quantity (∼10 mg) of paint from the side of the painted canvas to avoid intervention with the top surface of the painting and minimize sampling of varnish material. For all samples studied in this report, the binding material was dissolved in 0.6 mL of acetone-d_6 and extracted for 30 min in an ultrasonic bath. The solvent was then filtered through glass wool directly into a 5-mm NMR tube.

^{1}H and ^{13}C NMR 1D spectra were obtained on either a Bruker MSL-300 or a Bruker AMX-500 spectrometer using standard instrument software and pulse sequences,[25] at a probe temperature of 26 °C. Quantitative ^{13}C NMR spectra were acquired using low (30°) flip angle and a long relaxation delay (10 s). For the ^{13}C NMR spectra, a line broadening of 1 Hz and drift correction were applied prior to Fourier transformation. Polynomial fourth-order baseline correction was performed before manual integration of all NMR spectra. Chemical shifts in acetone-d_6 are reported relative to internal TMS. ^{1}H−^{1}H homonuclear gradient COSY 2D NMR spectra[19,25] were obtained using 256 increments of 1K data points, 16 scans, and 4 dummy scans with a recycle delay of 1 s. ^{1}H−^{13}C heteronuclear gradient multiple quantum correlation (gHMQC) and multiple bond correlation (gHMBC) 2D NMR spectra were obtained using 128 increments of 1K data points, 16 scans, and 4 dummy scans with a recycle delay of 1 s. The gHMQC experiment was optimized for one-bond ^{1}H−^{13}C couplings of 140 Hz by setting the evolution delay to 3 ms. The gHMBC experiment used an evolution delay of 60 ms optimized for long-range ^{1}H−^{13}C J-couplings of ∼8 Hz.[25] Before Fourier transformation, all 2D data sets were zero-filled to a 1K × 1K matrix, and a square-sinusoidal window function was used for processing. Details of the ^{31}P NMR protocol used for the quantification of diglycerides (DG), monoglycerides (MG) and free fatty acids (FA) in LO and b-LO can be found elsewhere.[26,27]

RESULTS AND DISCUSSION

The NMR spectra of filtered acetone extracts from an oil paint, in the absence of a varnish, are expected to contain mainly signals from the oleaginous binding medium. Figure 1 presents the ^{1}H and ^{13}C NMR spectra of the acetone-d_6 extract of fresh lamp black oil color and a five-year-old paint film of the same material. Although the paint contains the pigment component made of soot

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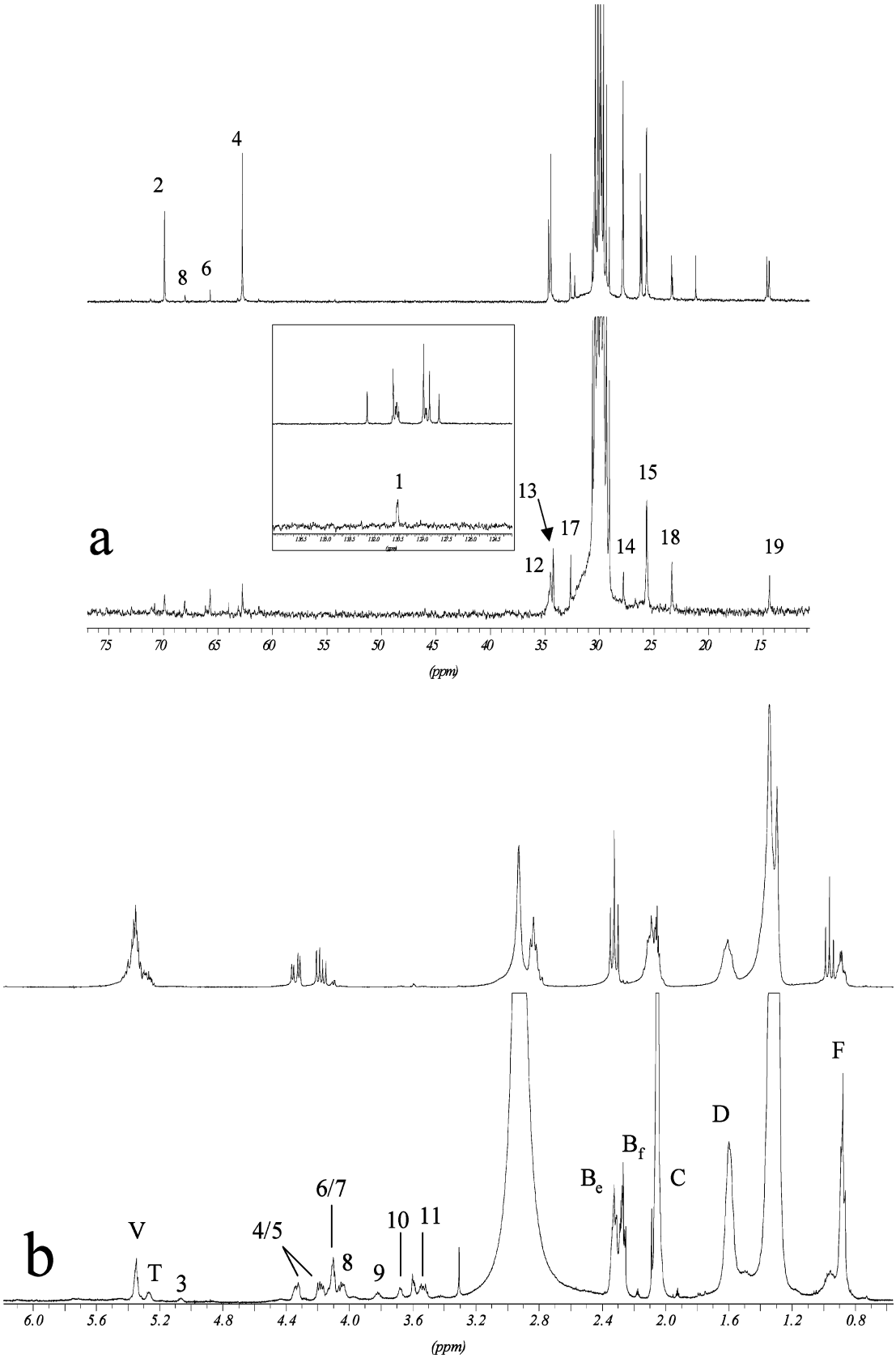

Figure 1. ^{13}C (a) and ^{1}H NMR spectra (b) of raw (top) and five-year-old (bottom) LB oil paint in acetone-d_6. Peak numbers and symbols according to Table 1. Peak C is overlapped by the residual solvent peak.

particles, no contribution to the NMR spectra of the acetone-d_6 extracts was observed as the particles were successfully removed by filtration. Table 1 summarizes the ^{1}H and ^{13}C NMR chemical shift data of triglyceride (TG), DG, MG, FA, diacid, and hydroxy (or oxo) acid (HA) moieties that contribute to the spectra of the aged paint. Peak assignment has been accomplished by a

Table 1. Chemical Shifts (δ) of the Main Resonances in the ^{1}H and ^{13}C NMR Spectra of Binding Material Extracted from Drying Oil Films, Oil Paints, and Paintings in Acetone-d_6 Solvent

	δ (ppm)			
	^{1}H	^{13}C	group	assignment
1 (V)	5.33	130.5	C*H*═CH	unsaturated vinyl protons
2 (T)	5.25	69.9	*CH*OCOR	triglycerides
3	5.05	73.0	*CH*OCOR	1,2-diglycerides
4	4.32/4.16	62.8	*CH*$_2$OCOR	triglycerides
5	4.34/4.13	63.1	*CH*$_2$OCOR	1,2-diglycerides
6	4.1	65.7	*CH*$_2$OCOR	1,3-diglycerides
7		66.1		1-monoglycerides
8	4.06	68.1	*CH*OH	1,3-diglycerides
			*CH*O─	hydroxy and oxo acids
9	3.8	70.8	*CH*OH	1-monoglycerides
10	3.66	61.2	*CH*$_2$OH	1,2-diglycerides
11	3.5	64.0	*CH*$_2$OH	1-monoglycerides
12 (B_e)	2.30	34.6	*CH*$_2$COOR	*sn*-2 esterified acids and diacids
		34.5		*sn*-1,3
13 (B_f)	2.24	34.2	*CH*$_2$COOH	free fatty acids and diacids
14 (C)	2.02	27.8	*CH*$_2$CH═	unsaturated fatty acids
15 (D)	1.57	26.3	*CH*$_2$CH$_2$COO─	all fatty acids and diacids
16 (E)	1.2−1.4	28−30	(*CH*$_2$)$_x$	all fatty acids and diacids
17	1.2−1.4	32.6	*CH*$_2$CH$_2$CH$_3$	oleic/linoleic acid
18	1.2−1.4	23.3	*CH*$_2$CH$_3$	oleic/linoleic acid
19 (F)	0.83−0.98	15.0	CH$_3$	all fatty acids

combination of ^{13}C-DEPT, gradient 2D NMR spectroscopy (Figure S-1, gCOSY; Figure S-2, gHMQC), and literature data where available.[12−14] The chemical shift data for TG and DG are in good agreement with literature data in a less polar solvent ($CDCl_3$).[28] The chemical drying of the paint by cross-linking is evident in the reduction of intensity of all peaks originating from linolenic and linoleic acid moeities.[28] The iodine value (IV), a measure of vinyl bond abundance in a given lipid mixture, can be easily calculated from the proton signal integrals of peaks V and F.[27] The intensity of peak F in the ^{1}H NMR spectra does not depend on the extent of TG hydrolysis or diacid formation, (the number of fatty acid end-methyl protons remains unchanged during these processes)[6] and thus can be used as a normalization factor for other peak integrals. Signals originating from various (oligomeric) glycerides, TG, 1,2-DG, 1,3-DG, and 1-MG, are well resolved in the region δ 61−73 (^{13}C, Figure 1a) and 3.5−5.25 (^{1}H, Figure 1b) of the paint film, while the fresh paint contains mainly TG and very few 1,3-DG, as expected.[27] Through NMR signal integration, the molar ratio of glycerides (TG + DG + MG = 1) can be calculated from both ^{1}H and ^{13}C NMR spectra. Thus, quantitative information on the extent of hydrolytic processes that have taken place in the oil paint during aging is obtained. The chemical shift of the methylene CH_2−COOH group of fatty moieties produces a signal (peaks B_f, 13 in Figure 1) separate from that of esterified CH_2−COOR groups (peaks B_e, 12 in Figure 1) in both ^{1}H and ^{13}C NMR spectra, as verified by gHMBC 2D NMR spectroscopy (Figure S-3 in Supporting Information). This provides an excellent means to quantify the ratio of free-to-total carboxyl groups in oil paint samples as B_f/B by ^{1}H and ^{13}C NMR spectral integration. It is worth noting that the direct use of quaternary carbon signals such as those of the carbonyl groups for quantitative integration is not experimentally realistic.[25] The ratio B_f/B does not depend solely on glyceride hydrolysis but is also affected by the extent of fatty acid chain scission that leads to the formation of C_8−C_9 diacids in oil paints,[29,30] since diacids contribute with two free carboxylic groups per molecule to B_f. Other signals in the ^{1}H NMR spectrum of the aged paint also depend on diacid formation and can be used to extract information on the extent of this procedure. The integral ratio $Di/FA = (3B - 1)/8F$ represents the ratio of diacids/fatty acids in the paint film extracts (see Appendix) and has a zero value for raw paints in which oxidative cross-linking has not been initiated. This ratio is expected to increase during aging because of diacid formation; however, diacids are themselves susceptible to further degradation to lower molecular weight products and volatiles. After subtracting the 1,3-DG methine proton contribution, signal 8 in the ^{1}H NMR spectrum can be used to evaluate the quantities of hydroxy acids present in the acetone extracts of the film samples, by normalizing its integral to that of the fatty acid methyl groups F, defining $HA/FA = 3(H - 8)/F$. Finally, the amount of triglyceride moieties in the extracts can be estimated by normalizing the integral of signal T (^{1}H NMR in Figure 1b) to F as $TG/FA = 9T/F$.

Drying Oils. To evaluate the applicability of the above analytical NMR approach to the study of solvent extracts of oil paintings, we first examined the cross-linking and film formation of some pure drying oils, which are the simplest model analogues of paints. Stand oils are processed drying oils produced by introducing a prepolymerizing step of heating in the absence of oxygen, leading to improved drying performance.[2] To examine the effect of oil processing on drying properties, two different sets of experiments were performed. In the first set, the composition of pure LO and b-LO was compared using ^{31}P NMR spectroscopy in conjuction with a well-known derivatization procedure[26] for the calculation of the absolute (μmol/g) DG, MG, and FA concentrations of the raw oils, while ^{1}H NMR was used to calculate the fatty acid composition and the IV of the oils.[27] It was found that the linolenic and linoleic acid content of b-LO was lower than that of LO, leading to a lower IV (169 vs 176), a result attributed to

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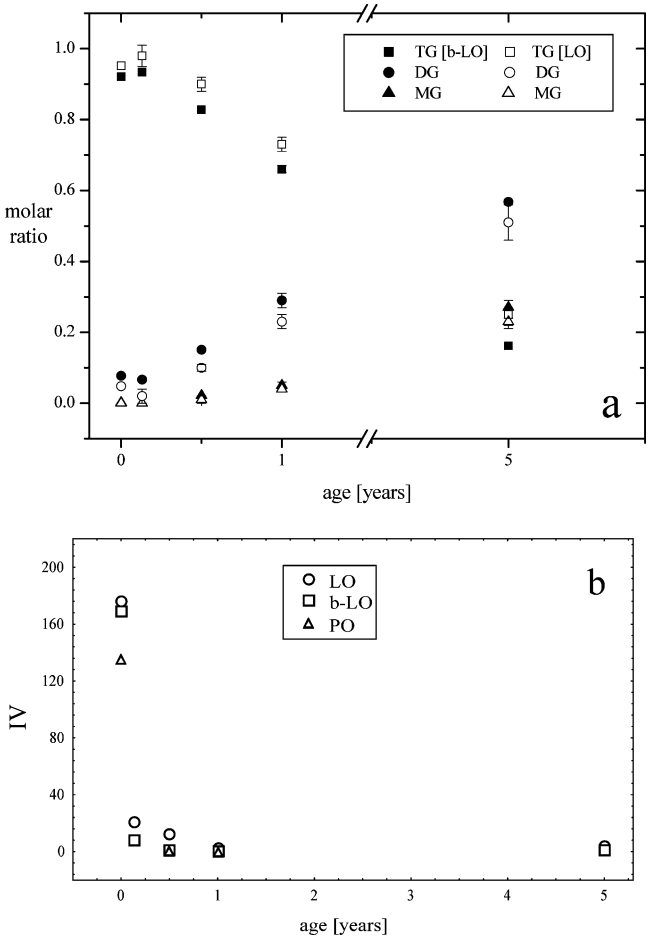

Figure 2. (a) Evolution of TG, DG, and MG in the acetone extracts of LO and b-LO) as a function of aging, as determined by ^{1}H and ^{13}C NMR spectroscopy. (b) Evolution of IV in the acetone extracts of LO, b-LO, and PO as a function of aging, as determined by ^{1}H NMR spectroscopy.

partial glyceride oligomerization and oxidation during processing.[2,31] Furthermore, the ^{31}P NMR measurements showed that in b-LO the DG and FA concentrations are much higher ([DG] = 124.8 μmol/g of oil, 77.3 μmol/g of oil in LO, and [FA] = 59.1 μmol/g of oil, 12.4 in LO). The equimolar increase in concentration for DG and FA indicates that they are produced by hydrolysis of triglycerides triggered by extensive heating of linseed oil during processing. The effect of heat-induced hydrolysis has recently been observed during linseed oil processing under various experimental conditions[31] and is further supported by the identification of small amounts (6.0 μmol/g of oil) of 1-monoglycerides, which are products of diglyceride hydrolysis, in our present b-LO sample, not observed in LO (data not shown).

The second set of experiments involved the preparation of model thin films of LO, b-LO, and PO on glass plates and their study as a function of aging under daylight conditions for five years. The evolution of IV, TG, DG, and MG in the acetone-d_6 extracts of the three types of oil film was followed by ^{1}H and ^{13}C NMR spectroscopy. Figure 2 presents the glyceride (a) and iodine value (b) evolution in the acetone-d_6 extracts of the LO and b-LO films as a function of aging time. As aging proceeds, the molar ratio of TG in the extracts is lowered, while the amounts of hydrolytic products DG and MG increase. The effect is more intense for b-LO compared to LO, while PO results were almost identical to LO (data not shown). It appears that the higher free acidity is responsible for the increase in the rate of hydrolytic procedures in the b-LO film. The double bond consumption during film hardening is very fast for all three films, and as a result, very few double bonds (low IV) remain in the binder mobile phase after about one year of aging. The values of other aging markers in the model films are reported in Table 2. The degree of hydrolysis of all the model oil films as drying proceeds is increasing, as reflected in the values of marker HFA (see Appendix), reaching values between 0.35 and 0.4 after five years of aging. The ratio Di/FA increases steadily as a function of aging for all three drying oil films, indicating that an increasing amount of diacids is present in their acetone extracts. This is also reflected on the constantly higher values of marker B_f/B compared to HFA. Hydroxy and oxo acids appear on the other hand to be less abundant, and only after several years of aging are appreciable amounts detected in the acetone extracts of the films (HA/FA < 0.1). Low amounts of alkanals and hydroperoxides were also detected in the ^{1}H NMR spectra of all the drying oil films. More detailed NMR analysis is, however, needed to isolate and identify the multitude of oxidized products of fatty acids in linseed oil films, especially since their concentration appears to be relatively small and variable with aging.

Finally, to test the extraction procedure, the five-year-old LO film was also extracted using MeOH-d_4 as a solvent and analyzed by NMR spectroscopy. No significant differences were found, as evidenced by the marker values reported in Table 2.

Model Paint Films. Samples from four different oil paint films, LB, TW, LW, and CR, left to age for a period of five years, were extracted and subjected to NMR spectroscopic analysis. LB and TW were also available as raw paints. The results of the study of all model paints are presented in Table 2, two separate analyses being performed for LB to check the repeatability of the analytical protocol. The initial IV values of 173 and 139 indicate that LB is based on linseed oil, while TW is based on an oil of low linolenic acid content, such as poppyseed oil. This was also verified by ^{13}C NMR spectroscopy and agrees with the product labels,"permanence 4*" for LB and "permanence 3*" for TW. The amount of triglycerides in the paint extracts is reflected in the ratio TG/FA, which takes values much lower than 1. This is expected because a relatively higher fraction of TG is incorporated in the solid polymer ("stationary") phase of the paint as aging proceeds, and thus, reduced quantities that can be extracted with a solvent remain in the "mobile" phase. The lowest amounts of TG are detected in LB, while the other three paints have similar TG/FA values, close to 0.5. The results reported in Table 2 indicate that hydrolysis and oxidation are ongoing processes taking place in all aged model paints studied. Lamp black paint appears to have suffered the highest levels of hydrolysis, with an HFA value close to 0.3, and has the highest ratio of diacids, as well as a significant amount of hydroxy and oxo acids. Titanium white appears to be the paint least affected by both hydrolysis and oxidation. Recently, an unexpectedly low degree of hydrolysis was reported for lead white pigmented oil paints,[6] while in another study, it was concluded that cadmium red has a delaying action on the drying

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Table 2. Characteristic Parameters (Age Markers) Determined from the ^{1}H and ^{13}C NMR Spectra of Binding Material Extracted from Drying Oil Films, Paints, and Original Paintings with Acetone-d_6 Solvent[a]

sample	age (years)	B_f/B[b]	HFA[b]	IV	Di/FA	HA/FA	TG/FA
LO	0.1	0.05 (0.01)	0.01 (0.005)	20.9	0.08	0.003	0.64
	0.5	0.13 (0.01)	0.03 (0.01)	12.3	0.15	0.01	0.89
	1	0.23 (0.02)	0.10 (0.01)	2.8	0.31	0.015	0.81
	5	0.47 (0.02)	0.32 (0.01)	4.9	0.62	0.09	0.30
	5[c]	0.48	0.35 (0.01)	4.0	0.47	0.09	0.31
b-LO	0.1	0.08	0.02	8.4	0.13	0.01	0.81
	0.5	0.21	0.06	1.4	0.38	0.02	1.1
	1	0.35 (0.04)	0.13 (0.01)	0.4	0.64	0.03	1.0
	5	0.53	0.37	1.3	0.59	0.08	0.23
PO	0.5	0.18	0.02	0.3	0.43	0.01	1.2
	1	0.29	0.10	0.1	0.56	0.05	1.1
LB	0	0.04	0.03	173	0.00	0.003	0.87
LB-1	5	0.46 (0.03)	0.28 (0.04)	15.4	0.19	0.10	0.28
LB-2	5	0.49 (0.02)	0.30 (0.01)	16.9	0.22	0.13	0.31
TW	0	0.00	0.01	139	0.00	0.00	0.97
TW	5	0.26 (0.02)	0.16 (0.01)	9.8	0.04	0.08	0.48
CR	5	0.39	0.18	0.93	0.18	0.10	0.55
LW	5	0.38	0.17	3.4	0.06	0.17	0.49
POYM[d]	~100	0.81 (0.01)	0.56 (0.01)	3.8 (0.6)	0.06 (0.04)	0.21 (0.03)	0.015 (0.008)
The Duke[d]	~300	0.89 (0.03)	0.48 (0.03)	2.0 (0.3)	0.27 (0.03)	0.12 (0.03)	0.016 (0.003)

[a] Standard deviations are given in parentheses. For abbreviations used, see the Experimental Section and text. [b] A plot of ^{1}H vs ^{13}C NMR-determined values of B_f/B and HFA has slope of 1.03, intercept of 0.04, $R^2 = 0.98$, so the average values of these parameters are reported, with standard deviation in parentheses. [c] Extraction with methanol-d_4. [d] Average values of three separate NMR analyses. POYM: Portrait of Young Man by Vandoros.

process.[32] The above observations are also suggested by the data presented in Table 2, although TW and LB were not included in either of the above studies. Clearly, a much wider sample range must be studied before definite conclusions can be made with respect to the effect of metal ions and pigments on oil paint hydrolysis and diacid formation.

Painted Artworks. Samples from two oil paintings dated to the early 20th (Portrait of Young Man) and the late 17th century (The Duke) were properly extracted and analyzed using the proposed NMR methodology in order to examine the usefulness of the parameters selected as aging markers for the study of samples from real paintings. Figure 3 compares the ^{13}C NMR spectra of the extracts of a series of aged linseed oil films with that of the late 17th century oil painting. As aging proceeds, the concentration of TG in the extracts drops, while the amounts of hydrolytic products (DG, MG, FA) increase; thus, glyceride signals appear in the glyceride region of the NMR spectra in Figure 3a, and the intensity of signal 13 attributed to free CH_2-COOH carbons is increased compared to esterified CH_2COOR (signal 12 in Figure 3b). Remarkably, no glycerides are evident in the ^{13}C NMR spectrum of the late 17th century oil painting, and only free CH_2COOH from (oxidized) free fatty acids and diacids can be observed. ^{1}H NMR with its higher sensitivity showed that glycerides *are* indeed present in both original paintings, but only in extremely small amounts not detectable by ^{13}C NMR. The ^{1}H−^{1}H gCOSY and ^{1}H−^{13}C gHMQC spectra of the acetone extracts of the painting by Vandoros are available as Supporting Information (Figures S-4 and S-5). MGs were found to be the most abundant glycerides in the two original paintings, in agreement with a recent GC/MS study of another 17th century painting.[7] The very low amount of TG present in the paintings' extracts is reflected in the values of marker TG/FA, which are ~50 times smaller than that of the five-year-old paint samples. Although the low TG content in the extracts after long aging times is expected (see Figure 2a), the extremely low concentration of DG and MG is evidence that these hydrolytic products are themselves hydrolyzed completely to produce glycerol and free fatty acids. The fact that no measurable amounts of glycerol were identified in the NMR spectra of the extracts of the two old paintings can be attributed to its slow evaporation from the painting during aging. van den Berg et al. were also unable to detect glycerol in a 17th century painting by transesterification Curie-point pyrolysis CG/MS.[7]

As evident from the high B_f/B and HFA values reported in Table 2, both original paintings are in an advanced state of hydrolysis. Free fatty acids comprise most of the fatty material extracted from the paintings, while the concentration of diacids is surprisingly low, especially in the "younger" ~100-year-old painting. This indicates that diacids in the course of extended aging are further degraded to low molecular weight products that slowly evaporate from the paintings, while saturated fatty acids survive the aging procedure. Another interesting observation is that the aged paintings have significant amounts of double bonds present even after such long periods of aging, and their 2D NMR spectra indicate they contain small traces of linolenic acid moieties (Figure S-5, Supporting Information). In support of this observation, oleic acid and linolenic acid have been recently detected in 19th century paintings by capillary electrophoresis.[33] At first, the relatively high IV values measured for the two old paintings appear to contradict the results of double bond consumption in Figure 2b, where IV values less than 1 are obtained after just one year of aging. One, however, should keep in mind that the model linseed oil films studied in this report were subjected to aging in an uncontrolled environment, under 14-h sunlight periods without the protection of a varnish coating, and their thickness was very

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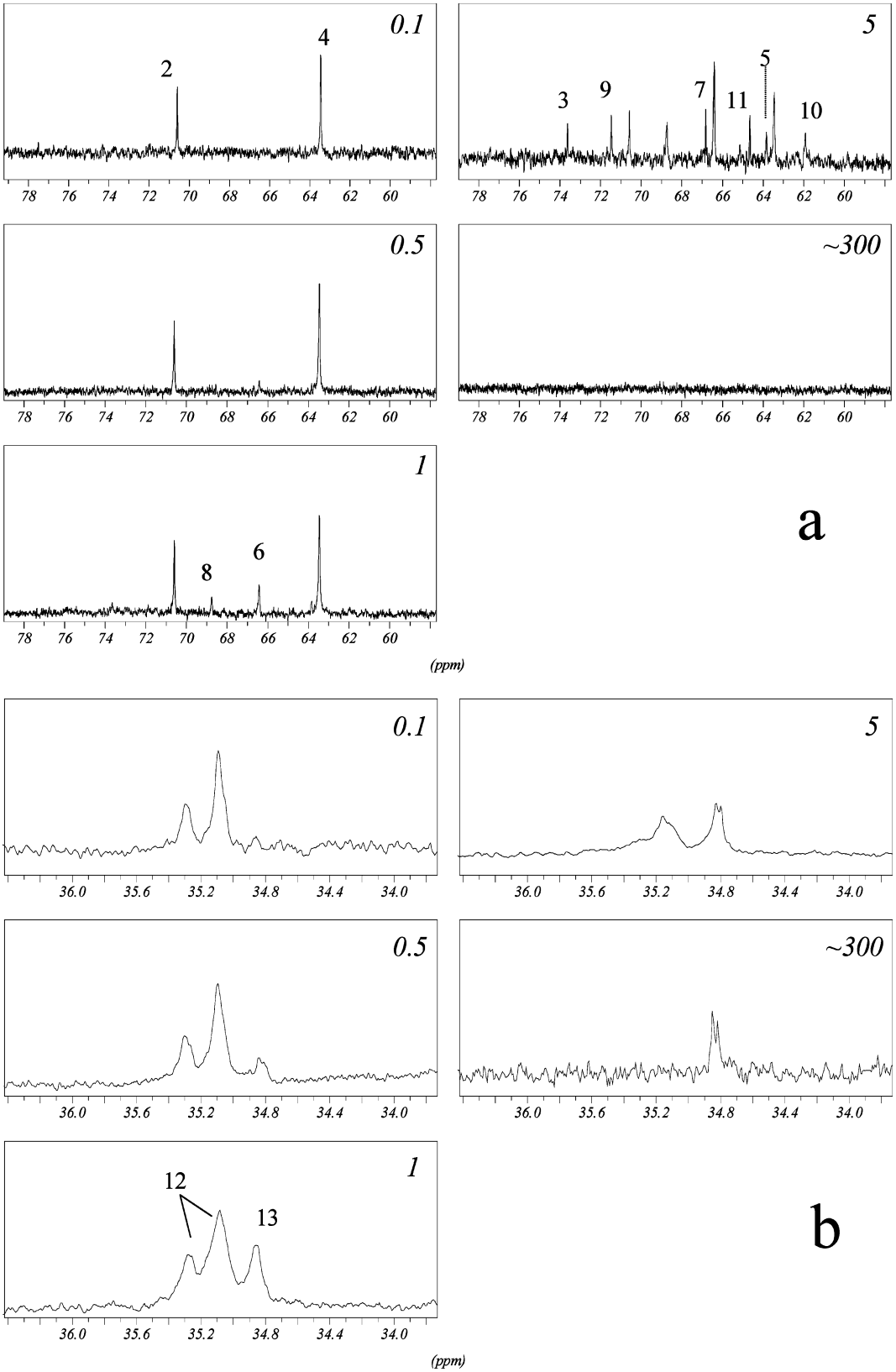

Figure 3. Expansions of the glyceridic and partial aliphatic regions of the ^{13}C NMR spectra of paint extracts in acetone-d_6, showing the evolution of glycerides (a) and signals 12 and 13 (b), the latter representing esterified and free carboxylic groups respectively, as a function of age in years (numbers in italics on top right of spectra). Peak numbering according to Table 1.

small. The high IV of the aged paintings corroborates the importance of layer stratigraphy in sampling[10] and indicates that binding material from deep layers of a painting is protected from direct light, allowing a small percentage of double bonds to be preserved even after prolonged aging. This has implications for the authentication of painted works of art, in that accelerated aging

might not be able to reproduce the chemical state of an original work aged under unknown conditions.[34]

Hydroxy derivatives of fatty acids are a class of compounds that have been identified by GC/MS on relatively young painter's films and in smaller concentrations in old paintings.[6,7] 9−10-Dihydroxyoctanoic acid is often detected when old oil paint layers are analyzed.[7] By comparing the integral of the methine CH(OH) proton (signal 8 in Figure 1b) of hydroxy acids to integral F of fatty acids, the molar ratio of hydroxyacids to total fatty acids in the extracts of the two original paintings was calculated as 0.21 ± 0.03 for the painting by Vandoros and 0.12 ± 0.03 for the older painting The Duke. These values are much higher than those of aged linseed oil films, but of the same order with the five-year-old model paints in Table 2. Overall, the parameter marker that best differentiates the two old original paintings from younger paints appears to be the ratio TG/FA, which assumes much smaller values in the former (by a factor of ∼50). Such low TG/FA values, combined with high values for B_f/B and HFA appear to be indicative of oil paintings of significant age.

An important issue with respect to the NMR analysis of works of art relates to the quantity of material required. In this study, it was demonstrated that using conventional NMR probes a few milligrams of paint are enough to provide a good characterization of the paint's "mobile phase", which represents 1−5% of the total paint sample weight. However, nowadays, cryogenically cooled NMR probes are available that offer on average a 4-fold increase in S/N ratio over that of conventional probes,[35] while capillary probes with 2.5-μL NMR-active sample volume have made possible the acquisition of ^{1}H NMR spectra of 1 μg of analyte (5 nmol) in ∼1 min.[36] It is estimated that, taking advantage of advanced probe technology, the paint sample size can be reduced to 0.1−0.2 mg, bringing NMR on par with current analytical techniques used in the study of works of art.

CONCLUSIONS

The results presented demonstrate the capabilities of NMR spectroscopy with respect to its use as an analytical tool to study the organic components in painted works of art. More specifically, by employing 1D and 2D NMR spectroscopy it was possible to characterize the chemical composition of the "mobile phase" (solvent-extractable component) of the binding medium from oil paintings and reveal important information regarding the ongoing processes of hydrolysis and oxidation. During aging, the triglyceride content of a paint extract appears to decrease. On the other hand, the concentrations of the di- and monoglyceride components reach a maximum in the extract, followed by a slow decrease with further aging, since these compounds suffer further hydrolysis to glycerol and free fatty acids. The amount of free fatty acids increases monotonically with aging and reflects the degree of hydrolysis of the oleaginous binding medium. Our studies with a series of model paint samples indicate that a low ratio of TG/FA in combination with high values of B_f/B and HFA represents an accurate set of markers to characterize the extent of aging of an oil paint.

The use of sophisticated cryoprobes and hyphenated LC NMR[35] techniques are expected to minimize the amount of material needed to perform NMR analysis of oil paintings and make NMR a more favorable technique for the analyst, in view of its rapidity and experimental simplicity. Thus, modern NMR spectroscopy has the potential to become an important analytical tool for the detailed study of painted artworks. Further work, involving the study of acrylic and tempera paintings, varnishes, and the utilization of hyphenated LC NMR techniques is in progress.

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SUPPORTING INFORMATION AVAILABLE

A listing of five additional figures. Figure S-1: ^{1}H−^{1}H homonuclear gradient COSY 2D NMR spectrum of the acetone-d_6 extract of aged LB paint; Figure S-2: ^{1}H−^{13}C heteronuclear gradient HMQC 2D NMR spectrum of acetone-d_6 extracts of aged LB paint. Figure S-3: Carbonyl region of the ^{1}H−^{13}C heteronuclear gradient HMBC 2D NMR spectrum of the methanol-d_4 extracts of a five-year-old linseed oil film, showing the assignment via the long-range ^{1}H−^{13}C coupling of methylene protons with free and esterified carbonyl groups. Figure S-4: ^{1}H−^{1}H homonuclear gradient COSY 2D NMR spectrum of the acetone-d_6 extracts of an early 20th century oil painting by Vandoros. Figure S-5: ^{1}H−^{13}C heteronuclear gradient HMQC 2D NMR spectrum of of the acetone-d_6 extracts of an early 20th century oil painting by Vandoros. Figure S-6: (a) Portrait of Young Man, by S. Vandoros, oil on canvas, early 20th century, private collection; (b) The Duke, unknown artist, oil on canvas, late 17th century, private collection. This material is available free of charge via the Internet at http://pubs.acs.org.

APPENDIX

Assuming that the acetone-d_6 extracts of a paint film contain a mol of esterified fatty acids, b mol of free fatty acids, and c mol of diacids, integral values in the ^{1}H NMR spectrum are expressed as

$$B_e = 2a \quad (1)$$

$$B_f = 2b + 4c \quad (2)$$

$$B = D = 2a + 2b + 4c \quad (3)$$

$$F = 3a + 3b \quad (4)$$

$$b = (F/3) - (B_e/2) \quad (5)$$

$$c = (B/4) - (F/6) \quad (6)$$

$$\mathrm{Di/FA} = (6B - 1)/8F \quad (7)$$

$$\mathrm{HFA} = (2\mathrm{DG} + 4\mathrm{MG})/6(\mathrm{TG} + \mathrm{DG} + \mathrm{MG}) \quad (8)$$

where TG, DG, and MG are the molar ratios of each type of glyceride.

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