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SPECIAL FEATURE: PERSPECTIVE

Organic Mass Spectrometry in Our Cultural Heritage

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INTRODUCTION

Experimentation has always represented an important aspect of figurative art. Artists have always looked for new materials in order to represent what is present in their souls, materials which are able to depict, as faithfully as possible, their feelings. Much effort has been spent, since antiguity, to find out, in the natural world, colour sources showing interesting chemical and physical properties. Adhesive properties and colour stability were the most desired characteristics. This inquiry work can be considered to be experimental research, even if rather empirical. It is surprising that, before Galileo's and Bacon's thoughts on experimental science, a similar approach was employed in the art field.

In the distant past, attention was mainly addressed to the botanical world, naturally rich in pigments. By adopting this approach the artist was faced with the all too evident instability of the material: once extracted from natural substrates and exposed to environmental agents (mainly to sunlight), the majority of vegetable paints change their colour.

As a consequence of such instability, artists' interests shifted to the mineral world. Many inorganic salts exhibit stable colours and their ability to mix and to cover the chromatic scale provided a powerful aid to the production of artistic works.

The use of inorganic powders created the problem of fixing them on the painting support (wall, wood or cloth). As an example, in the so-called fresco technique, aqueous solutions of the pigments are applied directly on the still wet plaster, which, once dried, incorporates the pigment as part of the plaster itself. The validity of the fresco technique is proved by the many paintings, many centuries old, still admirable in their splendour.

If the fresco technique is not used, a medium able to fix the mineral pigment on the chosen surface is necessary and many efforts were made to find suitable materials. The most commonly employed media, mainly used from the Middle Ages to the Renaissance, were of animal origin and proteinaceous materials represented a common choice. The resulting paints were called 'tempera' (distemper); their limitations were mainly due to their opacity, which causes a small depth of the picture. To overcome this problem, different compounds were usually added to the protein–pigment mixture, and each artist kept secret the paint formula employed in his school. For example, the Renaissance Venetian painters often used as paint media a mixture of beaten egg, white wine and garlic juice;[1] this recipe can be considered something between the magical and the empirical. However, considering that, once dried, the paint will contain some sugars (from wine) and some glycerides (from garlic), the painting will exhibit more brilliant colours (and consequently will gain in depth) with respect to those obtained using simply beaten egg as medium.

The Renaissance was a period in which many experiments were performed and, together with proteinaceous material (egg, glue from bones, skin and fish, casein), polysaccarides, resins, waxes, shellac and benzoin were extensively employed with the final aim of increasing brightness and depth.[2]

During this period, the Flemish painters found the best solution and their pictures showed aesthetic characteristics which make them unique. They kept their recipe secret and only after a half a century of spying did it become clear that the medium employed was mainly based on drying oils (from linseed and almond). Again, some experimental work was performed by different painting schools, mixing drying oils with the other proteinaceous media to obtain particular effects, or using different binders in different parts of the same picture.

These considerations provide clear evidence for the difficult but stimulating work of modern restorers, who necessarily need information on the pigments employed and on the media used in order to maintain homogeneity with the original painting. This work is further complicated by past restoration efforts, not always made using a correct approach.

The analysis of ligands has always been one of the most important goals of analytical chemistry in conservation studies.[3,4] These substances are mixed with pigments (oxides, salts) and, together with the processes

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due to oxygen and to light exposure, this promotes the alteration of the original macromolecules in what is termed 'ageing.' In this respect, organic mass spectrometry can give valuable information and, mainly in the last two decades, it has shown its power to do so.

Considering the low concentration, the heterogeneity and the high molecular mass of the media components, it follows that their direct analysis is very difficult. Two different approaches can be employed, as shown schematically in the flow chart in Fig. 1. The paint sample, usually in quantities of the order of 0.1–1 mg, must be chemically or thermally degraded. In the former case, acid hydrolysis represents the most commonly employed method. The mixture of the resulting low molecular mass compounds (amino acids, fatty acids, oxygen-containing heterocycles) is usually derivatized and analysed by gas chromatographic (GC) or GC/mass spectrometric (MS) methods.[4–9] Alternatively, a pyrolsis (PY) system, directly connected to a GC/MS system can be employed. The thermal degradation approach is often the most suitable owing to the small sample amount and the analysis time required and to the lack of required sample treatment.[10,11]

The first experiments performed in 1952 on an off-line PY/MS system led to the conclusion that the MS analysis of pyrolsis products of complex synthetic and natural macromolecules could be employed as a valid fingerprint of the original compounds.[12] This approach has subsequently been enhanced by the direct coupling of the pyrolsis chamber to a GC or a GC/MS system, avoiding or at least strongly reducing the possibility of reactions among pyrolysis products.

The thermally induced reactions are often highly complex and it is sometimes difficult to relate the pyrolysis products to the structure of the original macromolecule. However, the reproducibility of pyrolysis data allows the effective employment of this technique in structural characterization. In other words, different macromolecules (or mixtures of macromolecules) lead to pyrolysis products differing both in identity and/or in relative abundance. The methods currently employed to perform pyrolysis experiments are based on resistive or Curie point heating. The pyrolysis chamber can be connected on-line to an MS ion source or to the injector of a gas chromatograph, which can in turn be interfaced to different detectors, among which mass spectrometric detectors are surely the most effective.[11]

Resistive heating is obtained via the Joule effect using a calibrated resistor. Two different approaches are employed: in the first the sample in a quartz capillary is placed inside the resistive coil: by controlling the current which passes through the resistor, it is possible to regulate accurately the resistor temperature (the final pyrolysis temperature). It must be emphasized that the temperature control is performed on the resistor and that the temperature experienced by the sample will depend on many factors, such as the thickness of the quartz capillary walls and sample morphology. Alternatively, instead of a coil resistor, a platinum band resistor, on which the sample is directly deposited, can be used.[11]

A different approach is based on the use of ferromagnetic alloys in the form of thin rods placed inside an r.f. field. Depending on the alloy used, different temperatures (Curie points) can be reached. In this case the sample is deposited as a thin layer on the ferromagnetic rod. The final temperature is reached almost instantaneously (10^4 °C^{-1}) and the temperature of the sample is practically the same as that of the ferromagnetic alloy. This feature results in better reproducibility of the pyrolysis data.[11]

PY/GC/MS of a macromolecule allows more complete analysis of the less polar pyrolysis products. In addition, the highly polar compounds are adsorbed

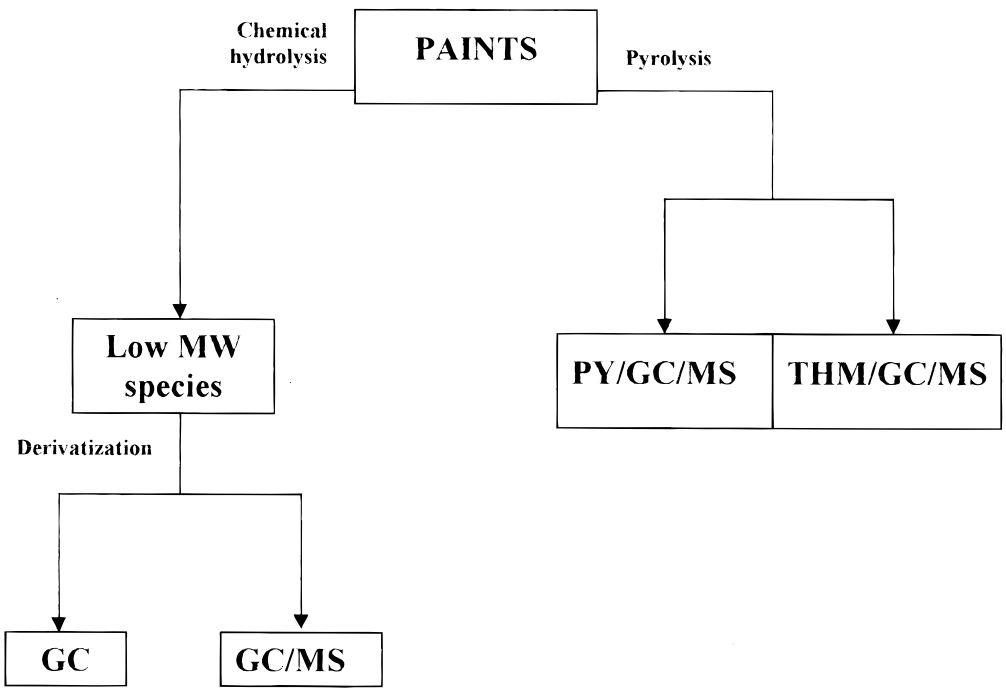

Figure 1. Possible approaches usually employed for the analysis of paint binding media.

either on the GC injector or on the first few centimetres of the chromatographic column. In order to allow a better determination of acids and phenolic compounds, a derivatization method, taking place simultaneously with the pyrolysis, has been developed.[13] This method, thermally assisted hydrolysis methylation (THM), consists of the addition of a small amount (5 μl) of a derivatizing agent (usually tetramethylammonium hydroxide (TMAH), 25% aqueous solution) to the pyrolysis sample. TMAH is known to decompose at moderately high temperatures, giving rise to reactive methylating species. This procedure is a development of a method originally aimed at derivatizing fatty acids while injecting them in the hot (360–380 °C) injector of a gas chromatograph[14] and is used for the analysis of various classes of substances, such as alcohols, phenols and fatty acids.[13]

Interesting results have been obtained using both approaches in the art field. In both cases a preliminary study on reference materials was required in order to identify pyrolysis products diagnostic of the different paint media. The studies were carried out on artificially aged media samples, despite possible limitations arising by transferring data obtained by analysing pure binding media to real samples, which do not necessarily contain only one medium and which experienced different environmental conditions.

PY/GC/MS AND THM/GC/MS OF STANDARD LIGANDS

Owing to the above described experimental work carried out by artists during the Renaissance period, particular attention was paid to the development of pyrolysis procedures able to differentiate the binding media employed in art objects of that time. We report here some experiences and results obtained by our groups in this field.[15]

A preliminary investigation carried out on artificially aged pure binding samples is summarized in Table 1. Binders can be subdivided in terms of their chemical nature. Linseed oil was the most commonly employed drying oil and, among proteinaceous materials, egg yolk and albumen were the most common.[15] Animal glues were sometimes employed either in the original paint or in early restorations. Polysaccharide compounds were more rarely employed. They were sometimes used to give a different aspect to a part of the painting. Resinous materials were added to the loose paint to achieve particular effects or were employed as a thin layer on the whole surface of the paint to protect it from environmental agents (in particular for the protection of white colour containing dyring oils as binders which, on ageing, tend to yellow). Some other materials, used as ligands, were of different origin (e.g. shellac is a resin obtained from insects). Another material, for which no investigation was undertaken, is represented by natural waxes of different origin. These materials are easily identified by mass spectrometry, being formed from complex hydrocarbon mixtures and long-chain esters.

The problem we faced in these preliminary investigations was the significance of data obtained by analysing pure, artificially aged ligands with respect to real paint samples. Each analysed binder led to complex pyrograms. Of course, the whole pyrogram cannot be used for direct identification of the ligand present in a real sample. We spent much effort in order to identify, among the many different pyrolysis products, those which could be considered as effective markers for the presence of specific binders in real samples.

The use of linseed oil is easily evidenced by the presence, among the pyrolysis products, of fatty acids, originating from the thermal degradation of triglycerides. However, it must be emphasized that these compounds are present in both drying oils and egg yolk. Nevertheless, the differences in the relative amounts of saturated and unsaturated long-chain fatty acids (palmitic, stearic, oleic and linoleic) can be used to determine which of these two ligands is present in the analysed sample.[15,16]

Using the PY-GC/MS approach (Curie point or filament), the fatty acid identification can be made by mass spectral library searches even if the fit values obtained are usually not very high. Definitive identification of a drying oil can be gained only by the presence of dicarboxylic acids, which are present only in trace amounts among the pyrolysis products of egg and glair (based on egg white). Dicarboxylic acids can be recognized only after derivatization due to the easy decarboxylation of the underivatized compounds. The identification of linseed oil can be easily achieved by THM/GC/MS, which leads to the production of the related methyl ester, exhibiting an easily detectable molecular ion.[11] THM/GC/MS experiments allowed the identification of the dicarboxylic octanedioic and nonanedioic acids, which are not revealed under normal pyrolysis conditions. In Fig. 2(a) and 2(b) the pyrolysis profiles obtained on analysing a 17 year old layer of linseed oil and egg yolk with green pigment are shown. Differentiation between linseed oil and egg yolk by Curie point PY/GC/MS without derivatization does not lead to straightforward results. In particular for egg yolk, some minor components due to nitrogen-containing heterocycles can be considered as markers of the proteinacous nature of the ligand.[17] Moreover, under such experimental conditions, azelaic acid is not detectable, which is reasonable considering the severe pyrolysis conditions and the high polarity of this compound.

The difference in the protein content of egg yolk and albumen is small, as evidenced by the amino acid content of the two binders reported in Table 2. However, it must be emphasized that egg white contains ovalbumin (a glycoprotein) as the principal component. From Table 2 it follows that both binders show a high degree of similarity, in terms of amino acid composition, with casein. The pyrograms obtained by the Curie point approach on casein, egg yolk and albumen showed some interesting differences[17,18] (Fig. 3(a), (b) and (c), respectively). In the casein pyrogram (Fig. 3(a)), a large number of pyrolysis products were detected. The components with retention times in the range 30–40 min are completely absent in the pyrograms of the other two binders (egg and animal glue)

Table 1 Analyzed binding media and pyrolysis methods used for the analysis

Type of sample	Sample No.	Binding medium	THM/GC/MS	Curie-point pyrolysis	Heated resistor pyrolysis
Drying oil	1	Linseed oil	✓	✓	✓
Proteinaceous binders	2	Egg yolk	✓	✓	✓
	3	Egg albumin	✓	✓	✓
	4	Milk casein	✓	✓	✓
	5	Bone glue		✓	
	6	Skin glue		✓	
	7	Rabbit glue	✓	✓	✓
	8	Fish glue	✓	✓	✓
Polysaccharides	9	Starch		✓	
	10	Arabic gum		✓	
	11	Tragacanth gum		✓	
Resins	12	Colophon		✓	✓
	13	Danmar		✓	✓
	14	Mastic		✓	✓
	15	Sandarac		✓	✓
	17	Copal			✓
	16	Venice turpentine			✓
	18	Elemi			✓
	19	Benzoin		✓	
Other	20	Shellac		✓	✓

and, consequently, they can be considered highly diagnostic for casein. The structure assignments obtained by a library search indicate that most of them are nitrogen-containing compounds, in agreement with the proteinaceous substrate. In contrast, egg yolk and albumin yield, in the 20–35 min range, practically identical pyrograms (Fig. 3(b) and (c)). However, in the case of albumen, the most abundant component is detected at 44.42 min and exhibits a mass spectrum completely different from those related to components at retention times of 43.60 and 43.69 min in casein and egg yolk pyrograms, respectively. Consequently, this component can be considered a highly valuable marker for the presence of egg white albumin.

Table 2 Amino acid contents (%) of some proteinaceous binding media[15]

Amino acid	Casein	Egg yolk	Egg white	Collagen	Keratin
Glycine	1.7	3.5	3.6	26.6	6.0
Alanine	2.7	5.6	6.3	10.3	3.9
Valine	7.2	6.4	8.3	2.5	5.5
Leucine	9.0	9.2	10.3	3.7	7.9
Isoleucine	6.0	5.1	6.2	1.9	3.8
Proline	13.2	4.5	4.5	14.4	6.7
Phenylalanine	5.1	3.9	5.2	2.3	3.7
Tyrosine	5.5	2.8	1.4	1.0	5.2
Serine	4.0	9.1	5.8	4.3	8.4
Threonine	2.7	5.6	3.7	2.3	6.6
Cystine	0.0	1.9	1.9	0.0	12.8
Methionine	2.3	2.3	1.2	0.9	0.6
Arginine	4.0	5.5	6.8	8.2	9.9
Histidine	3.6	2.4	2.4	0.7	3.0
Lysine	6.7	5.7	8.0	4.0	0.9
Aspartic acid	6.1	11.5	10.5	6.9	6.9
Glutamic acid	20.2	15.0	13.9	11.2	14.5
Hydroxyproline	0.0	0.0	0.0	12.8	0.0
Trytophan	0.0	0.0	0.0	0.0	1.9

In fibrous proteins, such as those present in animal glues, the linear polypeptide chains align themselves more or less parallel to each other. Many proteins belong to this class but those employed in the art field are mainly related to collagen and keratin substrates.[15] Collagen is the structural protein of connective tissues in animal and fish. It is insoluble in water but, once boiled, it leaks into the boiling solvent as gelatine owing to the partial degradation of the protein. Keratin is present in hair, wool, feathers, horn, nails, hoof and epithelial skin layers. Both of these proteins, whose amino acid contents are reported in Table 1, are commonly referred to as glues. The related Curie point pyrograms are strongly different from those of casein and egg proteins. Clear analogies are present between bone and fish glue and between rabbit and skin glue. However, some differences between keratin-based glues are also present, allowing the identification of the specific binders.[17]

A flow chart, based on THM/GC/MS analysis,[19] and showing the strategy to identify the presence of drying oil or different proteic binders in painting samples, is reported in Fig. 4. The method was developed by the THM/GC/MS analysis of 17-year old standard binding media mixed with different pigments to account for possible fluctuations. The flow chart shows peak heights relative to the sum of the five most intense peaks of the pyrogram with retention times longer than 4 min. Pyrrole was identified as a marker for the presence of animal glue in the analyzed samples. This compound is formed during the thermal degradation of hydroxyproline, an amino acid which is present in large amounts in animal glues only. The presence of a quantity of pyrrole higher than 50% of the chromatographic total area led to the conclusion that only

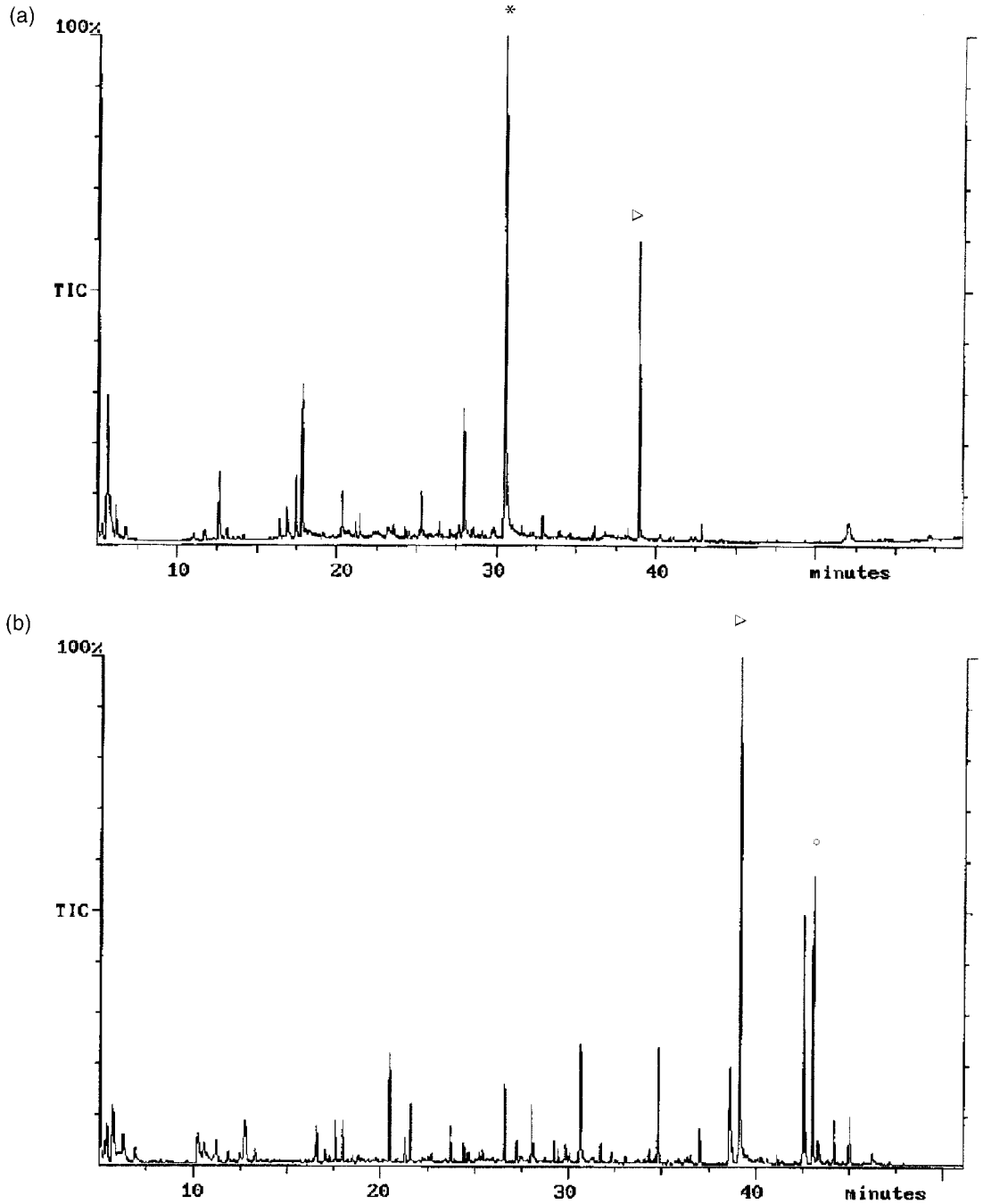

Figure 2. PY/GC/MS (heated filament) profiles of (a) linseed oil and (b) egg yolk. (∗) Azelaic acid; (△) palmitic acid; (○) stearic acid.

animal glue is used as a ligand. The presence of fatty acids (palmitic and stearic) in quantities higher than 60% was considered to be characteristic of the presence of egg yolk or drying oils. Albumen could be differentiated from casein by the presence of sulphur-containing pyrolysis products (methanethiol and dimethyl sulphide) which were present in larger amounts in albumen than in the other binding media. As discussed above, the presence of dicarboxylic acids allowed the identification of drying oils. Azelaic acid is a well known product of the degradation of unsaturated fatty acids. It represents a major peak in the pyrolysate of linseed oil (10–24%), whereas it is a minor fragment in the pyrolysate of egg yolk. However, the azelaic acid concentration generally increases with ageing and its amount depends on environmental conditions such as temperature, light, and pigment type and it is subject to wide variations in egg yolk.[15] The binding media can be identified with a great degree of confidence if the artist used only one type of substrate. When the ligand is formed by a mixture of substrates, the major components could be identified but their relative quantities could only be estimated.

From a chemical point of view, carbohydrates and natural resins are often encountered as museum materials owing to both their availability from natural

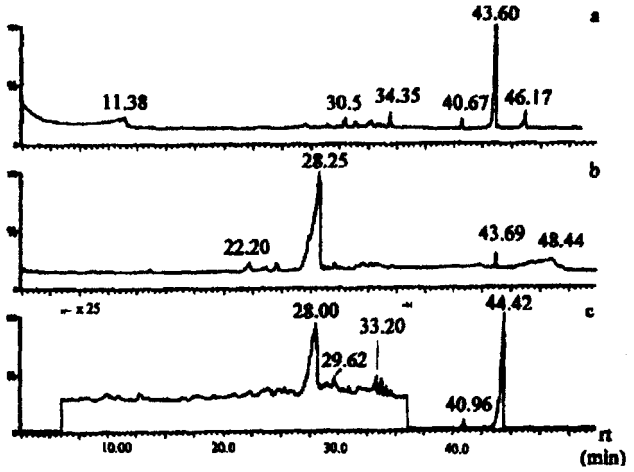

Figure 3. PY/GC/MS (Curie point) profiles of (a) casein, (b) egg yolk and (c) albumen.

sources and their good adhesive properties.[15,20–22] Among the former, the most important polysaccharides used as paint binders include cellulose, starch, plant gums and mucilage. Whereas the first two substances are of simple and regular composition, gums and mucilage are more complex. Usually plant gums were used as adhesives and binders on water colours (acquerelli) and miniatures whereas starch was used, often mixed with other substances (i.e. drying oils and resins), to obtain more flexible preparations (mestiche). Samples 9–11 in Table 1 belong to the polysaccharide chemical class. Gums 10 and 11 are amorphous materials originating from *Acacia senegal* and *Astragalus* (Leguminosae), respectively. They are polymers of different simple sugars, some of them containing carboxylic groups in the form of Ca or Mg salts. The main use of these substances is as adhesive binders in paintings on light supports (e.g. paper) and as mordents for gold. Often they were employed to form emulsions with oil, casein and egg. The total ion chromatogram of the pyrolysis products of starch, reported in Fig. 5, shows the presence of many components which, by a library search, were assigned to oxygen-containing compounds, analogous to those already described[23] in studies performed by in-source PY/MS of the same substrate. The pyrograms of arabic and tragacanth gums show specific components, due to the presence of different sugars in the polymeric chain.

Natural resins, spontaneously exuded by a variety of plants, were used as both adhesives and coatings. They are formed by complex mixtures of plant secondary products, e.g. metabolites produced by plants under physical stress as a defence against pathogens.[24] The resin composition depends on geographical and botanical origins, but terpenoids, including volatile terpenes (C_{10}), sesquiterpenes (C_{15}), triterpenes (C_{30}) and polyisoprenoids ($(C_5)_n$) are their main constituents.[15] Resins are encountered in ancient recipes for plaster adhesives, as protective films and solutions for consolidating museum objects such as small sculptures, paintings, jewellery and furniture.[25] Natural resins elude thorough characterization because of the enormous number of isomers in the terpenoid family and the involatility and macromolecular nature of many of them. Conventional analytical techniques cannot be used to characterize these compounds easily without complex sample pretreatment.

The pyrograms of natural resins show diagnostic components.[26] Colophony, derived from pine trees (Pinaceae) and being the solid residue obtained after the

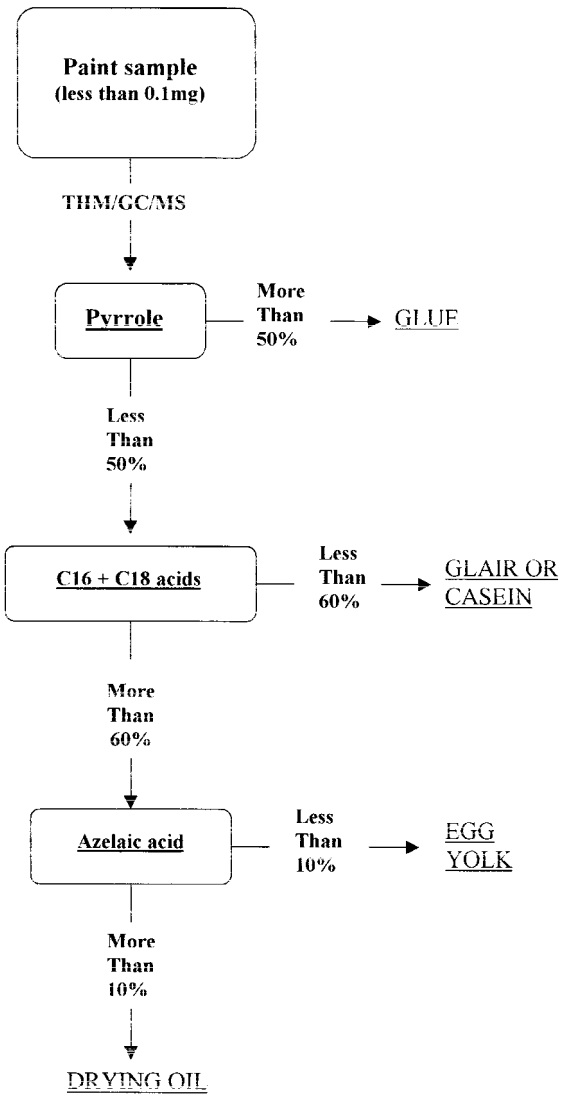

Figure 4. Strategy to individuate the binding media in painting samples using PY/GC/MS selected markers.

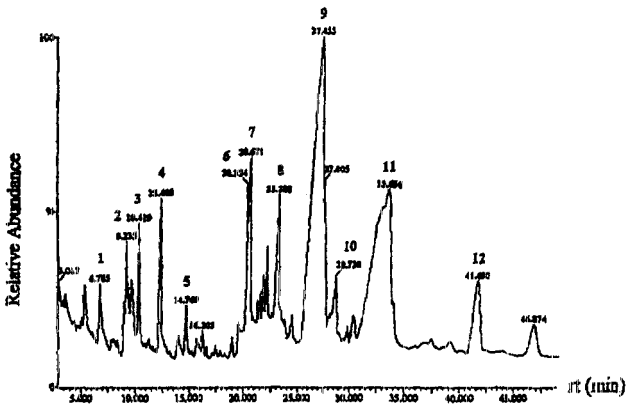

Figure 5. PY/GC/MS (Curie point) profile of starch.

distillation of turpentine oil, showed the presence of abietic acid and pimaric acid. Dammar, which originates from trees of the Dipterocarpaceae family (sample 12), was characterized by the presence of two peaks at 28.1 and 28.50 min, possibly positional isomers, whose mass spectra were consistent with a dehydrogenated sesquiterpene with the molecular formula $C_{15}H_{26}$ (molecular ion at *m/z* 202, base peak *m/z* 159) and a complex pattern of peaks of compounds of general formula $C_{15}H_{26}$ (molecular ion *m/z* 206 and base peak *m/z* 163) was also observed in the range 25.0–27.30 min. A phenolic compound, namely totarol ($C_{20}H_{30}O$, molecular peak *m/z* 286 and base peak *m/z* 271) was chosen as a marker for the identification of sandarac (obtained from *Tetraclinis articulata*, Cupressaceae), whilst mastic, which is the most important resin obtained from the genus *Pistacia* (Anacardiaceae), was characterized by a peak at a retention time of 28.20 min with a molecular mass of 206 and a base peak at *m/z* 191, consistent with a dihydrogenated sesquiterpene. Manila copal belongs to the Araucariaceae resins and, in particular, it derives from topical trees of the genus *Agathis*, which is the main resin-producing conifer genus in the southern hemisphere. It was identified by a series of peaks in the 23.20–30.0 min retention time range, belonging to sesquiterpenoid molecules with m_r 204 and base peak *m/z* 161. Venice turpentine is a product of *Larix decidua* (Pinaceae) and was used as a varnish ingredient, for example, in mixtures with sandarac resin. In the pyrogram of Venice turpentine, the presence of abietic acid was identified and this compound was chosen as a marker for this resin. Finally, elemi, which is produced by various genera of the family Burseraceae, usually contains large amounts of sesquiterpenes and has a pleasant citrus scent. It was definitively identified by the presence of elemicine (27.55 min) with characteristic ions at *m/z* 208 (molecular ion and base peak) and *m/z* 193 (50% of the base peak).

Benzoin has a chemical composition completely different from those of the resins discussed above, being mainly composed of esters of aromatic acids with aromatic alcohols. Its characterization can be easily achieved by the presence among its pyrolysis products of benzoic and cinnamic acid and conypheryl benzoate.[27] Finally, shellac represent the unique resin of animal origin employed in the art field, produced by the insect *Kerria lacca*. From a chemical point of view, this resin is composed of low molecular mass polyesters, waxes and water-soluble dyes. In this case, a peculiar pattern of fatty acids, evidenced by the typical ion at *m/z* 60 arising from McLafferty rearrangement, was chosen as a marker for shellac-containing samples.[26]

The preliminary research performed on artificially aged binder samples was essential to verify the validity of PY/GC/MS in the field of art analysis and to obtain markers allowing the unequivocal identification of different paint ligands. This last point has been achieved in terms of ionic species (either molecular or fragment ions) of characteristic pyrolysis products. This approach was highly effective with respect to PY/GC methods, allowing a less strict dependence of the analytical results on the experimental chromatographic conditions. Furthermore, the large amount of data obtained allowed the generation of a database which can be easily employed by comparison of results for real samples.

APPLICATION OF PY/GC/MS AND THM/GC/MS TO REAL SAMPLES

We report some results obtained by our groups on different museum objects of different historical periods. They represent a few examples of what is currenly being done all over the world as a consequence of the collaboration of mass spectrometrists with archaeological and art researchers.

PY/GC/MS of paint layers from two Egyptian objects was performed: a wooden sarcophagus (664–525 BC), a Theban specimen which belonged to Usai, Nekhet's son (XXVI dynasty), consisting of a large polychromatic sarcophagus containing a second one and, inside the latter, the still preserved mummy and a cartonage (Plate 1) containing the mummy of Nekhetubastetiru, 'the house lady,' daughter of the 'fourth Amon-Ra's prophet: Nekhtefmut (XXII and XXIII dynasty, 944–716 BC). The specimens are on exhibition at the Museo Civico Archeologico in Bologna. During restoration, some paint layers of different colours were sampled in order to characterize the ligands used.[28]

All except one of the 10 paint layers showed a homologous series of saturated and unsaturated hydrocarbons from C_8 to C_{24}, in which the alkene series give to the most abundant peaks surrounded by smaller peaks due to alkanes and dienes (Fig. 6). Such profiles are consistent with the presence of waxes as a binding medium. Waxes contain long-chain esters and hydrocarbons. Pyrolysis of such substances can produce hydrocarbons as the products of decarboxylation of fatty acids and of flash vaporization of the present hydrocarbon fraction. To confirm this hypothesis, pyrolysis of beeswax was performed using the same experimental conditions and it yielded a pyrolysis profile practically superimposable on that of the real sample. However, in one of the analysed samples such a profile was completely absent. Among the pyrolysis products of this sample, pyrrole was easily identified. As discussed above, this compound is a good marker for animal glue. Pyrrole was present also in four other samples, indicating that a mixture of wax and animal glue was present in these samples.

PY/GC/MS was also successfully employed to assess the geographical origin of some samples of amber archaeological objects at the Museo Civico Archeologico in Bologna.[29] Amber is a fossil resin with the unique feature of being formed mainly by terpenic polymers, in some cases cross linked by succinic acid. Determining the geographical origin of archaeological amber samples is of great importance in drawing the map of cultural and trade networks among ancient human settlements. Such an assessment has been achieved by comparing the pyrograms of museum objects with those obtained from standard amber samples of known geographical origin. The description of some archaeological objects is reported in Table 3. The samples were

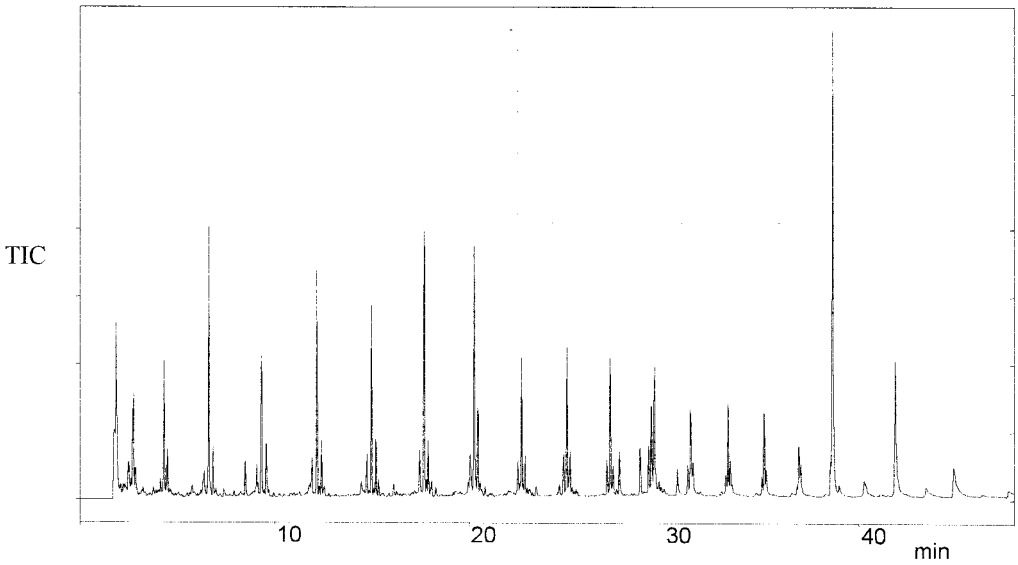

Figure 6. PY/GC/MS trace of an Egyptian sarcophagus paint layer showing a typical profile due to the presence of natural waxes.

collected in Etruscan tombs in the vicinity of Bologna, Italy. Standard amber samples (Table 3), when pyrolysed under the same experimental conditions, showed different pyrolytic profiles, allowing the characterization of their geographical origin. In particular, Baltic amber showed an intense succinic anhydride peak, which was absent in the other amber samples. These data were compared with those obtained from the analysis of museum objects. All the samples from the archaeological objects showed a PY/GC/MS profile similar to that obtained from Baltic amber, in which succinic anhydride was detected in large amounts. It is surprising to observe that, even in Etrurian times, a market in amber at the European level existed.

The Nascita di Venere painting by Botticelli (Plate 2) is surely one of the most representative pictures of the Italian Renaissance. The mythological theme and the female body intended as an expression of beauty and not of damnation, as in the Middle ages, represents a synthesis of aesthetic thought of the Renaissance period. THM/GC/MS was used to verify which of the proteic media usually employed by artists in that historical period was used by the painter in this masterpiece.[16] The analysis of a small sample of a green painted part of the painting (less than 0.05 mg) led to the chromatogram shown in Fig. 7. The presence of C_{16} and C_{18} fatty acids established that the ligand employed was egg yolk.

For restoration purposes, PY/GC/MS was used to analyse a sample from the ligneous roof in S. Giobbe Church in Venice and Giuseppe Nogari's oil painting shown in Plate 3 (Ritratto, private collection). The chromatographic profiles obtained were strongly different from those obtained on analysing standard aged reference materials, either because of ageing (about 300 years) or as result of environmental factors (in particular, the S. Giobbe paint was exposed to a brackish environment). What could not be recognized by the total ion chromatogram of the pyrolysis products could be identified from the reconstructed ion chromatogram of the diagnostic ions, allowing the determination of drying oil in the 'ritratto' and of egg yolk in the church roof.[18]

Analogously, the THM/GC/MS analysis of some other real painting samples,[19] namely paintings by Mentessi (Piazza S. Marco a sera, 1885, and La Pace,

Table 3 Description of amber archaeological objects and standard amber samples (Museo Civico Archerologico, Bologna)

	Sample No.	Inventory No.	Tomb	Object description
Archaeological objects	1	17328	GM nn	Pendant, woman shape
	2	17346	C361	Rectangular pendant, ram shape
	3	17348	C350	Pendant, woman shape
	4	17349	GM nn	Pendant, animal shape
	5	17393	C100	Pendant, animal shape
	6	17395	C nn	Pendant, ram shape
	7	—	BC56	Pearls from a necklace
Standard amber samples	8	—		Sicilian amber, uncertain origin, internal part, non-deteriorated
	9	—		Sicilian amber, uncertain origin, external part deteriorated
	10	16646		Sicilian amber, Simeto river
	11	—		Amber from Scanello, Bologna, Italy
	12	—		Amber from Mercato Saraceno, Forli, Italy
	13	2108		Baltic amber from Dobrzyn, Poland

Plate 1. Egyptian cartonnage of the mummy Nekhetubasteritu, Tebe, XXII-XXIII dynasty, 944-716 B.C., Palagi Collection (Nizzoli), inventary number KS 1972, Museo Civico Archeologico, Bologna, Italy (by kind permission of Museo Civico Archeologico, Bologna, Italy).

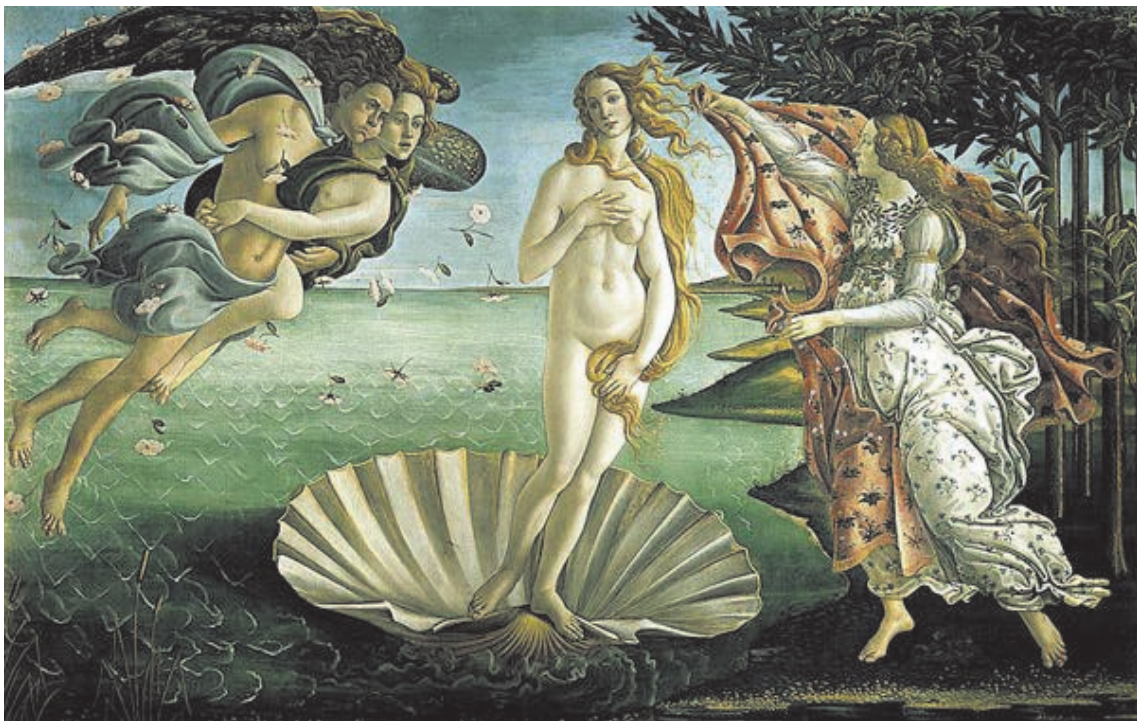

Plate 2. The "Nascita di Venere" by Botticelli, Galleria degli Uffizi, Florence, (by kind permission of "Ministero per i Beni Culturali e Ambientali").

Plate 3. "Ritratto" by Giusseppe Nogari (private collection).

Plate 4. "Nettuno" bronze by Giambologna (1566) in the centre of the city of Bologna, Italy.

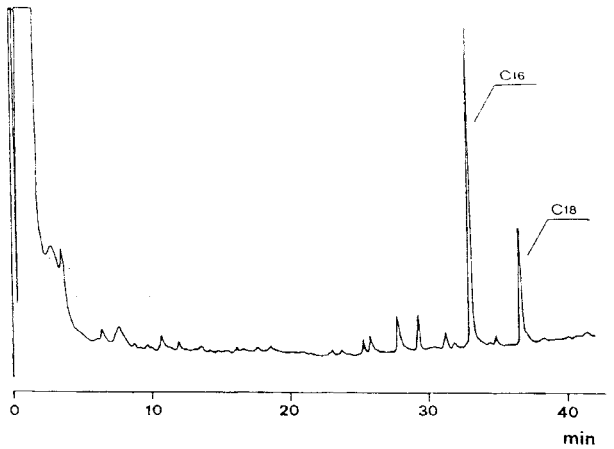

Figure 7. Pyrolysis profile of a sample from the Nascita di Venere painting showing the presence of the pyrolysis markers for egg yolk.

1907), by Cagnacci (S. Giuseppe, 1642–43) and a tempera from a wall in Palazzo Pepoli, Bologna, by an anonymous temperist of the 18th century, allowed the determination of the binders used by these artists (Table 4).

PY/GC/MS and THM/GC/MS were used for the chemical characterization of a sample of black crust from the stony central portal of St Denis Abbey, France.[30] The St Denis cathedral is the first example of a large gothic building in Europe and inspired the architecture of the cathedrals of the 12th century. The black patina present on the surface stone of small columns belonging to the reconstructed threshold of the church was analyzed to determine whether it was due to atmospheric pollution or to the degradation of a past restoration treatment. The formation of black crusts is a well known consequence of atmospheric pollution. Sulphur dioxide present in the polluted atmospheres of modern towns induces the transformation of calcite ($CaCO_3$) into gypsum ($CaSO_4 \cdot 2H_2O$) which precipitates with inclusion of carbon particulates. PY/GC/MS was used as a rapid technique to determine organic substances in the black crust. A homologous series of aliphatic nitriles (from C_8 to C_{17}) and some compounds containing nitrogen and sulphur (benzonitrile, propyltetrahydrothiophene and methyltetrahydrothiophene) were identified. Aliphatic nitriles are some of the pyrolysis products of bacteria and their presence in the black crust might be an indication of a biological origin of the pollution.[11] A further indication supporting this conclusion was obtained by THM/GC/MS, which produced a chromatogram dominated by a homologous series of fatty acid methyl esters (C_9–C_{18}). Sulphur-containing compounds might be related to rubber vulcanization and asphalt. PY/GC/MS and THM/GC/MS, in addition to other analytical techniques such as scanning electron microscopy/every-dispersive x-ray spectrometry and Fourier transform IR spectrometry, allowed us to rule out the hypothesis of a previous organic restoration on the surface of the cathedral.

The black patinas covering the Neptune statue, a renaissance outdoor bronze made by Gianbologna in 1566 and located in the center of Bologna (Plate 4), was analyzed by PY/GC/MS.[31] This type of analysis is important in elucidating the mechanism of formation, the binding role and the degradation problems related to the organic fraction present on the external surface of outdoor artefacts. The Neptune bronze was almost completely covered by a black patina, very thin and adherent to the surface of the statue, and no corrosion products, commonly found on outdoor bronzes exposed to atmospheric pollution, were found. On the bronze surface the authors found a homologous series of aliphatic hydrocarbons, carboxylic acids and other compounds (phthalic anydride and phthalimide) not previously found in similar samples and that could be due to a possible organic treatment (protective coatings) used in past restoration processes.

In conclusion, mass spectrometry and, in particular, PY/GC/MS, represents a valid tool in chemical analysis applied to our cultural heritage, allowing the identification of painting binders and of organic materials used in the arts. Knowledge of these data makes restorers' work easier. Mass spectrometry is currently being successfully employed in the art field and one can anticipate further and still more valuable applications.

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Table 4. Description of paint samples and identified ligands as obtained by THM/GC/MS

Sample No.	Author	Object	Ssmple	Ligand
1	Anon. Bologna	Tempera from a Wall in Palazzo Pepoli, Bologna	Dark brown paint layer	Egg yolk and some wax (previous restoration?)
2			Brown paint layer	Egg yolk, animal glue and some wax
3	Mentessi	Piazza S. Marco a sera	Light blue sky paint layer	Egg yolk and some drying oil
4		La Pace	Grey blue sky paint layer	Egg yolk and some drying oil
5			Blue sky paint layer	Drying oil
6			Blue sky paint layer	Egg yolk
7			Green paint layer	Egg yolk and some drying oil
8	Cagnacci	S. Giuseppe	Yellow paint layer	Drying oil
9			Flesh-coloured paint layer	Egg yolk
10			Brown paint layer	Egg yolk

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