Chapter X

Analysis of paint media, varnishes and adhesives

Liliane MASSCHELEIN-KLEINER

1. INTRODUCTION

The identification of film-forming substances is quite recent by comparison with the analysis of pigments.

Appreciable improvements were made in the past several years[1,2,3]. The latest investigations have been conducted on the use of selective staining techniques which enable the identification of vehicles within the painting's cross section.

Besides infrared spectroscopy, paper, thin-layer and gaschromatography, newer techniques such as high-performance liquid chromatography (H.P.L.C.), pyrolysis gas-chromatography, mass spectrometry are now available and afford considerable refinement in the characterization and authentification of agglutinants. Three techniques have been described in detailed monographs (I, II, III, IV, V).

Natural film-forming substances can be classified into five main categories[4] :

Proteins

Animal glue, egg white and yolk, casein, consist mainly of proteins. The latest are complex polymers made up of amino acids which act as building units.

Polysaccharides

They are water-soluble polymers which are found in a great variety of plant gums (arabic gum, tragacanth gum, plum and cherrygums, etc.) and mucilages (starch, guar, tamarind seeds, etc.). The building units are sugars.

These substances have been used as binding medium for aquarelle, gouache, miniatures and illuminated manuscripts.

Oils and fats

The majority of them consist of esters of glycerol with fatty acids. Drying oils contain polyunsaturated fatty acids which promote the oxidations and polymerisations responsible for the film formation.

Waxes

These compounds originate either from the animal (beeswax), vegetal (carnauba) or mineral kingdom (ozokerite). They contain long-chain hydrocarbons, acids, alcohols and esters.

Natural resins

They are normally exudated by plants to seal off mechanical injury. However shellac for example is produced by insect secretion. Natural resins are very difficult to identify because of the complexity of their composition consisting mainly of terpenoids.

2. Staining tests

Staining tests are certainly one of the oldest techniques which have been used for the identification of paint media[5].

Till the last few years, colour tests were generally considered with suspicion because of their poor selectivity. Interferences occurred with other organic and inorganic compounds which made the identification very confusing. The aging of the paint layers was often an additional difficulty[6].

Recent improvements now afford a resurgence of interest for this technique especially for the characterization of proteins and oils in mounted cross-sections of paint samples.

Some authors use the polished cross-section of paint fragment directly because the staining is more intense[8,12]. Others prefer using thin layers sliced with a microtome, about 40 to 80 μ thick. This avoids the infiltration of the stain into the cracks of the painting which would lead to a less clear stained image.

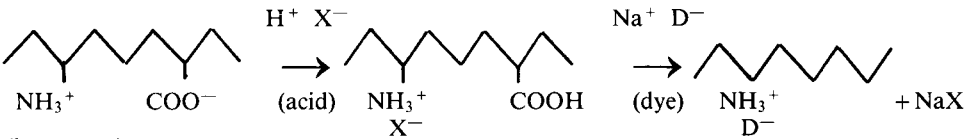
Gay also advices the use of different sections for each staining because of possible interferences.

Detailed procedure for the mounting of the sample is reported by Gay[7] and Johnson and Packard[8].

Besides specificity, the choice of the stain must be taken into account because of the nature and colour of the pigments.

2.1. *Proteins*

Most protein stains are acid dyes which means that they react with the NH_3^+ groups of the proteins, in the presence of acid, according to the reactions :



Procedures and results are summarized for the for the commonest dyes in table I.

TABLE I. — PROTEIN STAINS

Dyes	Solution	Staining time	Rinsing	Results
Light green SF CI acid green 5 CI 42095	0,1 % pH 8 and pH 1 (with acetic acid)		running water	all proteins[7,11]
Ponceau S CI acid red 112	saturated in 3 % acetic acid prepared a day before use	20 min.	running water	egg yolk : orange red animal glue : pink red[8]
Acid Fuchsin S CI acid violet 19 CI 42685	1 % in water	10 min. 15 min.	1) running water 2) water + acetic acid (99:1)	egg yolk and white and casein : light pink animal glue : dark red[11,12]
Amido black CI acid black 1 CI 20470	1 g + 450 ml conc. acetic acid + 450 ml (0,1 M)	5 min.	5 % acetic acid in water	egg yolk : dark blue[9,10,11,12]
AB 1 :	aqueous sodium acetate solution + 100 ml glycerol			
AB 2 :	1 g + 450 ml (N) acetic acid + 450 ml (0,1 M) aqueous sodium acetate solution + 100 ml glycerol	5 min. 10 min. when the ground layer is not sensitive to acids	1 % acetic acid in water	reveals all proteins
AB 3 :	1 g + 900 ml water + 100 ml glycerol	5 min.	1 % acetic acid in water	stains chiefly gelatine - casein

Moreover Martin differentiates egg-yolk from casein by the ammonium molybdate test[10]. The quantity of phosphorus in egg-yolk would be insufficient to be detected, as opposed to that in casein. This test does not appear fully reliable because phosphorus is present in many other materials.

A modified Ehrlich test is successfully used by Mills and White[44] to detect glue.

Kockaert proposes a Emich test for nitrogen and scales it down to enhance the sensitivity[45].

2.2. *Lipids*

For many years, oils were detected on the cross-section of paintings by saponification with diluted (10 %) potassium hydroxide[13,14].

Some attempts have been made by staining with solvent dyes soluble in oils, fats and waxes, such as Sudan black B or Oil red O : see table II.

TABLE II. — LIPID STAINS

Dyes	Solution	Staining time	Washing	Results
Sudan black B CI solvent Black 3 CI 26150	a) ethanol 60 % b) saturated in ethanol 60 % or	5-30 min.	ethanol 60 %	lipids : blue or black[7]
	a) propylene-glycol b) 0,7 Sudan black B + 100 ml propyleneglycol	5-30 min.	3 times + propylene-glycol + H_2O (85:15)	
Oil red O CI solvent red 23 CI 26100	a) isopropanol 60 % b) 0,5 g + 100 ml isopropanol dilute 6 ml of this sol. + 4 ml H_2O and filtrate before use	10 min.	isopropanol 60 %	lipids : red[7]

As a matter of fact, these staining tests are not always successful because the aging of oils very much weakens their ability to dissolve the dyes.

Kühn[46,47] often uses a hydrogen peroxide test for detecting drying oils. According to Gay[7] the presence of lipids can also be detected by heating the thin-section under the microscope : wax smelts from 60° C, dried oils from 160° C, egg-yolk from 200° C.

Matteini, Moles and Tosini[15] recently proposed a topochemical identification of dried oils based on their high retention of organic solvents

containing basic nitrogen atoms, such as n-butylamine. Retained amines are localized by the formation of dithiocarbamate and chromatic detection with silver nitrate which gives black silver sulfide.

The different steps of the test are :

a) 5 min. interaction with vapour n-butylamine ;

b) elimination of excess amine : 15 min. at 80° C ;

c) formation of amine dithiocarbamate with vapour CS_2 : 5 min.

d) elimination of excess CS_2 by cold ventilation : 2 or 3 min. ;

e) reaction with silver nitrate concentrated solution : 10 min. max. ;

f) washing in distilled water.

Proteins and resins give negative results, egg yolk stains a brown colour instead of becoming black, beeswax does not react.

Interferences were noticed with sulfides containing pigments such as vermilion, orpiment, cadmium yellow and red, lapis lazuli and artificial ultramarine.

2.3. *Natural resins*

Terpenic resins still present difficulties of detection. Martin[10] proposes a staining of free acids with bromocresol purple (0,1 % in ethanol) but the embedding material must be eliminated and oils also interfere.

Smelting tests can help in some cases[7].

2.4. *Conclusions*

All the above tests need great care in interpretation. They must be supported by a long experimentation with standards because they are based on visual appreciation of differences in shades or in intensity of colour given by a single dye in several media.

Dark paint layers however cannot be examined with this method.

Nevertheless staining tests constitute a useful tool for the localization of proteins, drying oils and emulsions of proteins and oils, on fragments of paintings.

Our general feeling is, however, that these analyses should be confirmed by some other analytical techniques.

3. Infrared absorption spectroscopy

The infrared absorption spectrum of molecules results from transitions between vibrational and rotational energy levels. It gives information on the functional groups and also provides 'Finger Prints' of molecules.

Feller[16,17] was one of the first to use infrared spectroscopy for the analyses of painting varnishes.

The method needs previous purification of the sample to ensure as far as possible that only one layer has been taken, and because uncertainties occur when analysing complex mixtures of organic and inorganic substances.

Nevertheless, it allows a useful differentiation between the classes of natural organic materials[1,2].

Some refinements in the instrumentation were made using beam condensers[2], microscopical system with reflecting optics[23], diamond cell[24] and I.R. Fourier transform spectroscopy[25]. The common aim of these attempts is to obtain an infrared spectrum with the best resolution from the smallest sample possible.

3.1. *Polysaccharides*[20-27]

The sample is extracted with warm water. The I.R. spectrum of the dried residue shows only the absorptions of the OH group : OH stretching frequencies between 3200-3600 cm^{-1}, OH deformation and C-O stretching frequencies between 1050-1200 cm^{-1} and 1410-1260 cm^{-1}. The band and 1630 cm^{-1} could be due to water[21].

The infrared spectrum is nearly the same for all vegetable gums.

3.2. *Proteins*

They are to be found either in the water soluble part of the sample (animal glue) or in the insoluble (egg proteins - casein).

They can be characterized by the I.R. absorptions of the peptidic bond, CO-NH, near 1650 cm^{-1} (CO absorption Amide I) and 1550 cm^{-1} (NH_2 deformation, Amide II).

It is not possible for the moment to identify the type of proteins.

Birstein and Tul'chinsky[22] studied the aging of gelatin with IR spectroscopy. They observed a decreasing of intensity for the band at 1400 cm^{-1} and related it with a decreasing of free $—COO^-$ groups during the evaporation of water.

3.3. *Lipids*

When possible the sample is purified by extraction with hot chloroform.

Glycerides and particularly vegetable oils present an intense absorption ν(C—O) of esters in 1165 cm^{-1} with two weaker absorptions at 1250 cm^{-1} and 1110 cm^{-1}.

Waxes were very early studied by Kühn[18] with IR spectroscopy. Long hydrocarbon chains in solid state give a doublet for the rocking $\delta_r(CH_2)$ at 725-715 cm^{-1}. I.R. spectroscopy can detect beeswax in a varnish even at very low concentrations such as in a matt varnish layer[50].

3.4. *Natural resins*

After Feller[16], Masschelein and Kleber[19] used IR spectroscopy for the characterization of terpenic resins. Carboxylic functions of the resin acids absorb at 1700 cm^{-1} : $\nu(C—O)_{COOH}$ acid and at 2650 cm^{-1} ν (OH) of acid dimers. The absorption bands between 1300-1200 cm^{-1} can sometimes help the differentiation of resins[19].

Low and Baer[26] were able to distinguish dammar from mastic using a Fourier transform spectrometer.

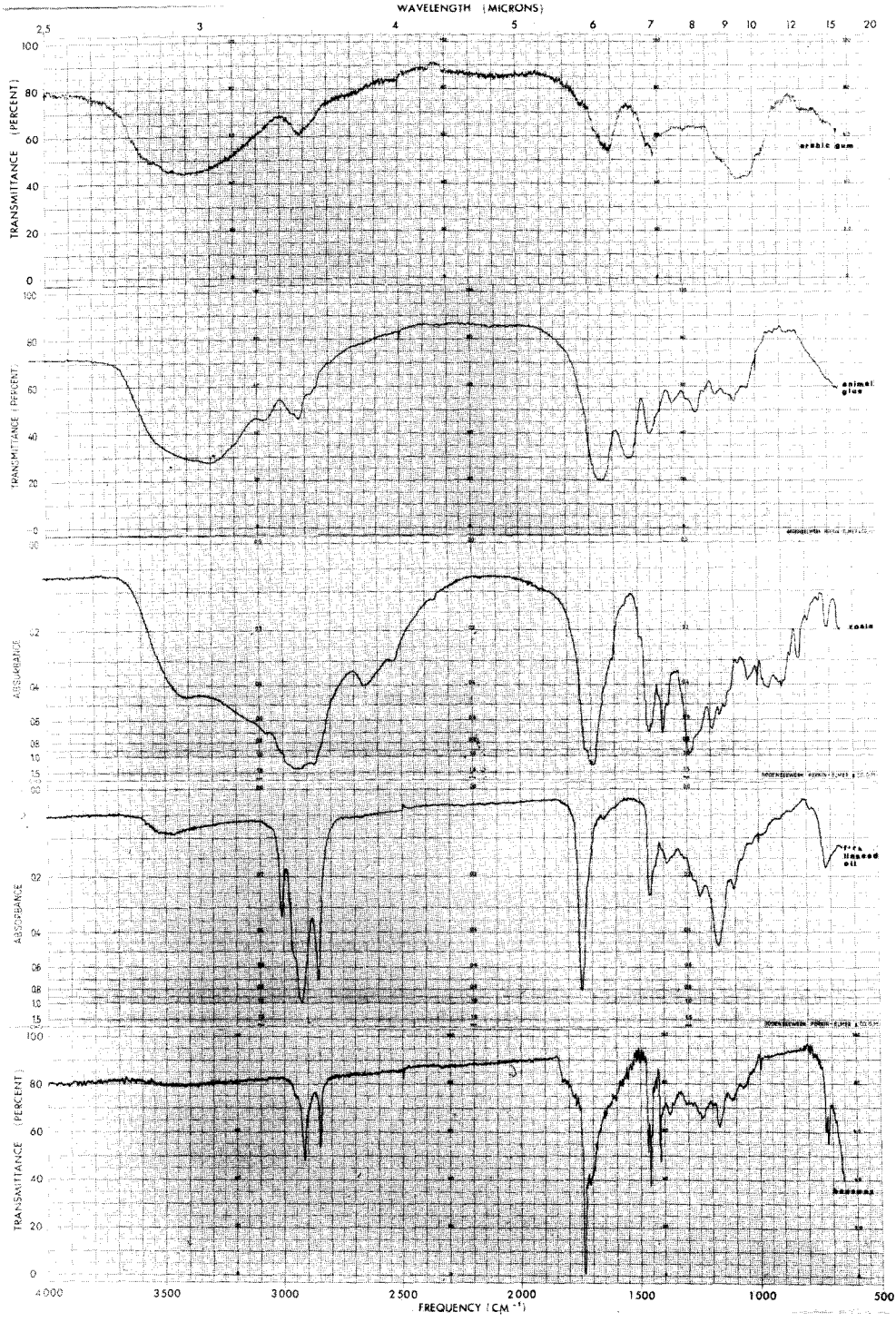


Fig. 1. Infrared spectra of natural binding media.

3.5. *Conclusions*

Although progresses in instrumentation is promising, usual IR spectrometers only enable for the moment a general distinction between categories of natural media (Fig. 1). IR spectroscopy is also very useful for characterizing synthetic resins[28].

4. THIN-LAYER CHROMATOGRAPHY (T.L.C.)

In T.L.C. the mobile phase is a solvent migrating on a plate covered by a thin-layer of adsorbent. The sample in solution is applied at the lower edge of the plate. The individual components of the sample are carried upwards by the solvent at different rates depending on absorption, partition and/or ion-exchange processes. The separated spots are then detected by some suitable means

Historically, chromatographic separations were at first performed on paper[29,30,31] but the limitations of this method are now clearly recognized.

Thin-layer chromatography presents considerable advantages when compared with paper chromatography : higher resolution, speed, sensitivity and possibility of using corrosive reagents (acids).

4.1. *Polysaccharides*

The polymers need at first to be hydrolysed into sugar units[1,2,27,32]. We get the best results by using diluted sulfuric acid (0,7 M) in a sealed tube, 72h at 120° C. Neutralization is then performed with barium carbonate which has the advantage of coprecipitating most inorganic impurities[33].

TABLE III. — THIN-LAYER CHROMATOGRAPHY OF POLYSACCHARIDES

Reference	Adsorbent	Solvent	Detection reagents
2	silica G alumina G (1:1) or precoated Kieselgel 60F254 (Merck)	propanol + ethyl acetate + water + ammonia 25 % (30:5:15:5)	naphtoresorcinol (0,2 % in 10 % ethanolic phosphoric acid)
32	sil. Kodak K 301 V	butanol + ethanol + water (57:27:16)	idem
27	microcristalline cellulose (GDR)	ethylacetate + pyridin + water (100:35:25) (3 runs at 37° C)	anilinephtalate

4.2. *Proteins*

The same hydrolysis technique reported in 4.1 can be used to break down proteins into amino acids. Fig. 2, shows the thin-layer chromatography of aminoacids and hydrolyzed proteins[2].

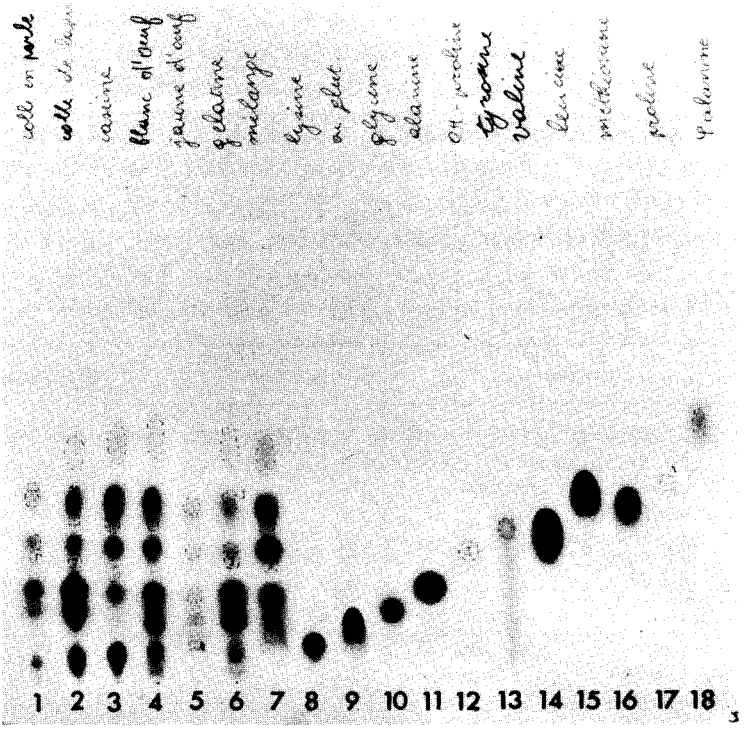


Fig. 2. Thin-layer chromatography of amino acids and hydrolyzed proteins. Sil G + Alum G (1:1), phenol/water (3:1), ninhydrine + collidine.
1. animal glue ; 2. rabbit glue ; 3. casein ; 4. white of egg ; 5. yolk ; 6. gelatine ; 7. mixture of amino acids ; 8. lysine ; 9. glutamic acid ; 10. glycine ; 11. alanine ; 12. hydroxyproline ; 13. tyrosine ; 14. valine ; 15. leucine ; 16. methionine ; 17. proline ; 18. phenylalamine.

TABLE IV. — THIN-LAYER CHROMATOGRAPHY OF PROTEINS

Reference	Adsorbent	Solvent	Detection reagents
2	silica G alumina G (1/1)	phenol + water (3:1)	ninhydrine-collidine
32	K 511 V Kodak (polycarbonates) impregnated with pH 6.8 buffer	ethanol + water + ammonia (85:13:2)	ninhydrine 2 % in ethanol
27	silufol UV_{254} Kavalier	n-butanol + ethylacetate + water (60:20:20)	ninhydrine 0,5 % in ethanol
33 dansyl amino acids deriv.	MN polyamide Macherey-Nagel + MN cellulose 300 Macherey Nagel (8:2)	isopropylbenzene + acetone + methanol (20:3:3) (twice)	UV light (350 mμ)

4.3. *Lipids*

Thin-layer chromatography is not very satisfying for oils, but suitable for waxes[36].

It was reported that the unsaponifiable fraction (presumed to be mainly sterols) could be used to characterize drying oils and yolk[34]. However, Mills and White[35] showed that sterols largely disappear during the ageing process thus making any identification very doubtful.

TABLE V. — THIN-LAYER CHROMATOGRAPHY OF LIPIDS

Lipids	Adsorbent	Solvent	Detection reagents
Oils 35	silicagel 60F254 Merck (precoated) or idem impregnated with silver nitrate	ether + hexane 1:1 (two runs)	I_2 vapour
Waxes 36,2	silicagel (Merck)	heptane + diethylether + acetic acid (90:10:2)	antimony pentachloride (50 % in CCL_4)

4.4. *Natural resins*

Much work has been done in this field[2]. When dealing with aged samples or resin/oil mixtures, polar oxidation products and polymers very much hinder the elution and make any identification very questionable, especially for diterpenoid resins.

TABLE VI. — THIN-LAYER CHROMATOGRAPHY OF NATURAL RESINS

Reference	Adsorbent	Solvent	Detection reagent
37	silicagel 60F254 Merck no. 11798 with concentrating zones precoated	benzene + methanol 92:8	vanadium pentoxide

4.5. *Conclusions*

T.L.C. is certainly the cheapest method enabling identification of sugars and amino acids even at very low concentrations. Beeswax is also very well identified. Aged oils and resins give generally less satisfying results.

5. GAS CHROMATOGRAPHY (G.C.)

In G.C., the mobile phase is a gas travelling through a column which contains a stationary phase, generally a viscous liquid adsorbed onto an inert

support. The sample is flash evaporated at one end of the column. Its components are carried through the column at different rates depending on the partition coefficients between the gas and the stationary phase. The time required by each constituent to reach the detector at the end of the column, is called its 'retention time'.

Most substances need to be converted to more volatile nonionized derivatives before they can satisfactorily be separated by gas chromatography. This drawback is now largely compensated by the high sensitivity of the method. Most authors use a flame ionization detector (F.I.D.).

5.1. *Polysaccharides*

The polymers need to be broken down into sugars just as for thin-layer chromatography.

TABLE VII. — GAS CHROMATOGRAPHY OF SUGARS

Ref.	Volatile derivatives	Column	1) injector 2) detector Temp. ° C	Oven Temp. ° C	Apparatus
2	Silylated sugars : TMS derivatives	2,5 % silicone gum rubber E301 on chrom. G (AW-DMCS) 80-100 mesh length : 2 m ext. diam. : 3 mm	1) 200° C 2) 400° C carrier gas : He : 30 ml/min.	160° to 200° C 1,7° C/min.	PE 800 (FID)
38	Silylated sugars : TMS derivatives	3 % OV_1 or 3 % OV_{17} on chrom. AW.DMCS 100/120 mesh length : 3 m int. diam. : 2,2 mm st. steel, packed	N_2 : 1,5 bar	120° to 300° C ? ° C/min.	Girdel 3000 (FID)

Depending on the preliminary treatments, each sugar shows several anomers due to mutarotation. This must be taken into account for the identification of the G.C. fractions.

5.2. *Proteins*

With amino acids there is additional difficulty that they have two functions, the acid groups and the amine groups, which must be converted into volatile derivatives. Inorganic contaminants are disturbing and must be eliminated e.g. by dialysis or ion-exchange[71].

TABLE VIII. — GAS CHROMATOGRAPHY OF PROTEINS

Ref.	Volatile derivatives	Column	1) injector 2) detector Temp. °C	Oven Temp. °C	Apparatus
39	Trimethylsilyl (TMS)	10 % UCC W982 on silanized Chrom. W packed 2 m st. steel 2 mm ID	1) 195° C 2) 400° C He : 30 ml/min.	90° C to 220° C at 2° C/min.	HP 5750 FID
40	N-ester trifluoro-acetates (N-TFA)	2 % OV_{17} ± 1 % OV_{210} on Supelc. 100/120 mesh and 0,65 % EGA on 80/100 mesh chrom. W 1,5 m × 4 mm ID glass, packed		60° C 3 min. 6°/min. to 235° C 60° C to 225° C 6° C/min.	Bendix 2500 FID Varian 2100 Packard 7300
71	N-acetyl, methyl esters	1 % XE60 cyanosilicone on 100/120 mesh Diatomite CQ 9 ft × ¼ inch	1) 200° C 2) 250° C A : 45 ml/min.	80° C to 3° C/min.	Pye FID

5.3. *Lipids*

G.C. is particularly satisfactory for the identification of fatty acids. They used to be converted into the methyl esters by either of the various methylation methods : with diazomethane[42], with BF_3-methanol[43] or with sodium methoxide[37,41]. The latest method has the advantage of avoiding the previous alkaline hydrolysis. Fig. 3 shows the separation of fatty acids and methyl esters from linseed oil[48].

TABLE IX. — GAS CHROMATOGRAPHY OF LIPIDS

Ref.	Volatile derivatives	Column	1) injector 2) detector Temp. ° C	Oven Temp. ° C	Apparatus
42	Methyl esters (diazomethane)	1,5 % Apiezon L + 1,5 % E_{301} silicone on 80-100 mesh silanized celite 9ft packed glass	carrier gas : A 1,75 kg/cm^2	150° C to to 210° C 4°/min.	Pye Pan-chromato-graph FID
44	Methyl esters	3 % OV_1 silicone		110° C to 190° C 5° C/min.	Pye 104

TABLE IX. — GAS CHROMATOGRAPHY OF LIPIDS (cont.)

Ref.	Volatile derivatives	Column	1) injector 2) detector Temp. ° C	Oven Temp. ° C	Apparatus
41	Methyl esters (methoxide-methanol)	15 % DEGS × on gas chrom. W 12 f′ × ⅛″	N_2 36 psi	190° C	PE 800 FID
48	Idem	DEGS capillary column 50 m	1) 310° C 2) 300° C He = 5 ml/min.	145° C	HP 5750 G FID
50	Waxes	1 % OV_1 on acid wash. diatomite 100/120 mesh 5 ft × ¼ inch od. glass, packed	1) 40° C above lower limit 2) 385° C	180° C to 380° C 3° C/min.	Pye FID

Hydrocarbons from paraffin and waxes are easily detected in the same way[37,50].

Mills[42] showed that different drying oils can be differentiated by the rates between palmitic and stearic acid. They are now systematically investigating the paint media of National Gallery paintings[44]. They report, for example, the predominant use of walnut oil in the earlier Italian oil paintings and the increasing use of linseed oil from the 16th century on. White identified linseed, poppy-seed and walnut oils in a series of portraits of the 18th century painter, Thomas Bardwell[49].

5.4. *Natural resins*

G.C. associated or not with pyrolysis and mass spectrometry is actually the most promising analytical approach to these complex mixtures of terpenoids.

TABLE X. — GAS CHROMATOGRAPHY OF TERPENOID RESINS

Ref.	Volatile derivatives	Column	1) injector 2) detector Temp. ° C	Oven Temp. ° C	Apparatus
52	Methyl esters (diazomethane)	1 % XE60 on 100/120 mesh diatomite CQ 274 × 0,84 cm glass, packed	1) 220° C 2) 250° C A : 45 ml/min.	192° C	Pye FID
37	Methyl esters (Na methoxide)	3 % SP 2250 on supelcoport 100/120 mesh 100/120 mesh 225 cm, 2 mm i.d. glass, packed	He : 60 ml/min.	100° C to 290° C 4°C/min. then 60 min. at 290° C	H.P. 5750 G (FID)

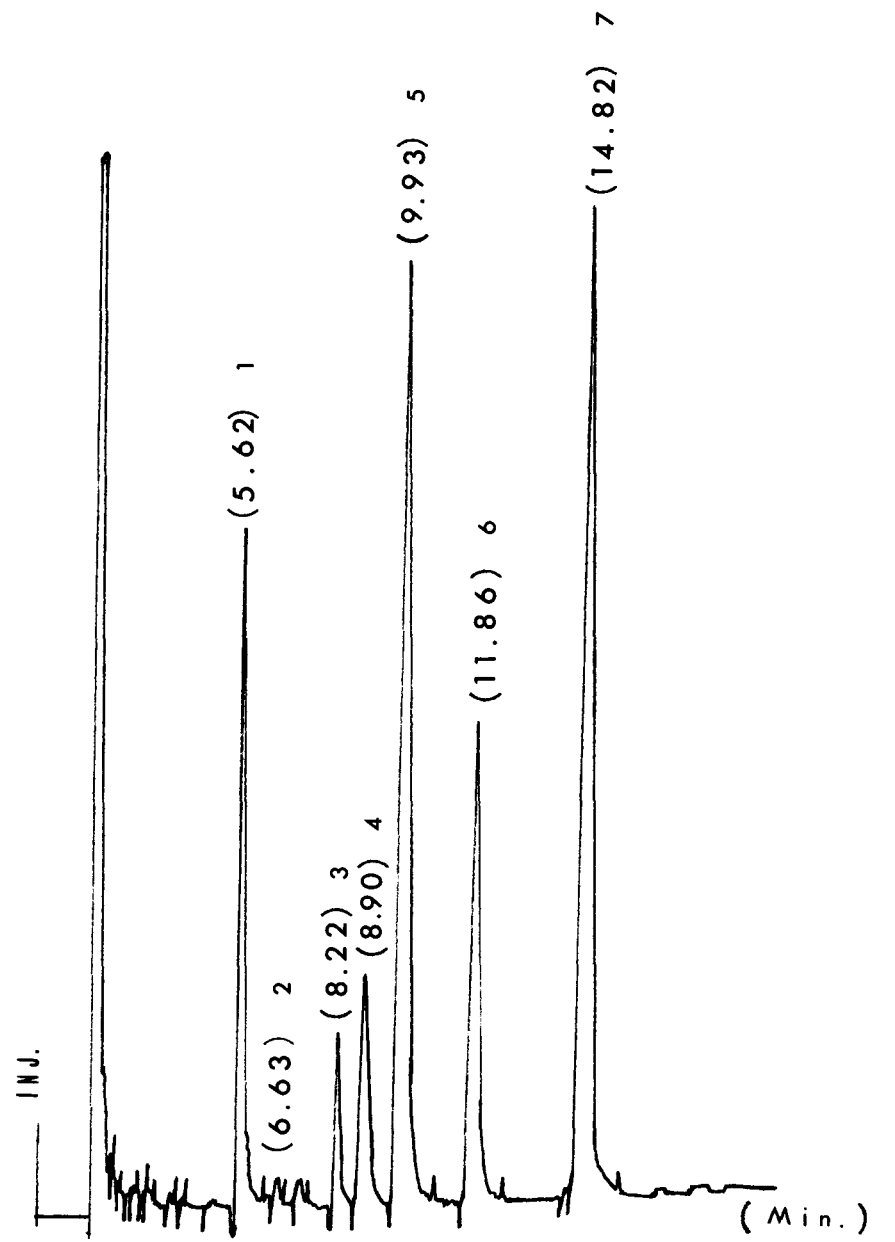


Fig. 3. Gas chromatography of linseed oil.
1. palmitic acid ; 2. suberic acid ; 3. azelaic acid ; 4. stearic acid ; 5. oleic acid ; 6. linoleic acid ; 7. linolenic acid.

6. Pyrolysis gas chromatography (P.G.C.)

Very encouraging results have been achieved during the last few years with P.G.C. altough its reproducibility needs the accurate checking of many parameters. Indeed, polymer decomposition seems to be very much influenced by the pyrolysis temperature, the rate of temperature rises in the sample during pyrolisis, sample size, geometry of the cell, inhomogeneities in the wire, carrier gas-flowrate... All these factors have a marked effect on the resultant pyrogram and should be controlled[56]. Nevertheless P.G.C. enabled Breek and Froentjes[55] to conclude that five Vermeer and P. de Hoogh fakes attributed to Van Megeren had a polymerized phenol formaldehyde binder identical to the synthetic resin Van Megeren stated he had used.

Witt-Rogers recently[57] used a Pyroprobe 100 Solids pyrolyser for identifying linseed oil, egg white and yolk, mastic and dammar resins in painting layers.

White[61] reported some initial experiments with resin/oil varnish mixtures.

Table XI. — Pyrolisis gas chromatography

Ref.	Pyrolyser	Pyrolisis T° C	Column	1) inj. 2) det.	Oven T° C	G.C.
55	CDS Pyroprobe 190 solids pyrolyser Pt spiral in a quartz tube	10 sec. at 700° C	10 % apiezon L on chrom. W.AW 60/80 mesh 2,5 m, ⅛ in. OD stainless steel	1) 200° C 2) 200° C	2 min. at 90° C then to 190° C in 16° C/min.	PE 3920 FID
56	CDS Pyroprobe 100 solids pyrolyser quartz tube in a coil probe	20 sec. at 600° C interface at 200° C T° rise : 20° C/m. sec.	10 % UC-W 98 on chrom. W 80/100 mesh N_2 : 57 ml/min.	1) 205° C 2) 225° C	78° C	HP 5750 FID
62	Wire	5 sec. at 770° C	3 % OV—1 preoxidised, 5′ × ¼″.		90° C to 250° C 5°/min.	
63 64	CDS Pyroprobe 150, Pt spirale in quartztube	10 sec. at 600° C (ramp off) Interface 250° C	50 m SE30 capillary (0,5 mm ID)		80° C to 220° C in 6° C/min. Interf. 250° C	Finnigan 9500

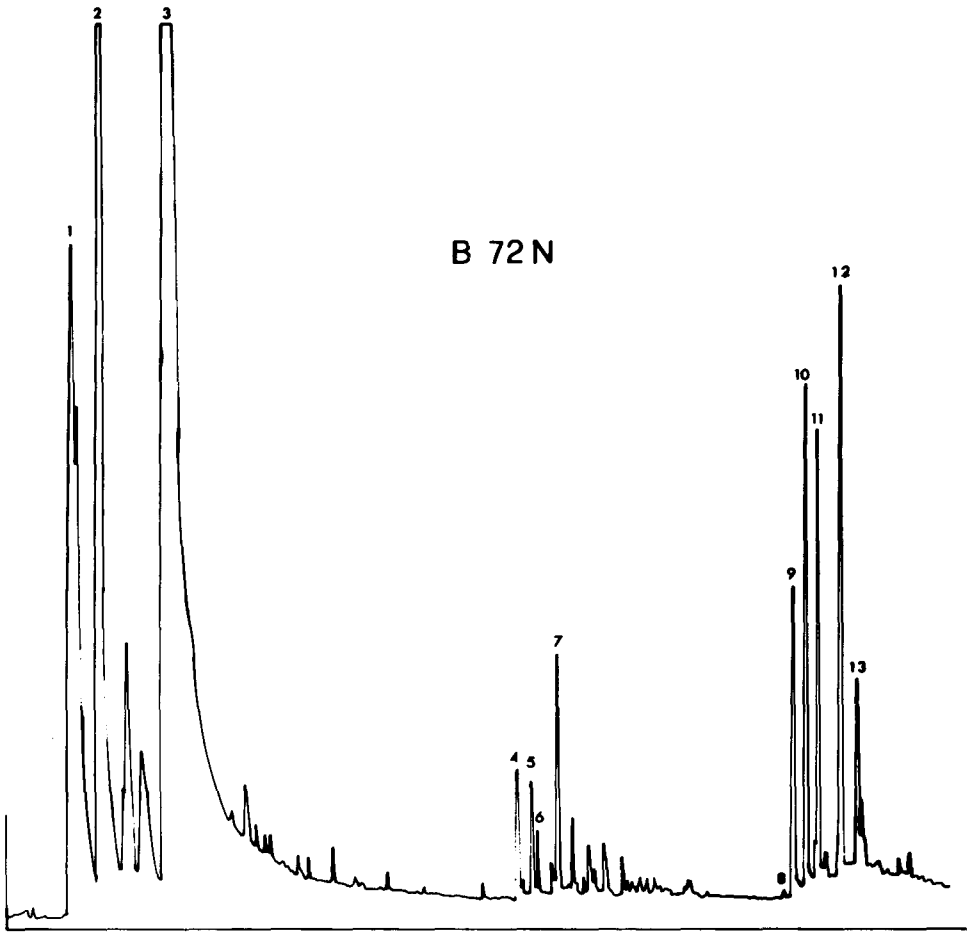


Fig. 4. Pyrogram of Paraloid B 72 N.
1. toluene ; 2. methyl acrylate (MA) ; 3. ethyl metacrylate (EM) ; 4. dimer of MA ; 5. dimerisation product of MA + EM ; 6. dimerisation product of EM ; 7. dimer of EM ; 8. phenyl benzoate ; 9. trimer of MA ; 10. trimer of 2 x MA + 1 x EM ; 11. trimer of 2 x MA + 1 x EM ; 12. trimer of 2 x MA + 1 x EM ; 13. trimer of 1 x MA + 2 x EM.

7. Mass spectroscopy (M.S.) and combined G.C.-M.S.

In most usual systems a substance is vaporized at very low pressure (10^{-7} mm Hg) and simultaneously ionized by an electron beam ; the resulting molecular ions break up into fragments of a lower mass. These ions are then accelerated by an electric field and segregated by deflection in a magnetic field. The resulting curve path is proportional to the ratio : particle's mass/ particle's charge (m/e).

Molecular weight and masses of the broken units can be determined in this way. The molecular fragmentation pattern provides not only a 'finger print', but the whole structure of the initial molecule can also be deduced by reassembling the fragments.

Mass spectrometry has been proved to be a powerful tool for identifying gas chromatography peaks[58,59,60]. This combined system is called G.C.-M.S.

TABLE XII. — G.C.-M.S.

Ref.	Derivatives	G.C. Column	G.C. Interface	G.C. Oven T° C	G.C. Appar.	M.S. 1) scan. speed 2) ioniz. potential	M.S. Appar.	M.S. Data syst.
58	TMS sugars	OV—101 capillary 20 m × 0,25 mm ID		130° C to 250° C 2° C/min.		1) sec./decade	DuPont 21-492B	DuPont 094-B2
59	TAB amino acids	3 % OV—101 on HP chrom. W			Varian 1500	1) 8 sec./decade 2) 70 eV	CEC 21-110	Jeolco JEC - 6
60	TMS amino acids	1 m × 0,4 cm glass, packed						
61	Methyl esters of fatty acids	OV—17 6′		90° C to 300° C 10° C/min.		4 sec./ spectrum	400 sp/ run	Hitachi RMV/6L data Syst. IBM 1800
64	Pyrolysed synthetic resins	SE_{30} capillary 50 × 0,5 mm ID	250° C	80° C to 220° C 6° C/min.	Finnigan 9500	1) sec./scan. 2) 70 eV	Finnigan 3000	Finnigan 6000
70	Methyl esters of fatty acids	SE 30 quartz capillary 50 m		90° C to 210° C 4° C/min.		1) 0.5 sec./ decade 2) 70 eV	Kratos MS 25	Data General Nova 3
	T.M.S.E. cholesterol	?						
	Methyl esters of resin acids	SE 30 quartz capillary 6 m		120° C to 260° C 5° C/min.		1) 1 sec./decade 2) 70 eV	id.	id.

The G.C.-M.S. system is highly promising for all organic compounds whether natural or synthetic[63,64,70].

8. LIQUID CHROMATOGRAPHY (L.C.)

The mobile phase is a liquid migrating through a column containing the stationary phase which can be either a non-miscible viscous liquid (liquid-liquid chrom.) or a solid (liquid-solid. chrom.).

TABLE XIII. — TYPES OF LIQUID CHROMATOGRAPHY (L.C.)

Stationary phase	Mobile phase	Type of liquid chrom.
Ions- exchangers	Buffers with gradient of pH	Ion-exchange
Polar	Non-polar	Normal-phase
Non-polar	Polar	Reverse-phase
Chemically bonded (Polar or non-polar)		Bonded-phase
Controlled pore size gels		Gel permeation Size exclusion

In the last 15 years performances of L.C. were remarkably improved in resolution and speed.

Amino acid analyzers were the first 'High performance liquid chromatographs' (H.P.L.C.). The stationary phases consisted of ion-exchange resins.

Keck and Peters showed the possibilities of the method for protein analysis[65]. Sack, Tack and Peters have identified gelatine in this way, in the medium of an ancient Egyptian painting[66]. Roelofs had some promising results with binding media[67].

The high cost of the apparatus is the only reason why this technique has not yet found many actual applications in the study of painting materials.

TABLE XIV. — HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Ref.	Derivatives	Column	Solvent	Colum T° C	Detector	Appa-ratus
65	Amino acids	Ion-exchange (polystyrene sulfonic acid)	Buffer citrate : pH gradient from 2.875 to 5.000	60° C	Photometer (ninhydrine)	Techni-con
68	PTH amino acids	μ Bondapak C 18 300 × 4 mm (bonded reverse phase)	A : buffers diethylene triamine DETA + trichloro-acetic acid TCA (20 mM) B : DETA + TCA + 60 % (v/v) acetonitrile pH : 4.2 5 % B to 100 % B in 33 min. (4,5 ml/min. 4000 p.s.i.)	20° C (8.0 mM)	CE 212 OV photometer at 268 nm	Waters

9. Differential thermal analysis

The Doerner Institute started in 1976 a research program on the application of DTA in the field of art and archaeology. Preuszer[69] found that oil-media present two exothermal reactions, one at about 300° C, the other at about 400° C. The ratio of these two reactions varies linearly with the logarithm of the age. A sample older than 150 years gives mainly the reaction at 400° C, a recent one, mainly the reaction at 300° C.

However, the described method seems difficult to be reproduced. Further experiments need to be done in order to evaluate the influence of additives, such as pigments or other binding media.

10. General conclusions

The analytical approach of paint media depends on two main factors : the scientific staff in charge and the financial capacities of the laboratory.

Identification of organic painting materials requires the skill of specialized chemists. Even very expensive instruments like G.C.-M.S. or H.P.L.C. don't solve the problems by themselves. Art objects present very special difficulties which cannot be surmounted by routine procedures.

However, it is always advisable to use more than one method for each sample. This will afford more confidence in the interpretation of the results.

A permanent dialogue between the chemist and the conservator, the art historian or the archaeologist is indispensable not only during the researches but still more for using the results.

11. Notes

I. L.J. Bellamy, *The infrared spectra of complex molecules*, London, 1962.

II. K. Randerath, *Thin-layer chromatography*, Verlag Chemie and Acad. Press, New York, 1963.

III. H.P. Burchfield and E.E. Storrs, *Biochemical applications of gas chromatography*, Acad. Press, New York, 1962.

IV. W.L. Budde and J. Weichellberger, *Organic analysis using gas chromatography - Mass spectrometry*, Ann Arbor Science, 1979, 242 p.

V. L.R. Snyder and J.J. Kirkland, *Introduction to modern liquid chromatography*, J. Wiley, New York, 1979, 863 p.

1. J.S. Mills, *The identification of organic materials in art objects with particular reference to painting*, I.C.O.M. Leningrad-Moscou, 1963.

2. L. Masschelein-Kleiner, J. Heylen et F. Tricot-Marckx, *Contribution à l'analyse des liants, adhésifs et vernis anciens*, in *Studies in Conservation*, 13, 1968, p. 105-121.

3. J.S. Mills, *The identification of paint media, an introduction*, in *Conservation and restoration of pictorial art*, edited by N. Bromelle and P. Smith, London-Boston, 1976, p. 69-71.

4. L. MASSCHELEIN-KLEINER, *Liants, vernis et adhésifs anciens*, Cours de Conservation, Brussels, Institut royal du patrimoine artistique, 1978.
5. A. EIBNER, *L'examen microchimique des agglutinants*, in *Mouseion*, 20, 1932, p. 5-23.
6. P.L. JONES, *Some observations on methods for identifying proteins in paint media*, in *Studies in Conservation*, 7, 1962, p. 10-17.
7. M.C. GAY, *Essais d'identification et de localisation des liants picturaux par des colorants spécifiques sur coupes minces*, in *Annales du Laboratoire de recherche des Musées de France*, 1970, p. 8-24.
8. M. JOHNSON et E.P. PACKARD, *Methods used for the identification of binding media in Italian paintings of the fifteenth and the sixteenth centuries*, in *Studies in Conservation*, 16, 1971, p. 145-164.
9. E. MARTIN, *Note sur l'identification des protéines par la coloration au Noir Amide*, in *Annales du Laboratoire de recherche des Musées de France*, 1975, p. 57-60.
10. E. MARTIN, *Some improvements in techniques of analysis of paint media*, in *Studies in Conservation*, 22, 1977, p. 63-67.
11. J. MILLS and R. WHITE, *Organic analysis in the arts : some further paint medium analysis*, in *National Gallery Technical Bulletin*, 2, 1978, p. 71-76.
12. L. KOCKAERT and M. VERRIER, *Application des colorations à l'identification des liants de Van Eyck*, in *Bulletin de l'Institut royal du patrimoine artistique*, XVII, 1978/79, p. 122-127.
13. P. COREMANS, R.J. GETTENS and J. THISSEN, *La technique des Primitifs flamands*, in *Studies in Conservation*, 1, 1952, p. 1-29.
14. J. PLESTERS, *Cross-sections and chemical analysis in the study of paint samples*, in *Studies in Conservation*, 2, 1956, p. 1-47.
15. M. MATTEINI, A. MOLES and I. TOSINI, *Topochemical reactions for the recognition of oil media in paint fragments*, ICOM Committee for Conservation, 6th Triennial Meeting Ottawa (1981), 81/1/6.
16. R.L. FELLER, *Dammar and mastic I.R. analysis*, in *Science*, 120, 1954, p. 1069-1070.
17. ID., *Special report on identification and analysis of resins and spirit varnishes*, Mellon Institute, sept. 1958.
18. H. KÜHN, *Detection and identification of waxes including Punic Wax by IR spectroscopy*, in *Studies in Conservation*, 5, 1960, p. 71-80.
19. R. KLEBER and L. MASSCHELEIN-KLEINER, *Contribution à l'analyse des composés résineux utilisés dans l'art*, in *Bulletin de l'Institut royal du patrimoine artistique*, VII, 1964, p. 196-218.
20. L. MASSCHELEIN-KLEINER and F. TRICOT-MARCKX, *La détection de polysaccharides dans les matériaux constitutifs des œuvres d'art*, in *Bulletin de l'Institut royal du patrimoine artistique*, VIII, 1965, p. 180-191.
21. C.N.R. RAO, *Chemical application of IR spectroscopy*, Academic Press, New York, 1963, p. 619.
22. V.J. BIRSTEIN and V.M. TUL'CHINSKY, *IR spectroscopic analysis of aged gelatins*, ICOM Committee for Conservation, 6th Triennial Meeting Ottawa, 1981, 81/1/9.
23. E.H. VAN'THUL-EHRNREICH, *Infrared microspectroscopy for the analysis of old painting materials*, in *Studies in Conservation*, 15, 1970, p. 175-182.
24. J.C. MacCAWLEY, *Diamond cell IR spectroscopy in the analysis of paints and pigments*, ICOM Committee for Conservation, 4th Triennial Meeting Venice, 1975, 75/4/5.
25. M.J.D. LOW and N.S. BAER, *Application of infrared Fourier transform spectroscopy to problems in conservation*, in *Studies in Conservation*, 22, 1977, p. 116-128.
26. ID., *Dammar and mastic IR analysis*, ICOM Committee for Conservation, 5th Triennial Meeting Zagreb, 1978, 78/16/5.
27. V.J. BIRSTEIN, *On the technology of Central Asia wall-paintings : the problem of binding media*, in *Studies in Conservation*, 20, 1975, p. 8-19.

28. J. HASLAM and H.A. WILLIS, *Identification and analysis of plastics*, D. Van Nostrand Inc., New Jersey, USA, 1965.

29. J.S. MILLS and A.E.A. WERNER, *Paper chromatography of natural resins*, in *Nature*, 169, 1952, p. 1064-1068.

30. K. MAČEK and M. HAMŠIK, *Paper chromatography. A new method for the characterization of binding media*, in *Umeni* (Prague), 2, 1954, p. 58.

31. M. HEY, *The analysis of paint media by paper chromatography*, in *Studies in Conservation*, 3, 1958, p. 183-193.

32. F. FLIEDER, *Mise au point des techniques d'identification des pigments et liants inclus dans la couche picturale des enluminures de manuscrits*, in *Studies in Conservation*, 13, 1968, p. 49-86.

33. L. MASSCHELEIN-KLEINER, *An improved method for the thin-layer chromatography of media in tempera paintings*, in *Studies in Conservation*, 19, 1974, p. 207-211.

34. M. JOHNSON, H.E. ROSENBERG and R.P. SKOWRONSKI, *Thin-layer chromatography of sterols on magnesium trisilicate*, in *Studies in Conservation*, 16, 1971, p. 165-167.

35. J.S. MILLS and R. WHITE, *The identification of paint media from the analysis of their sterol composition, a critical view*, in *Studies in Conservation*, 20, 1975, p. 176-182.

36. A. CARLIER, F. PUISSIEUX, C. MIET and C. LE HIR, *Analyse des pommades. II. Application de la chromatographie sur couches minces à l'identification des huiles et des cires*, in *Annales de la pharmacie française*, 24, 1966, p. 336-346.

37. L. MASSCHELEIN-KLEINER and P. TAETS, *Contribution to the study of natural resins in the art*, ICOM Committee for Conservation, 6th Triennial Meeting, Ottawa, 1981, 81/16/3.

38. F. FLIEDER, R. BARROSO and C. ORUEZABAL, *Analyse des tanins hydrolysables susceptibles d'entrer dans la composition des encres ferro-galliques*, ICOM Committee for Conservation Venise, 1975, 75/15/12.

39. L. MASSCHELEIN-KLEINER, *Contribution to the study of aged proteinaceous media*, in *Conservation and restoration of picturial art*, p. 84-87.

40. C.W. GEHRKE, K. KUO and R.W. ZUMWALT, *GLC separation of protein amino acid*, in *Journal of Chromatology*, 57, 1971, p. 209-217.

41. N. STOLOW, *The application of gas chromatography in the investigation of works of art*, in *Application of science in the examination of works of art*, Museum of Fine Arts Boston, Proceedings of the seminar sept. 7-16 1965, p. 172-183.

42. J.S. MILLS, *The gas chromatographic examination of paint media. Part 1. Fatty acid composition and identification of dried oil films*, in *Studies in Conservation*, 11, 1966, p. 92-107.

43. C. PAQUOT, *Méthodes d'analyse des matières grasses et dérivés*, in *Union internationale de chimie pure et appliquée*, 1979, 6th ed., p. 2-301.

44. J. MILLS and R. WHITE, *Analysis of paint media*, in *National Gallery Technical Bulletin*, 1, 1977, p. 57-59 ; 2, 1978, p. 71-76 ; 3, 1979, p. 66-67 ; 4, 1980, p. 65-68.

45. L. KOCKAERT, *Notes sur les émulsions des Primitifs flamands*, in *Bulletin de l'Institut royal du patrimoine artistique*, XIV, 1973-74, p. 133-139.

46. H. KÜHN, *A study of the pigments and the grounds used by J. Vermeer*, in *National Gallery of Art, Report and Studies in the history of Art*, 1968, p. 55-202.

47. H. KÜHN, *Untersuchungen zu den Malgrunden Rembrandts*, in *Jahrbuch der Staatlichen Kunstsammlungen in Baden Württemberg*, II, 1965, p. 189-210.

48. L. MASSCHELEIN-KLEINER (unpublished).

49. R. WHITE, *An examination of Thomas Bardwell's portraits, the media*, in *Studies in Conservation*, 20, 1975, p. 109-113.

50. ID., *The application of gas chromatography to the identification of waxes*, in *Studies in Conservation*, 23, 1978, p. 57-68.

51. B. RAMER, *The technology, examination and conservation of the Fayum portraits in the Petrie Museum*, in *Studies in Conservation*, 24, 1979, p. 1-13.

52. J.S. MILLS, *Diterpenes of Larix oleoresins*, in *Phytochemistry*, 12, 1973, p. 2407-2412.

53. J.S. MILLS and R. WHITE, *Natural resins of art and archaeology. Their sources, chemistry and identification*, in *Studies in Conservation*, 22, 1977, p. 12-31.

54. R. WHITE, *An examination of varnish from three 18th century musical instruments*, ICOM Committee for Conservation, 5th Triennial Meeting Zagreb, 1978, 78/16/1.

55. R. BREEK and W. FROENTJES, *Application of pyrolysis gas chromatography on some of Van Meegeren faked Vermeer and Pieter de Hooghs*, in *Studies in Conservation*, 20, 1975, p. 183-189.

56. J. NAVEAU, *Sensibilité paramétrique de la pyrolyse couplée à la chromatographie gazeuse*, in *Journal of Chromatology*, 174, 1979, p. 109-122.

57. G. DE WITT-ROGERS, *An improved pyrolytic technique for the quantitative characterization of the media of works of art*, in *Conservation and Restoration of Pictorial Art*, 1976, p. 93-100.

58. P. ARPINO, J.P. MOREAU, C. ORUEZABAL and F. FLIEDER, *G.C.-M.S. of tannin hydrolysates from ink*, in *Journal of Chromatology*, 134, 1977, p. 433-439.

59. K.R. LEIMER, R.H. RICE and C.W. GEHRKE, *Complete M.S. spectra of N-Trifluoroacetyl-n-Butyl esters of amino acids*, in *Journal of Chromatology*, 141, 1977, p. 121-144.

60. K.R. LEIMER, R.H. RICE and C.W. GEHRKE, *Complete M.S. spectra of the per-trimethyl silylated amino acid*, in *Journal of Chromatology*, 141, 1977, p. 355-375.

61. N.R. WEISS and K. BIEMANN, *Application of mass spectrometric techniques to the differentiation of paint media*, in *Conservation and Restoration of Pictorial Art*, p. 88-92.

62. R. WHITE, *A review with illustrations of methods applicable to the analysis of resin/oil varnish mixtures*, ICOM Committee for Conservation, 6th Triennial Meeting Ottawa, 1981, 81/16/2.

63. E. DE WITTE, M. GOESSENS-LANDRIE, E.J. GOETHALS and R. SIMONDS, *The structure of « old » and « new » Paraloid B72,*, ICOM Committee for Conservation, 5th Triennial Meeting Zagreb, 1978, 78/16/3.

64. E. DE WITTE and A. TERFVE, *The use of pyrolysis-gas chromatography-mass spectrometry technique for identification of high molecular weight products*, in *Science and Technology in the service of conservation*, IIC Congress Washington DC, september 1982.

65. S. KECK and T. PETERS, *Identification of proteincontaining media by quantitative amino acid analysis*, in *Studies in Conservation*, 14, 1969, p. 75-82.

66. J.P. SACK, F.C. TAHK and T. PETERS, *A technical examination of an ancient Egyptian painting on canvas*, in *Studies in Conservation*, 26, 1981, p. 15-23.

67. W.G. ROELOFS, *Some experiments with high performance liquid chromatography in analysing binding media in objects of art*, ICOM Committee for Conservation, 4th Triennial Meeting, Venice, 1975, 75/5/5.

68. W.D. ANNAN, *Separation of phenylthiohydantoin-amino acids by high performance liquid chromatography*, in *Journal of Chromatography*, 173, 1979, p. 194-197.

69. F. PREUSZER, *Differential thermal analysis of paint samples*, ICOM Committee for Conservation, 5th Triennial Meeting, Zagreb, 1978, 78/20/2.

70. J. MILLS and R. WHITE, *Organic mass-spectrometry of art materials : work in progress*, in *National Gallery Technical Bulletin*, 6, 1982, p. 3-18.

71. R. WHITE, *The characterization of proteinaceous binders in art objects*, in *National Gallery Technical Bulletin*, 8, 1984, p. 5-14.

12. RÉSUMÉ

Les liants, vernis et adhésifs utilisés dans les peintures anciennes groupent des substances organiques filmogènes de natures très diverses : gommes polysaccharides et mucilages (polymère de sucres), protéines (polymères d'acides aminés), huiles siccatives (esters d'acides gras), cires (hydrocarbures à longue chaîne et esters d'acides gras, etc.), résines naturelles (composés terpéniques), etc.

Leur identification a beaucoup progressé ces dernières années grâce à la mise au point de nouvelles méthodes d'analyse et au perfectionnement de méthodes plus anciennes comme celle des tests colorés.

Les données opératoires et les résultats obtenus dans l'étude des peintures anciennes sont passés en revue pour les principales méthodes analytiques utilisées : tests de coloration, spectrométrie d'absorption infra-rouge, chromatographie en couche mince, chromatographie en phase gazeuse sans et avec pyrolyse, spectrométrie de masse combinée ou non avec la chromatographie en phase gazeuse, chromatographie en phase liquide haute performance, analyse thermique différentielle.

Plus encore que par le passé, ces analyses exigent un personnel spécialisé et un équipement coûteux. Il est de plus essentiel que les résultats soient discutés et interprétés après un dialogue systématique avec les restaurateurs et les historiens d'art. En outre, il est souhaitable d'étayer les conclusions en utilisant pour chaque cas plusieurs méthodes analytiques.

Manuscript received may 1982.