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The Application of FTIR-Microscopy to the Analysis of Paint Binders in Easel Paintings

JENNIFER PILC AND RAYMOND WHITE

For a period of approximately thirteen years from 1979, analytical investigations into the constitution of paint binders within the Scientific Department relied, primarily, on the use of gas-chromatography combined with mass-spectrometry (GC–MS). Prior to this, there had been a history of use of gas-chromatography for this same purpose since 1965. These methods were occasionally supplemented by dispersive infra-red analysis, an analytical technique which first came into general use in the early 1950s when the machines became commercially available. Using this technique it is possible to examine the different varieties of chemical bond vibrations that occur between atoms within a molecule, by measurement of the characteristic energies associated with such movements. The information obtained enables the analyst to suggest a possible identification of the material present.

Despite improvements over the next few decades, dispersive instruments remained slow, limited in resolution and somewhat insensitive, and examination was confined to the relatively macroscopic sample, often just extracts of materials resulting from conservation treatment (such as wax-based lining adhesives) or extracts of cleaning swabs. The size of a paint sample normally taken was too small for successful analysis. The fact that paint scatters infra-red radiation to a significant degree and has a particularly high opacity coupled with the difficulty in locating the specimen close to the detector means that very little useful infra-red energy arrives at the detector. Thus the variations in absorption signal detected and amplified were often of the same order of magnitude as the thermal and electronic noise in the spectrometer.

Further limitations are placed on this 'global' examination of the paint binder by obstruction of key diagnostic regions of the infra-red absorption spectrum of the organic binding medium by those of mineral pigments; these are, of course, an integral part of the paint.

Non-dispersive infra-red spectrometers, usually referred to as Fourier transform infra-red (FTIR) spectrometers, examine infra-red radiation that has passed through the specimen, but – in contrast with dispersive machines – has not been split up into frequency bands by a prism or diffraction-grating. The beam is reflected from a moving mirror and combined with a reference beam that has not passed through the specimen. When optically combined, the two beams give rise to a continually changing interference pattern, recorded as an interferogram. This pattern can be de-convoluted (or 'unravelled' into discrete absorption bands) by a mathematical process known as Fourier transformation to yield a spectrum or absorption pattern; this shows the degree to which the sample absorbs different wavelengths of incident infra-red radiation and is displayed as a series of peaks on a graph (see, for example, Fig. 1a). The technique is very quick and permits the acquisition of hundreds of scans of the absorption spectrum of a material in just a few minutes. These may be combined to give an averaged spectrum in which random signal noise may be substantially reduced. This is particularly important where very small samples are to be examined.[1]

In addition, FTIR has the advantage of enhanced resolving-power over a conventional infra-red spectrometer; that is, it is better able to resolve two neighbouring absorption peaks into their individual components, rather than rendering them as one broad envelope.

Introduction of infra-red microscopes,

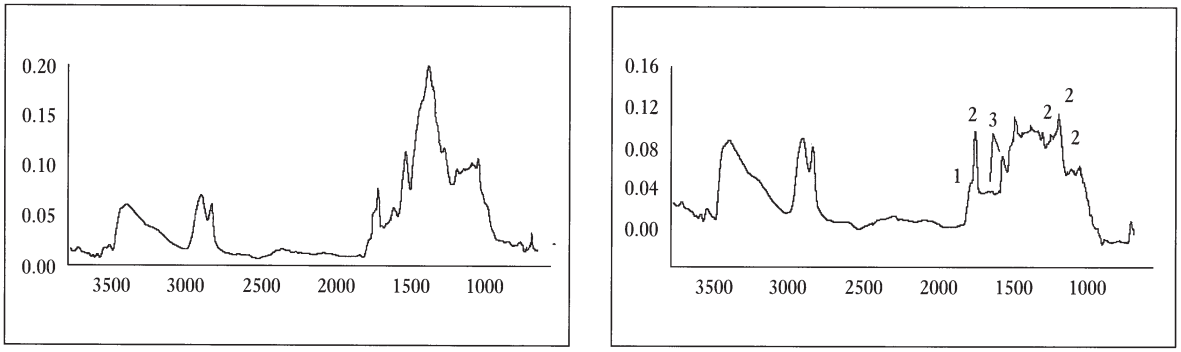


Fig. 1. FTIR analyses of white paint of headdress from Rogier van der Weyden's *A Portrait of a Lady*, as a silver chloride-embedded, microtomed thin cross-section:

(a) Spectrum of medium-rich region using IR microscope in transmission mode. This spectrum contains some contribution from adjacent pigment.

(b) Corresponding pigment-subtracted spectrum. This spectrum shows more clearly some of the medium-specific features of the sample. The band (marked 1 at 1778cm^{-1})is more clearly shown and, in conjunction with the presence of ester-associated bands (marked 2), is indicative of the use of a drying oil paint medium. Other bands, marked 3 (1620 and 1536cm^{-1}), are the result of carboxylate ion formation caused by reaction of the pigment with the oil medium.

connected directly to the main optical bench of the FTIR spectrometer, has heralded a new era in the infra-red analysis of paint, as it has become possible to produce good spectra from very small samples. Most importantly, it has become feasible to obtain *dimensionally* resolved infra-red measurements, across the layer structure of a paint sample, for example. Combined with the enhanced facilities for display and data processing readily available with Fourier transform manipulated data and by utilising background subtraction, samples of the order of 8μm in diameter may be successfully examined.[2] The subtraction of the spectrum of an inorganic pigment particle from that of a surrounding or occluded region of organic binder can result in a passably good spectrum of the paint medium alone. For the first time it would appear that the analytical possibilities begin to approach those for the investigation of mineral pigments, which have long been commercially available to the analyst of inorganic materials.

The natural, chemical components present in paint binders and other constituents of easel paintings, such as varnishes and adhesives, are invariably present as complex mixtures, even in the freshly produced work. The molecular structure of many of these individual components are themselves complex. This qualitative complexity invariably increases with age, as a result of oxidative changes, the joining of molecular units by polymerisation and cross-linking and molecular scission of some components. GC analysis is able to separate out these components and provide a qualitative and

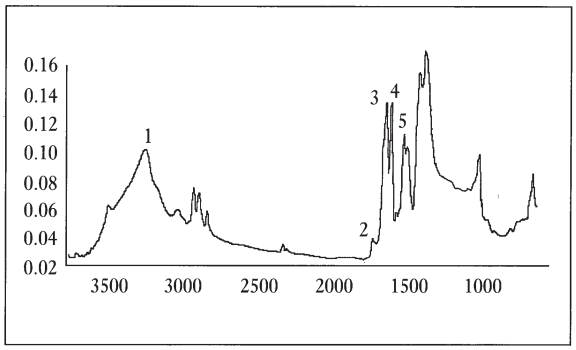


Fig. 2. Spectrum recorded of the lower layer of less dense underpaint in the same section. After subtraction and following multiple derivative analysis of the spectral curve. Note the presence of an absorption maximum at 3290cm^{-1} (marked 1), the result of absorption by N–H groups. The ester carbonyl absorption (2, 1739cm^{-1}) is minor and little or no evidence exists for the presence of the 1776cm^{-1} band. Prominent features, such as the Amide I carbon-oxygen absorption at 1658cm^{-1} (marked 3), the Amide II amino group deformation (1633cm^{-1}, marked 4)) and the doublet at 1548 and 1520cm^{-1} (Amide II band, marked 5) would point to the presence of egg tempera paint medium. The other bands are residual absorptions from the pigment.

quantitative display of many of them. GC–MS is able to take this process one stage further, in that it can 'fingerprint' the complex mixture in a qualitative and quantitative way and is able to give a characterisation of the individual components at a *molecular* level. However, a potential complication associated with such techniques occurs in the case of paint that has not been

Fig. 3 Rogier van der Weyden, *A Portrait of a Lady* (NG 1433), *c.*1460. Oak, painted area 36.2 × 27.6 cm.

sampled as one discrete layer. Chromatographic techniques are only able to give an overall analysis and quantitation of all the components present in a sample. If this truly represents one layer only, there is no problem; with samples containing more than one paint layer, it is not possible to assign components of the paint medium to a specific layer. Only by re-sampling each layer, one at a time, would this assignment become possible.

By contrast, infra-red spectrometry is not able to give as detailed a molecular 'fingerprint' of the chemical components that may be present in, say, a paint binder. This type of spectrometry looks only at certain aspects of the molecular structure and gives information on the bond absorption of, predominantly, functional groups. (Examples of the latter include alcohol groups (–OH) and amino groups ($-NH_2$).) It is rather like viewing fragments of an outdoor scene through a few narrow windows, when standing well back in the room. Thus the technique may only suggest the presence of alcohol groups or ester groups (possibly associated with oils or waxes), amino or amide groups (associated with protein-containing materials, such as egg tempera or glue) or carboxylic acid groups (as might occur in terpenoid resins). Regrettably, the infra-red spectrum of a complex mixture contains so many individual bond absorptions that these overlap and merge into broad envelopes.[3] The enhanced resolving power of the FTIR spectrometer is able to offset this problem to a degree and when combined with examination of the first and multiple derivatives (rates of change and acceleration of the spectral curve), as illustrated below, some diagnostic underlying features and partially overlapping absorptions may be split into discrete bands.

Normally, in the Scientific Department, fragments of paint are examined by FTIR-IR microscopy before analysis by GC–MS, that is, our approach to analytical problems has become bivalent, broadly speaking. The former technique is able to provide limited information concerning the class of chemical components within the sample; it can make a spatial assignment and give the precise location of a given functional group (component) within the layer structure of the sample and functional group. FTIR analysis is not able to give specific information about the source of a drying oil and experience to date suggests that it is unlikely to yield information concerning the prepolymerisation of drying oils. This information is obtainable, in the case of heat prepolymerisation of oils, from GC and GC–MS analysis.

The more detailed quantitative and molecular information from GC–MS analysis can thus be interpreted in the light of data from the IR system (and vice-versa), to enable a more detailed characterisation of the paint to be made. The two instrumental methods are in no sense employed as rivals; rather, each complements the deficiencies of the other, such that the sum of the information obtained from each instrument greatly outweighs the individual contributions, when taken in isolation.

From a purely practical point of view, detailed assignment of every infra-red band to specific bond-types within a molecular complex is time-consuming and almost invariably riddled with uncertainty. One bond often gives rise to a series of peaks resulting from stretching vibrations of the bond, symmetrical and asymmetrical, coupled with various overtones, scissor-like vibrations and multiple-bond com-

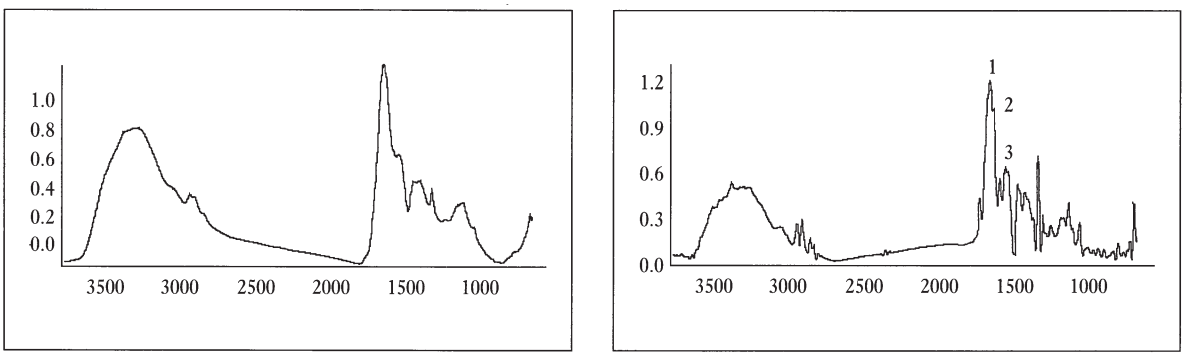


Fig. 4 Absorption spectra obtained in transmittance mode with the infra-red microscope from a sample of red paint of the hem of the Virgin's red dress in Dieric Bouts's *The Entombment*:

(a) Spectrum, after background subtraction.

(b) Reconstructed spectrum following examination of the spectral derivative curves, showing improved resolution and clearer maxima for poorly resolved band-shoulders. It is now just possible to distinguish the Amide I carbon-oxygen band and the Amide II amino deformation band at 1654cm^{-1} (1) and 1630cm^{-1} (2) respectively. The enhanced spectrum also has revealed the presence of a split, Amide II band (marked 3) at 1540 and 1518cm^{-1}.

posite vibrations. Even with one pure molecular component, this may produce a very 'cluttered' spectrum. It is more efficient, from the point of view of identifying materials, to look for certain diagnostic bands that have been found empirically to be associated closely with a specific source. If the circumstances of the analysis so demand, then it may be necessary to isolate particular functional group absorptions and make a more detailed assignment or investigation of any partially resolved or underlying bands. A need for more detailed interpretation might occur where two samples of the same paint were being compared, but one had suffered a higher level of oxidation – resulting in, say, an increase in carboxylic acid groups – than the other. An example is given below in Fig. 9.

Sample preparation and use of equipment

Tiny paint fragments from under frame-rebates or from the edges of cracks and losses may be sampled with the point of a scalpel in the usual way or may be scraped, depending upon requirements. These are placed on a sodium chloride or potassium bromide disk and first examined in transmission or reflectance mode under the microscope, without any further preparation, using a conventional optical objective (100x total magnification). Having determined the general area of interest, the infra-red lenses (total magnification 150x, 320x) are selected as required. The area whose spectrum is to be measured is delimited by the use of an

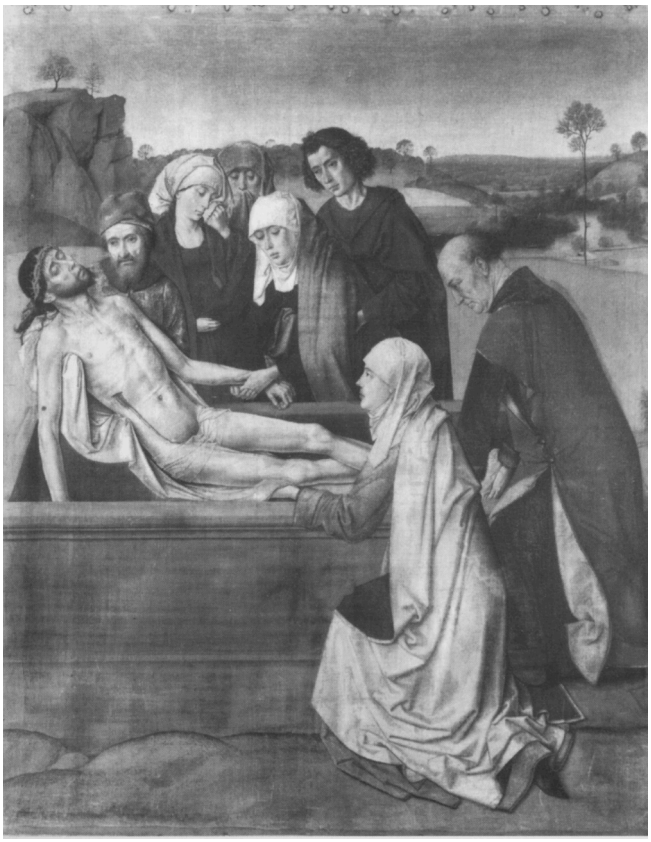


Fig. 5 Dieric Bouts, *The Entombment* (NG 664), 1450s? Canvas, painted area 90.2 × 74.3 cm.

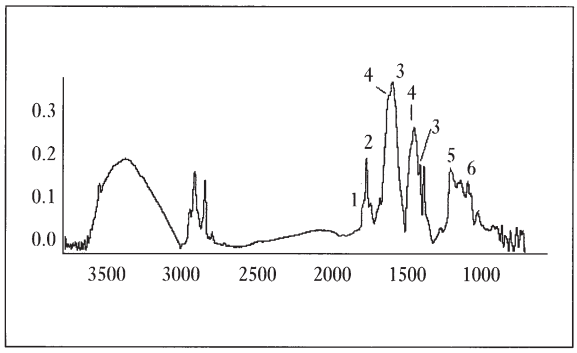
Fig. 6 Garofalo, *An Allegory of Love* (NG 1362), 1530s. Canvas, 127 × 177.8 cm.

illumination masking device, based on adjustable knife-edge apertures. A further set of knife-edge apertures (remote field apertures) is located on the detector side of the microscope stage, below the condenser.

Best spectral results are obtained in transmission mode, that is, where the modulation of the infra-red beam is monitored after its passage through the sample. Excessive scattering of the beam at the sample/air interface will degrade the spectrum somewhat. It may be difficult to focus on a region of interest that has a thickness (optical path length) such that the most intense absorption bands of the sample have not gone to saturation. This occurs when all the incident radiation at a frequency has been removed before it has completed its passage through the sample. This produces unreliable peak-maximum data, since the band will be flat-topped, preventing determination of the frequency of the true absorption maximum. Even more alarmingly, the amplifier in the spectrometer is liable to interpret 'noise' in the system as meaningful absorption peaks and will render the saturated peak as a multiplet of sharp peaks. Better quality spectra are obtained if some form of cross-sectional preparation is undertaken. This makes location of optical features within the sample easier, particularly at high magnifications where the infra-red Cassegrain-type lens used has a very limited depth of field.

Paint sections embedded in the synthetic resin mixtures used for conventional optical microscopy have been used in some laboratories.[4] The contribution from the surrounding synthetic polymer to the overall spectrum of paint medium and pigment is diminished by focusing on the embedding plastic alone, recording its spectrum and removing its contribution by computer subtraction from the overall spectrum. Our own experience has been that careful monitoring of this process is critical, calling for great precision, expertise and a judicious choice of normalising absorption. During grinding and polishing of the section or during the cutting of a thin section, the intense surface tension effect involved can smear an extremely thin film of embedding resin over the section surface. The contribution of this film to the overall infra-red spectrum can swamp – and certainly rival – the contribution of the paint medium itself. Two situations arise from computer subtraction of this type of 'background' contaminant: either a spectrum results that continues to be heavily contaminated by the synthetic resin component, or the resultant spectrum has been over-subtracted in some measure. Sometimes a result is obtained that is selectively depleted of critical band information, or a convincing spectrum of plausible-looking absorption bands, which are in fact noise, results. This transition from one extreme to another is extremely sharp and difficult to control.[5]

As a result of these problems, an alternative to the usual epoxy and polyester synthetic



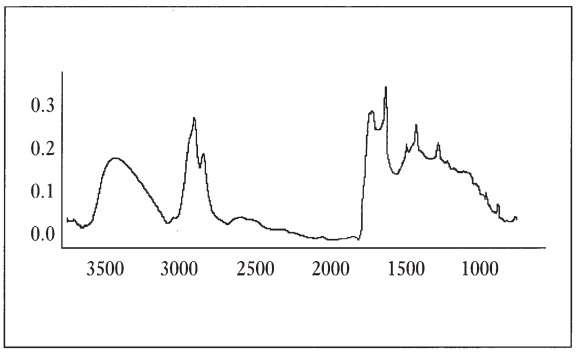
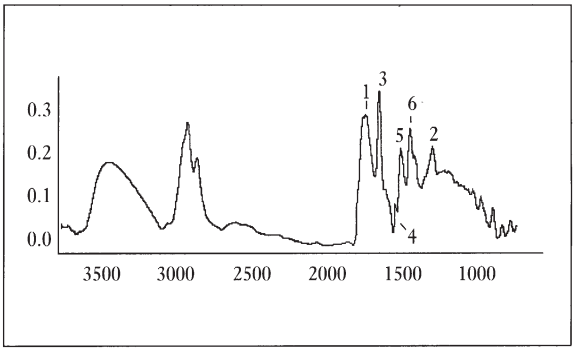


Fig. 7. FTIR analyses of green paint from foliage in Garofalo's *An Allegory of Love*:

(a) Spectrum of the lower more opaque green layer, following partial subtraction of some of the pigment (verdigris) contribution. The absorption at 1777cm^{-1} (1), suggestive of drying oil, can be seen quite clearly; this is adjacent to the ester carbonyl band at 1739cm^{-1} (2). Bands, which are the result of fatty acid carboxylate formation with copper ions can be seen at 1579 and 1382cm^{-1}(3). Shoulders resulting from the presence of verdigris are apparent at 1601 and 1456 and 1427cm^{-1}(4). No resin acid bands (such as those at 1246cm^{-1}) are present in this layer. Prominent bands at 1145 (5) and 1050cm^{-1} (6) are probably due to carbon–oxygen bond stretching (–(C=O)–O–R and –O–CH_2–) within the oil glycerides.

(b) Spectrum of an area of the upper, glaze-like green layer, which corresponds quite well with a 'copper resinate' standard.



(c) The same spectrum, following resolution enhancement by multiple derivative analysis. Note the virtual absence of the 1776, 1164 and 1050 cm^{-1} bands, common to old paint bound with a drying oil medium; there is little evidence for the presence of fatty acid carboxylate bands at 1586cm^{-1} in this spectrum. Absorption peaks due to resin acids and resin acid copper salts can be observed at 1698 (1, typical of an asymmetric C=O stretch of a resin acid), 1246 (2) and 1608cm^{-1} (3, carboxylate). A weak band at 1499cm^{-1} (4) is probably due to an aromatic ring breathing from dehydroabietic acid, present in the 'copper resinate'; the 1464cm^{-1} band (5, methylene and methyl group C–H scissors) and the doublet structure of the methyl group asymmetric scissors (6, 1394/1364cm^{-1}) suggest the presence of isopropyl substituents, such as encountered in the abietane-based diterpenoid resin acids.

resins used to produce sections for optical examination has been sought. Ideally, infrared-transparent materials which have no absorption bands in the frequency range of 4000cm^{-1} to 400cm^{-1} would suit the purpose well. Experiments with various hydrocarbon-based polymers and polymer mixtures have indicated that, although only partially absorbing in this region, the absorption pattern is limited to carbon–hydrogen bond stretching bands and their overtones. By contrast, inorganic materials such as sodium chloride and potassium bromide are completely transparent in the diagnostic region. They have the additional physical property that they undergo cold flow with the application of pressure.

For many years, materials have been ground with potassium bromide powder and pressed with a hydraulic ram into discs with smooth surfaces which minimise scattering of the infrared beam. An attempt was made to embed intact paint samples using finely divided, dry potassium bromide. During this process it was not uncommon for the embedded paint fragments themselves to disintegrate. Nevertheless, some were successful and these could be cut on a glass microtome to reveal an exposed, cross-sectional surface of the paint. Attempts to expose a cross-sectional surface by cutting thin sections were, however, generally unsuccessful as the glassy state of the potassium bromide rapidly reverted to a more brittle and crystalline state. The fragment and the surrounding potassium bromide would then simply crumble to powder, or the fragment would be pulled bodily from the embedding block during cutting. It seemed that the presence of moist air accelerated this process markedly.

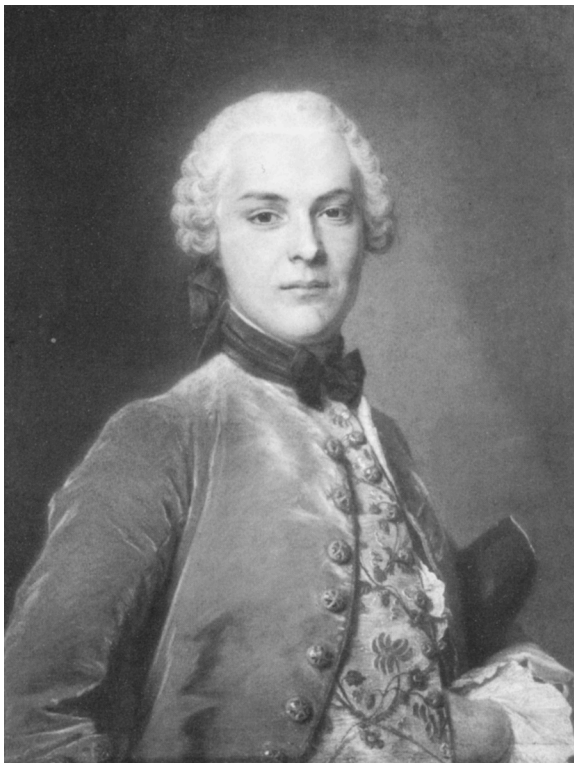


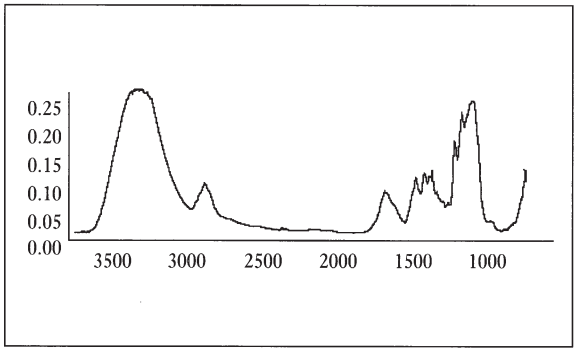
Fig. 8 Maurice-Quentin de La Tour, *Henry Dawkins* (NG 5118), *c.*1750. Pastel on paper, mounted on canvas, 66.7 × 53.3 cm.

Silver chloride is transparent to infra-red radiation throughout the absorption region of interest. It has the added advantage over potassium bromide in being soft and malleable: indeed, it is distinctly plastic and can be squashed with the pressure of the fingers. As minimal pressure is needed to induce cold flow, disruption of the paint structure is less likely. An infra-red grade of silver chloride can be purchased in amorphous lumps, which can be cut into very fine pieces with a scalpel. This is best carried out immediately before use. A die-holder is packed uniformly with finely cut silver chloride to a depth of about 4mm. A depression is made in the top surface and the paint fragment is pressed gently into it. The fragment is covered by a further layer of silver chloride to a depth of about 4mm, a steel die-pellet is placed on top and gentle pressure is applied by hydraulic press or hand-screw for one minute, resulting in a glass-like cylinder. For convenience, the remaining steps are best carried out in subdued, low UV-content light, since silver chloride is photo-sensitive. This will not affect the absorption or reflection characteristics of the final thick or thin section, but it will make location of the sample within a darkened block more difficult until its exposure by cutting.

The block may be cut using a scalpel or microtome. The microtome is preferable since the sample is held very firmly and the cutting is controllable, giving cross-sections of uniform thickness. The block is mounted in the jaws of the sample holder and thin slivers of the block trimmed to expose the paint in section. Thin sections may then be cut, gently removed from the blade of the knife with a brush and transferred to an infra-red-transparent disc. Curled sections may be flattened by gentle rolling with an appropriate tool. If the section is too thick and causes excessive absorption in the infra-red beam, the path length may be adjusted by placing the thin section between the windows of a diamond compression cell[6] while examining it under the infra-red microscope.

When examining very small areas, such as regions of paint binder trapped between pigment particles, one must bear in mind that there are quite dramatic changes in chemical composition from one area to another. In a paint which contains an emulsion of two components, for example, it is important to be aware of the limitations inherent in the precise definition of the measurement area. The area of the sample *actually* illuminated by incident infra-red radiation and measured by the instrument is somewhat larger than the optical image of the mask would suggest. The intensity profile of the image of the masking-blades does not follow a simple 'step' function, but is modified by diffraction bands, which effectively give unwanted energy in the geometrical shadow of the mask. To minimise this problem, caused by 'leakage' from the diffraction-limited image, dual remote-image masking can be used.[7] As a further precaution the remote field apertures should be reduced by about two per cent beyond the desired sample area. This may not be possible if the energy throughput to the detector is insufficient.

An advantage of the use of inorganic embedding agents such as silver chloride is that the embedded sample has not been contaminated with any organic material and can be re-used for GC and GC–MS or some other form of organic analysis. This is not the case with samples embedded in synthetic resin polymers. As



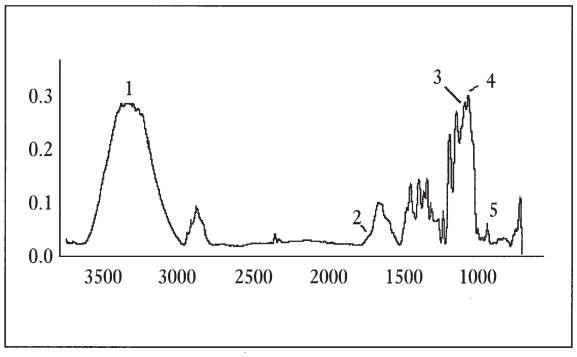
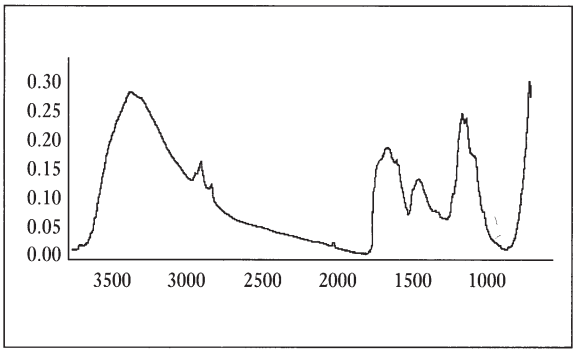
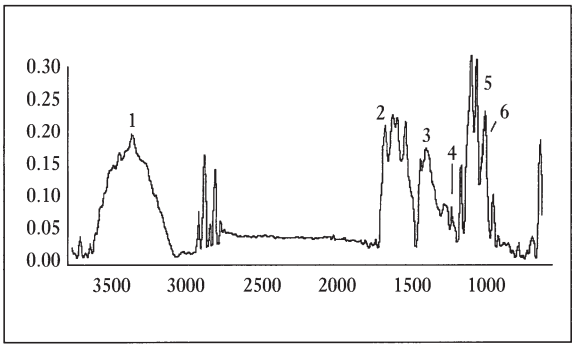


Fig. 9 Spectra of unmounted paint fragments from the coat of the sitter in Maurice-Quentin de La Tour's *Henry Dawkins*.

(a) Cochineal lake paint of jacket, lower layer protected by faded paint above, background subtracted.



(c) Similar, but faded paint of jacket, from an unprotected area, background subtracted.



(b) Reconstructed spectrum, improving resolution. The broad band (1) between 3300 and 3500cm^{-1} is produced by stretching of the bond within hydroxyl groups. A weak stretching band (2, 1710cm^{-1}) associated with carboxylic carbonyl groups within the material result from the presence of uronic acid components; small amounts of these are present naturally in natural polysaccharide gums. Within the complex region from 1000 to 1250cm^{-1}, we can see a variety of carbon-oxygen bond vibrations; those at 1052cm^{-1} (3) may be assigned to secondary alcohol C–OH bond stretching, while that marked 4 (1035cm^{-1}) may be assigned to primary alcohol groups. Secondary alcohol functions are attached to many of the carbon atoms incorporated within the pyranose or furanose ring structure of the component gum saccharides. An absorption at 904cm^{-1} (5) may be interpreted as a pyranose ring vibration.

(d) Reconstructed spectrum, improving resolution. Comparison of this exposed, faded sample with that of the protected paint, described above, seems to give evidence of a substantial amount of oxidation. Once again there is a broad absorption, peaking at about 3400cm^{-1} (1). The band labelled 2 (1709cm^{-1}) is much enhanced; it may be assigned as a carbonyl stretching absorption in a carboxylic acid. This interpretation may be supported by intensified acid C–O stretching bands at 1440 (3) and 1250cm^{-1} (4). These carboxylic acid groups appear to have been produced as a result of oxidation of primary alcohol groups within the polysaccharide molecule, in view of the relative reduction of the C–OH stretching band at 1032cm^{-1} (6, assigned to primary hydroxyl groups) compared with the corresponding band at 1052cm^{-1} (5) for secondary hydroxyls. The susceptibility of primary alcohol groups within polysaccharides has been noted from studies involving photolytic oxidation of cellulose.[19]

a precaution, however, it is advisable to carry out lipid assays on each new batch of silver chloride for possible background contamination caused by handling.

Applications, results and discussion

Figs. 1a and b illustrate the use of the infra-red microscope on a cross-section prepared from a sample of white paint from the headdress of the sitter in Rogier van der Weyden's *A Portrait of a Lady*, NG 1433 (Fig. 3), dated about 1450/60; this was embedded in silver chloride and cut with a glass knife microtome. In this case, it was difficult to be sure that only a single layer of paint had been taken. GC–MS of an intact fragment of the white paint indicated the presence of drying oil, but the drying glyceride component was diluted with non-drying egg lipids. This suggested that both drying oil and egg tempera were present in the sample. The infra-red microscope was focused on an area of white paint from the principal layer of the prepared cross-section; the resulting spectrum is displayed in Fig. 1a. This represents predominantly medium entrapped between the pigment particles, with some contribution from the pigment itself. Fig. 1b is the result after subtraction of some of the remaining lead white pigment bands. Subtraction in this way can help to clarify some of the medium-specific absorption bands, even if it is not feasible to obtain a totally pigment-free spectrum of the paint binder. Carbonyl bands from the ester groups, associated with the glycerides, are quite prominent; the diagnostic band centred at 1778cm^{-1} (probably caused by a lactone, formed by long-term ageing) can be seen more clearly in the subtracted version. Strong carboxylate absorptions, resulting from the reaction of the medium with the pigment, are prominent at 1620 and 1536cm^{-1}. Overall, the spectrum indicates the presence of a drying oil in this principal layer.

A spectrum of the underpaint is shown in Fig. 2, after subtraction of the lead white pigment. There is little in the way of lactone absorption at 1776cm^{-1} and only weak features associated with ester bands at 1739cm^{-1}; these are due to non-drying fats from egg yolk. There is a prominent, broad maximum in the region of 3290cm^{-1}, typical of N–H bond stretches in proteinaceous materials. Other features include bands at 1658cm^{-1} (Amide I, C–O stretch), 1633cm^{-1} (Amide II, NH_2 deformation band) and a doublet at 1548 and 1520cm^{-1}(Amide II band), split as a result of the influence of the pigment on the tertiary structure. The latter is a function of hydrogen bonding between amino acid groups within the peptide chain of the protein; such bonding is susceptible to the effects of pigment (mineral) additives. All these infra-red absorption features point to the use of a proteinaceous binder in this instance.

Comparison of these results with subsequent GC–MS analysis of the remainder of the sample, following mechanical separation of the upper and lower layers under the optical microscope, gave final confirmation of the presence of drying oil in the principal layer, identifying it as walnut oil; the presence of non-drying lipids from egg tempera was confirmed in the underpaint.

Fig. 4a shows the absorption spectrum obtained in transmittance mode from a sample of the hem of the Virgin's red dress in Dieric Bouts's *The Entombment*, NG 664 (Fig. 5). This work is a rare surviving example of a *Tüchlein*, a relatively small painting on canvas, executed in an aqueous medium.[8] The rather broad absorption envelopes result from overlapping bands; in some cases, underlying bands are suggested by partial shoulders. Fig. 4b is a reconstructed spectrum, showing underlying absorption maxima, detected by examination of the first and fourth order mathematical derivative curves, a technique used with great success in optical spectroscopy for pigment identification.[9] Program packages are available which are able to carry out another, related type of resolution enhancement in just a few seconds by a single-step process known as Fourier Self-Deconvolution.[10]

Overall, the spectrum indicates that the paint medium is based on a proteinaceous material. It can be quite difficult to distinguish between old proteinaceous binders; many aspects of the background-subtracted spectrum presented in Fig. 4a are very close to those of egg tempera medium. Application of multiple derivative techniques to improve resolution can help to make the distinction between proteins clearer. Fig. 4b indicates that there is no underlying 1776cm^{-1} lactone band and little evidence of

glyceride bands that might be associated with non-drying egg fats. In egg tempera, the C–H bands in the 2800 to 3000cm^{-1} region may be better resolved and more prominent than they are in animal glue. Here, they were poorly resolved. Glue distemper often has a less clearly resolved Amide I and Amide II band, resulting in an envelope with a maximum at about 1546cm^{-1} from the inclusion of a poorly resolved intermediate band at 1590cm^{-1}, as present in this sample. The surest distinction between types of protein present in paint is obtained by the combined information from the infra-red spectrum and GC analysis.

The results of infra-red examination were confirmed by various pieces of evidence: GC–MS analysis clearly indicated the presence of very low levels of non-drying lipids; solubility examination showed that the paint was susceptible to leaching of the medium by weakly acidic water; finally this extract provided a positive reaction when subjected to a Modified Ehrlich Test. The latter is indicative of the presence of hydroxyproline residues within the protein's molecular chain. Hydroxyproline is absent from egg tempera and, for that matter, casein, but is characteristic of animal glue, the medium used for this painting.

Interesting results were obtained from a sample taken from an area of green foliage in Garofalo's *An Allegory of Love,* NG 1362 (Fig. 6).[11] After embedding the sample in silver chloride and examining a thin section, the presence of two green paint layers was observed. The upper layer was more glaze-like than the lower, the green pigment being verdigris in both cases. A spectrum of the lower layer is displayed (Fig. 7a), together with one of the upper glaze layer (Fig. 7b).

The spectrum of the underpaint has been reconstructed after multiple derivative analysis. The strong acetate bands at 1601, 1456 and 1427cm^{-1} are characteristic of verdigris; bands associated with the glyceride carbonyl groups (1739cm^{-1}), together with lactone bands at 1776cm^{-1}, suggest the presence of a drying oil. One can also detect fatty acid carboxylate bands at 1579 and 1382cm^{-1}, resulting from antisymmetric and symmetric stretching of carbon–oxygen bonds, due to some reaction between the copper-containing pigment and the drying oil. No bands that might be assigned to resin were present. GC–MS analysis of this layer showed that the medium consisted of walnut oil without any addition of natural resin.

In the case of the green glaze layer above, the paint exhibited the infra-red characteristics of a drying oil medium in some areas; other regions, however, produced a spectrum similar to that illustrated in Fig. 7b. Fig. 7c shows the same spectrum following multiple derivative enhancement. This trace is closely comparable with that given by a test sample of 'copper resinate', prepared by warming verdigris with wood rosin.[12] In the region examined, there is no evidence for the 1776 and 1741cm^{-1} bands associated with the presence of drying oil. Bands at 1698-1713cm^{-1} may be interpreted as carboxylic and carbonyl bands associated with resin acids, such as are found in some Coniferae resins – in those from the genus *Pinus*, for instance. These bands, as well as other 'resin' bands at 1246 and around 840cm^{-1}, appear to be absent from the surrounding oil medium. GC–MS analysis of a sample of this paint layer, carefully separated from that below, indicated the presence of some walnut oil, but with an input of pine resin. The latter technique is not able to answer the question that would naturally follow if this had been the only method of analysis used: is the glaze paint based on an oil–resin medium, with verdigris pigment added? An example of the formation of a green glaze paint by the addition of resin, in the form of varnish, to the mixture of verdigris and drying oil during grinding is described by Armenini[13] and examples of such paint have, in fact, been found.[14] In this case, however, the combined information from both IR-microscopy and GC–MS seems to point to the somewhat unexpected conclusion that a different method of preparation was probably used: verdigris was dissolved in colophony with gentle warming; the green product was then spread out thinly and allowed to dry. It was then ground and employed as a pigment in a walnut oil medium.[15] Although 'copper resinate' glazes are known to discolour and become brown, this paint gave little evidence of discoloration when examined in section.

Finally, a pastel, a rather rare type of work for the National Gallery Collection, *Henry Dawkins*, by Maurice-Quentin de La Tour (NG 5118), is illustrated in Fig. 8.[16] The paper support

has at some time been attached to a backing canvas; FTIR suggests this has been done with animal glue. This example has been chosen to show the ability of FTIR-IR microscopy to cope with quite polar, highly functionalised media, such as polysaccharides. It demonstrates the improvements in the quality of spectra which are achieved when multiple-derivative examination of the spectral curve is made. The infra-red results also illustrate the vulnerability of the pastel's medium to photolytic damage.

The samples were taken from the sitter's coat, which is a chestnut brown colour, although unexposed paint from the protected edge of the picture revealed the original colour to be a brilliant crimson. This same colour was revealed under the brown exposed surface paint. The fragments of paint were placed on sodium chloride disks and examined in transmittance mode without any further sample preparation. The infra-red absorption spectrum, following background subtraction, is illustrated in Fig. 9a; this may be compared with the resolution-enhanced spectrum, Fig. 9b. Exposed brown paint gave a spectrum shown in Fig. 9c, with the corresponding resolution-enhanced version in Fig. 9d.

These results are typical of those obtained from polysaccharide materials, such as gum. The dyestuff in the pigment was that obtained from cochineal,[17] but it is interesting to note that no bands were detected for the dyestuff. Probably so little cochineal colorant is needed to give a strong absorption in the visible part of the spectrum that the infra-red bands of the hydroxyanthraquinone dyestuff are simply too weak to be observed. Comparison of the resolution-enhanced spectrum of the exposed and faded material (Fig. 9d) with the corresponding trace for the protected sample (Fig. 9b) reveals an interesting difference, rather more clearly than in the ordinary, subtracted spectra. The sample from the top of the paint layer shows some degree of oxidation to produce a higher proportion of acidic compounds (enhanced carboxylic band at 1709cm^{-1}). It is most likely that this is the result of photolytically induced oxidation of primary alcohol functions within the polysaccharide to the corresponding uronic acids.

Although the use of honey is sometimes recommended for use with gum in the preparation of pastels, gum alone was detected in this case.[18]

Instrumental and spectral acquisition details

Spectra were acquired on a Nicolet 710 Series FTIR bench connected to a Nic-Plan infra-red microscope, fitted with a MCT Type A detector, cooled by liquid nitrogen. Measurements were made with the microscope in transmission mode, using a 32x Spectra-Tech Reflachromat Cassegrain objective and a tube factor of 10x. A total of 128 scans were made of each sample and the interferogram averaged. The scan velocity for the detector was 40cm s^{-1}, with a resolution of 8cm^{-1} and Happ-Genzel apodisation. Both spectrometer and microscope were purged with air, which had been cleaned of particulates and hydrocarbons and was carbon dioxide and water-free, at between 1–2 litres min^{-1}. Acquisition and post-run processing were carried out using Nicolet 'SX' and 'PC-IR' software. The spectrometer was controlled by a Nicolet 625 computer system.

Specimen blocks were trimmed and cross-sections cut using an LKB Pyramitome glass knife microtome with a 35° knife; the cutting thickness was usually set to 10μm.

Notes and references

1. M.J.D. Low and N.S. Baer, 'Application of Fourier transform spectroscopy to problems in conservation. I. General principles', *Studies in Conservation*, 22, 1977, pp. 116–28. R.J. Meilunas, J.G. Bentsen and A. Steinberg, 'Analysis of Aged Paint Binders by FTIR Spectroscopy', *Studies in Conservation*, 35, 1990, pp. 33–51. J.S. Mills and R. White, *The Organic Chemistry of Museum Objects*, 2nd edn, London 1994, pp. 20–2.
2. R.G. Messerschmidt and M.A. Harthcock eds, *Infra-red Microspectroscopy*, New York 1988. J.E. Keaton and A.J. Sommer, 'IR Microspectroscopy. Routine IR Sampling Methods Extended to the Microscopic Domain', *Analytical Chemistry*, 64, no.19, 1992, 931A–940A. J.S. Mills and R. White, cited in note 1, pp. 185–90.
3. J.S. Mills and R. White, cited in note 1, p. 21.
4. J-S. Tsang and R.H. Cunningham, 'Some Improvements in the Study of Cross-Sections', *Journal of the American Institute for Conservation*, 30, 2, 1991, pp. 163–77.
5. However, recent experience using a *resolution-enhanced* spectrum with a microtomed sample of a cochineal lake (painted out in January 1975 in linseed drying oil, sampled and embedded six months later in 'Durcupan'(Araldite) epoxy resin and sectioned shortly afterwards) was more encouraging. The section was stored in the dark between two glass slides for nineteen years, and coincidentally appears to indicate that in some cases more consistent and less arbitrary spectra may result. Unfortunately, at present this process is very time-consuming, without recourse to software such as that mentioned above. See note 10.
6. We employ a Spectra-Tech micro-compression cell, fitted with two 1mm diamond windows.
7. The remote aperturing system fitted to this microscope is the Nicolet Redundant Aperture System.
8. D. Bomford, A. Roy and A. Smith, 'The Techniques of Dieric Bouts: Two Paintings Contrasted', *National Gallery Technical Bulletin*, 10, 1986, pp. 39–57.
9. C.M. Wakeford and R.H. Wardman, 'The Identification of Blue Pigments in Paints by Derivative Spectroscopy', *Journal of the Oil and Colour Chemists' Association*, 72, (1), 1989, pp. 22–8.
10. This is included in an acquisition and processing package entitled *Omnic*, developed and available from The Nicolet Corporation. We understand that the process may be controlled and monitored by adjustment of both enhancement and bandwidth factors.
11. R. White and J. Pilc, 'Analyses of Paint Media', in this *Bulletin*, pp. 86–95.
12. The laboratory sample had been prepared in the 1960s following information given in W. Sandermann, *Naturharze, Terpentinöl, Tallöl: Chemie und Technologie*, Berlin 1960, pp. 209–17, and L. Masschelein-Kleiner, 'Perspectives de la chimie des liants picturaux anciens', *Bulletin de l'Institut Royal du Patrimoine Artistique*, VI, 1963, pp. 109–26.
13. G.B. Armenini, *De' veri precetti della pittura*, Ravenna 1587, p. 126.
14. See, for example, Raphael's *Procession to Calvary*, NG 2919, sample 4 reported in this *Bulletin*, pp. 86–7, and note 9, p. 92.
15. This is mentioned in H. Kühn, 'Verdigris and Copper Resinate', *Artists' Pigments. A Handbook of Their History and Characteristics*, 2, ed. A. Roy, Washington, New York and Oxford 1993, pp. 148–58. Originally published in *Studies in Conservation*, 15, 1970, pp. 29–36; revised by A. Roy.
16. R. White and J. Pilc, 'Analyses of Paint Media', in this *Bulletin*, pp. 86–95.
17. Analysis of the dyestuff was carried out by J. Kirby, using HPLC.
18. The gum was found throughout the thickness of the paint layer, indicating that this was indeed a binder and not a fixative. Apparently the artist had been unable to find a suitable fixative for his work, which he kept between two pieces of glass for protection. He was in the habit of rubbing the sleeve of his coat over the finished work in an attempt to secure the colour to the support: see A.-J. Pernety, *Dictionnaire portatif de peinture, sculpture et gravure, avec un traité pratique des differentes manières de peindre*, Paris 1757, p. cxxvii. Pernety says that pastels were prepared by mixing the ground pigment with honeyed water, to which a little gum had been added; see p. 444. Other authors suggest that water alone was adequate, except with certain pigments. Carmine was one such, which required the addition of gum to the water. See R. de Piles, *Élémens de peinture pratique*, rev. edn by C.-A. Jombert, Paris 1776, p. 289. By the 1780s fixatives were certainly employed; see P.R. de C...C., *Traité de la peinture au pastel*, Paris 1788, pp. 307–29.
19. J. S. Mills and R. White, cited in note 1, p. 73.