

# Notes on Pigments

## Chronology of Artist's Pigments

First recorded date	Pigment	Fell into Disuse
Before 1300	Asphaltun (Bitumen)	
" "	Azurite	1825
" "	Blue Verditer	
" "	Bone White	
" "	Bone Black	
" "	Calcined Bone	
" "	Chalk	
" "	Charcoal	
" "	Cinnebar (Vermilion)	
" "	Copper Resinate	
" "	Egyptian Blue	
" "	Gambouge	
" "	Green Earth (Terre Verte)	
" "	Gypsum	
" "	Indigo	1800
" "	Iron Earths (ochres & sienna's)	
" "	Ivory Black	
" "	Lead Tin Yellow	1750
" "	Lead White	
" "	Litharge	
" "	Madder	
" "	Malachite	
" "	Massicot	
" "	Minium (Red Lead)	
" "	Mosaic Gold	

"	"	Orpiment
"	"	Realgar
1550		Red Lead
"	"	Saffron
"	"	Terre Verte
"	"	Ultramarine (Lapiz Lazuli)
"	"	Verdigris
"	"	Vermilion
"	"	Vermilion (dry process)
"	"	Umbers
700		Bismuth White
1400		Cochineal
1500s		Smalt
1549		Graphite
1565		Van Dyke Brown
1600s (late)		Naples Yellow
1610		Prussian Blue & Yellow Ochre
1700		King's Yellow, Sepia, Bistre
Early 18thC		Scheele's Green
1778		Vermilion (wet process)
1780		Cobalt Green synthesised
c.1780		Turner's Yellow
1781		Zinc Oxide
1781		Discovery of Chromium
1797		Indian Yellow
c. 1800		Cobalt Blue
1802		Cerulean Blue
1805		Barium Chromate

First recorded date	Pigment	Fell into Disuse
1809	Calcium Carbonate (precipitated)	
1800s	Emerald Green	
1814	Cadmium Yellow	
1817	Chrome Yellow	
1818	Synthetic Ultramarine	
c.1824	Viridian	
1825	Zinc White	
1825	Alizarin	
1826	Cobalt Green (known since 1786)	
1834	Strontium Yellow	
1836	Barium Sulphate	
1840	Antimony Vermilion	
1842	Zinc yellow	
1847	Prussian Blue & Cadmium Yellow	
1850	Iron Oxides (mars yellow)	
1850	Ultramarine Green	
1854	Coal Tar Pigments (mauvre)	
1856	Cobalt Violet	
1861	Chromium Oxide	
1862	Carbon Blacks (Commercially produced)	
1864		

## Refractive Indices of some Pigments, Fillers, Binding Agents & Dilutents

	Pigment/ Filler etc	Refractive Index
<b>Whites</b>	Titanium White	2.50 - 2.60
	Lead White	1.94 - 2.09
	Zinc White	2.00
	Plaster	1.53 - 1.62
<b>Blues</b>	Chalk	1.50 - 1.64
	Aluminium Hydrate	1.50 - 1.56
	Cerulean Blue	1.84
	Azurite	1.73 - 1.84
<b>Greens</b>	Cobalt Blue	1.74
	Prussian Blue	1.56
<b>Yellows</b>	Lapis Lazuli (Ultramarine)	1.50
	Smalt	1.49 - 1.52
	Green Earth	2.50 - 2.70
	Chromium Oxide	2.50
	Cobalt Green	1.94 - 2.00
	Malachite	1.65 - 1.88
	Orpiment	2.40 - 3.02
	Cadmium Yellow	2.35 - 2.48
	Naples Yellow	2.01 - 2.28
	Yellow Ochre (natural)	2.00 - 2.40
	Raw Sienna	1.87 - 2.17
<b>Reds</b>	Indian Yellow	1.67
	Vermilion	2.81 - 3.14
	Realgar	2.46 - 2.61

Pigment/ Filler etc	Refractive Index
<b>Browns</b>	Red Lead 2.42
	Cadmium Red 2.64 - 2.77
	Raw Umber 1.87 - 2.17
	Van Dyck Brown 1.62 - 1.69
<b>Dilutents</b>	Water 1.330
	Oil of Turpentine 1.470
<b>Aqueous</b>	Gum Arabic 1.344
	Egg Tempera 1.346
<b>Oils</b>	Poppy Oil 1.477
	Walnut Oil 1.480
	Linseed Oil 1.484
<b>Resins</b>	Dammar 1.515
	Mastic 1.536
	Shellac 1.516

## Historical Notes on Some Pigments;

### 'How to make an Excellent Ultramarine Blue'

Libri colorum (The Book of Colours) compiled by Jean Lebègue, 15<sup>th</sup> Century

Take lapis lazuli and grind it fine on a porphyry stone. Then make a mass or paste of the following ingredients: for a pound of lapis, 6 ounces of Greek pitch, two of mastic, one of spike or linseed oil, and half an ounce of turpentine; bring it all to the boil in a pot until almost melted, then filter and gather the product in cold water. Stir and mix well with the powdered lapis lazuli and let sit for a week or so. The longer it rests the better and finer the blue will be. Next knead the paste with the hands, sprinkling it with warm water; the blue will come out with the water. The first, second and third rinsing should be done separately. When you see the blue fall to the bottom of the container, throw out the water and keep the blue. (The difficulty rests in extracting only the blue element, the lazurite, from the minerals that make up lapis lazuli.)

## Experiment 2 : A Simple Test for Copper (II) Ions

### Test on suspected malachite $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$

Sample Conclusion	Test	Result	Result
2 suspected malachite	A small drop of dilute (2M) <b>hydrochloric acid</b> was added to the crystals	<b>effervescence</b> was seen at 100x magnification under to microscope	The presence of a <b>carbonate</b> in the pigment sample.

Sample Conclusion	Test	Result	Result
2 suspected malachite	A crystal of potassium hexacyanoferrate (II) was added to the solution	A <b>pink – brown precipitate</b> Formed	<b>Copper (II) ions present</b>

## Experiment 3 Simple chemical test for ultramarine

- Crystals of sample 1, suspected of being ultramarine, were placed on a microscope slide
- With the addition of 3M hydrochloric acid the sample turned white immediately
- This indicated the presence of  $\text{S}_3$  ions which are converted to  $\text{H}_2\text{S}$  by the acid
- The characteristic sulphur smell was also noted

### Experiment 1: Confirmation of lead in a Lead White

1. A few crystals of the unknown white pigment were placed on a microscopic slide with a dissecting needle.  
*In order to discover if the sample was a carbonate the following test was carried out;*
2. A tiny drop of dilute (1M or 2M) nitric acid was placed near the crystals. The slide was then observed under incident light of a microscope set to 100x magnification. The acid coming into contact with the crystals was observed and any changes noted.
3. The solution was then evaporated off on the hot plate.
4. Once dry the sample was once again viewed under the microscope any observations recorded.
5. *Finally in order to confirm or discount the presence of lead the following test was carried out;*
6. The sample was redissolved with a drop of distilled water and with the aid of a microspatula, a crystal of potassium iodide was dropped onto the sample.
7. Any changes in the sample were recorded.

### Experiment 1: Confirmation of a carbonate and lead in lead white

Sample Conclusion	Test	Result	Result
B (Suspected to be lead white from week 3)	A small drop of dilute (2M) <b>nitric acid</b> was added to the crystals	<b>effervescence</b> was seen at 100x magnification under to microscope	The presence of a <b>carbonate</b> in the pigment sample.

- After placing the solution on the hot plate and evaporating off the liquid, the sample was looked at again under the microscope and crystals observed. Fine dendritic (finger-like), colourless crystals were seen and drawn as seen below;

of

The drawing was referenced with images of Lead nitrate crystals and the sample confirmed as the latter.

### Experiment 1: Confirmation of a carbonate and lead in lead white

lead white + dilute nitric acid  $\Rightarrow$  lead nitrate + carbon dioxide + water



Lead nitrate + water  $\Rightarrow$  potassium iodide + water (?)

### Experiment 3 Simple chemical test for ultramarine

- Crystals of sample 1, suspected of being ultramarine, were placed on a microscope slide
- With the addition of 3M hydrochloric acid the sample turned white immediately
- This indicated the presence of  $S_3$  ions which are converted to  $H_2S$  by the acid
- The characteristic sulphur smell was also noted

### Experiment 4: Simple Test for Prussian Blue

- Crystals of sample 5, suspected of being prussian blue, were placed in a microscopic slide
- To test for  $Fe^{3+}$  ions, a drop of sodium hydroxide (NaOH) was added to the sample and the results observed under the microscope
- After a few seconds the sample turned a red/ brown colour
- The colour change was due to the  $Fe^{3+}$  ions being converted to  $Fe(OH)_3$  by the NaOH as follows



### Experiment 2: A simple test for copper (II) ions

1. A few particles of the sample suspected of being malachite were mounted on a microscopic slide.
2. These were placed on a Vickers M10 microscope with  $45^\circ$  incident light focus on the sample.
3. A drop of dilute (3M) hydrochloric acid was placed on the pigment sample and observations recorded.
4. A dissecting needle was then used to place a crystal of hexacyanoferrate (II) (potassium ferrocyanide) onto the latter solution. The reaction was observed and conclusions drawn.

8. A few crystals of the unknown white pigment were placed on a microscopic slide with a dissecting needle.

*In order to discover if the sample was a carbonate the following test was carried out;*

9. A tiny drop of dilute (1M or 2M) nitric acid was placed near the crystals. The slide was then observed under incident light of a microscope set to 100x magnification. The acid coming into contact with the crystals was observed and any changes noted.

The solution was then evaporated off on the hot plate.

### Experiment 2: Spot tests for Calcium in Chalk

- 1.
- 2.
- 3.
4. As Before
1. *Once the presence of a carbonate was confirmed the following test was carried out to detect the presence of calcium ions:*
2. The sample was re-dissolved in a drop of distilled water and a tiny drop of dilute (2M) sulphuric acid was added.

3. The sample was then looked at under the microscope at 100x magnification and results recorded.

### Experiment 2: Spot Tests for Calcium in Chalk

#### ❖ Identifying the presence of a carbonate

Sample	Test	Result	Conclusion
D (Suspected to be chalk white from week 3)	A small drop of dilute (2M) <b>nitric acid</b> was added to the crystals	<b>effervescence</b> was seen at 100x magnification under to microscope	The presence of a <b>carbonate</b> in the pigment sample.

#### ❖ Identifying calcium nitrate

Test	Result	Conclusion
The solution was then evaporated to a dryness on the hot – plate and the slide then examined under the microscope	No crystals were seen but a gel-like precipitated was observed in a droplet formation	Calcium nitrate is so hygroscopic all you should see are tiny droplets of liquid.

#### ❖ Recognising acicula crystals of calcium sulphate dihydrate to confirm that the pigment sample contained calcium ions.

Calcium carbonate + dilute nitric acid  $\Rightarrow$  calcium nitrate + carbon dioxide + water

$\text{CaCO}_3$

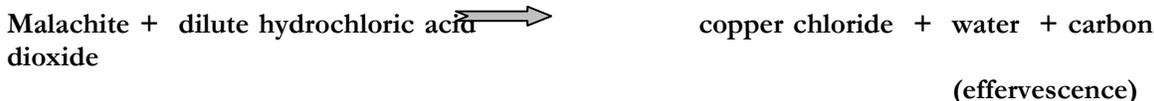
Calcium nitrate + water + dilute nitric acid  $\Rightarrow$  calcium sulphate dihydrate + water

### Experiment 2: A simple test for copper (II) ions

5. A few particles of the sample suspected of being malachite were mounted on a microscopic slide.
6. These were placed on a Vickers M10 microscope with 45° incident light focus on the sample.
7. A drop of dilute (3M) hydrochloric acid was placed on the pigment sample and observations recorded.
8. A dissecting needle was then used to place a crystal of hexacyanoferrate (II) (potassium ferrocyanide) onto the latter solution. The reaction was observed and conclusions drawn.

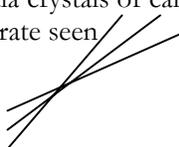
### Experiment 2 A simple test for copper (II) ions

Sample 2 suspected of being malachite effervesced when dilute hydrochloric acid was added to it. The reaction for the equation is as follows;



### Experiment 2 A simple test for copper (II) ions

Sample 2 suspected of being malachite effervesced when dilute hydrochloric acid was added to it. The reaction for the equation is as follows;

Test	Result	Conclusion
The residue from the previous test was dissolved by adding a drop of <b>de-ionised water</b> and a tiny drop of dilute <b>(2M) sulphuric acid</b> .	Acicula crystals of calcium sulphate dihydrate seen 	The pigment contained calcium ions.

### Experiment 2: Spot Tests for Calcium in Chalk

- A few pigment particles of Sample 4 (suspected of being yellow ochre) were placed on a microscope slide
- A Vickers M10 microscope with 45° incident light was focussed on the sample
- A drop of concentrated (3M) hydrochloric acid was placed on the sample and observed as it touched the particles
- After the results were recorded a crystal of potassium hexacyanoferrate (II) (Potassium ferrocyanide) was placed on the sample and observations noted



### Experiment 3: Confirmation of Iron (III) ions in yellow ochre

#### Experiment 3: Confirmation of Iron (III) ions in yellow ochre

- A few pigment particles of Sample 4 (suspected of being yellow ochre) were placed on a microscope slide
- A drop of concentrated (3M) hydrochloric acid was placed on the sample and observed as it touched the particles
- Most of the particles were seen to dissolve under the microscope but no other observations were noted. A possible reaction for the addition of the hydrochloric acid could be as follows: Showing that another compound has
- After the results were recorded a crystal of potassium hexacyanoferrate (II) (Potassium ferrocyanide) was placed on the sample and observations noted

## **Experiment 2: Confirmation of Iron (III) ions in red ochre**

### **METHOD 1**

- The sample suspected of being red ochre was viewed under the Vicker's microscope with 45° incident light.
- A drop of concentrated (3M) hydrochloric acid was placed on the sample and the results observed.
- No reaction appeared to be taking place so the sample was placed on the hot plate until it dissolved.
- A drop of distilled water was then placed on the sample.
- Using a microspatula a crystal of potassium hexacyanoferrate(II) (potassium ferrocyanide) was placed on the sample and any results recorded.

### **METHOD 2**

- The sample suspected of being red ochre was again set up on the Vickers microscope.
- A drop of concentrated hydrochloric acid was placed on the pigment particles and observations noted
- The sample was then diluted with a drop of distilled water
- A crystal of potassium thiocyanate was placed on the sample and the reaction and results noted.
  
- A drop of distilled water was then dropped on the sample
- Using a microspatula a crystal of potassium iodide was placed in the solution and any reactions/ observations noted.

## **Experiment 1 : Microscopical Analysis**

- It was relatively simple to distinguish the red pigments analysed and the Becke test seemed to be more clear for these crystals as compared to the yellows.

## **Experiment 2 : Confirmation of Iron (III) ions in red ochre**

### **Method I**

- Sample 1 was suspected of being red ochre.
- A few particles of the pigment were placed on a microscopic slid and a drop of 3M hydrochloric acid was added to the sample
- No reaction appeared to take place so the sample was placed on the hotplate for a few minutes.
- After this time it appeared that the pigment sample had dissolved slightly but no other change was noted. The reaction for the reaction could have been as follow:

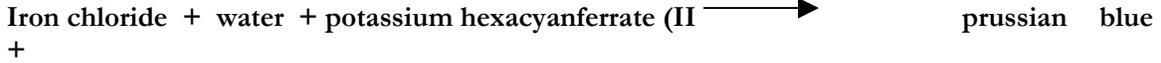
## **Experiment 3: Confirmation of lead in red lead**

- A few crystals of the sample suspected of being red lead were placed on a microscope slide and looked at on the Vickers microscope using 45° incident light

- In the fume cupboard a drop of hydrochloric acid was then placed on the pigment particles and observations noted



- A drop of distilled water was then added to the latter sample
- A crystal of potassium hexacyanoferrate (II) (potassium ferrocyanide) was added to the sample and a dark blue precipitate formed. The dark blue precipitate formed was prussian blue or **Fe<sub>4</sub>[Fe(CN)<sub>6</sub>]<sub>3</sub>**

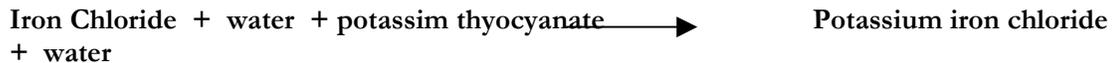


**Potassium chloride**

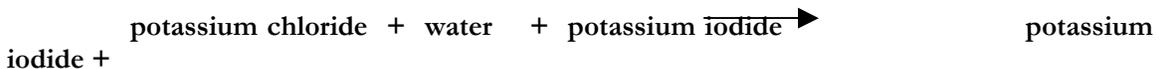
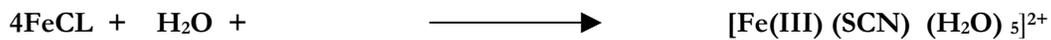


### Method II

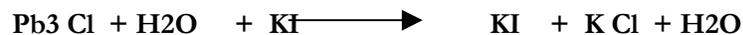
- As before a few crystals of sample 1 were placed on a microscope slide
- A drop of concentrated hydrochloric acid was placed on the sample and then on the hotplate
- The sample partially dissolved on the hotplate. The result appears to be the same reaction that took place in the first part of method 1, however a more obvious reaction took place when concentrated hydrochloric acid was used instead of 3 molar.
- The sample was then re-dissolved in a drop of distilled water and a crystal of potassium thiocyanate added to the sample with a microspatula. The sample immediately turned a blood red colour indicating the presence of iron (III) ions in the original sample. The red coloured compound made was made as follows;



**Hydrogen chloride**



**potassium chloride**  
**+ water**



## *Instrumental/ Analytical Analysis Techniques for Pigments*

### **Methods which Identify Elements:**

**EDX** (Energy Dispersive X-Ray Analysis)

**XRf** (X-Ray Fluorescence)

**NAA** (Neutron Activation Analysis)

**LMA** (Laser Microanalysis)

**PIXE** (Particles Induced X-Ray Emission)

**PIGE** (Particle Induced Gamma Radiation Emission)

### **Methods Which Identify Compounds:**

**HPLC** (High Powered Liquid Chromatography)

**Raman Microscopy**

**FTIR** (Fourier Transform Infra-Red Analyser)

**Reflectance Spectroscopy**

**XRD** (X-Ray Diffraction)

**Notes on Fibers**

## Chronology of Paper

Approx Date of Intro	Fiber/Pulp	Fell into Disuse
From 15 <sup>th</sup> Century (?)	Rags (Cotton & Linen)	To the Present Day
From 1800s	Books with rag pages	" "
1845	Rags & soda woodpulp	1890
1800	Straw	1870 - 90
From 1853 USA 1875	Soda woodpulp	To the Present Day
From 1869 USA 1875	Mechanical woodpulp (ground pulp)	To the Present Day
	Mechanical pulp with rags added	1869 - 80
1857 (UK)	Esparto	1890 (UK)
Early 1860	Esparto with rags added	1890
From 1880	Esparto with woodpulp	Approx 1883
From 1872 1889 USA	Sulphite woodpulp	To the Present Day
1884 From 1907 in North America	Sulphate woodpulp (Kraft pulp)	To the Present Day
From 1884	Bagasse	
From 1920	Cotton Linters	To the Present Day
		" " "

### **Important Dates in the History of Western Papermaking**

European first production of paper approx	1150 (Spain)
	1580 (Holland)
Double watermarks added	1483
Blotting paper introduced	1465
Cardboard produced	From 1580
Stamping Mills for fiber preparation developed	From 1760
Hollander beater for fiber preparation developed	From 1760
Blotting paper revival after slump in its manufacture	1800
Paper machines developed	From 1803
Esparto Grass paper	1861

### **Japanese paper;**

#### **Caveat;**

Commonly today woodpulp and unbleached kraft pulps in particular are often added to the more expensive native Japanese bast fibers.

### **The Manufacture of Washi**

General procedure for white bark in all bast fibers (e.g. kozo, gampi, mitsumata etc) as follows;

1. Soaking in water
2. Boiling
3. Washing and bleaching by exposure to the sun
4. Speck and particle removal
5. Beating
6. Papermaking

7. Removing water from the sheet through pressure
8. Drying on boards

(From the 'Handbook on the Art of Washi')

### **Shrinkage Test Indicating Ages in Years of Various Parchments**

(Mc Crone Research Institute Microscopy Course for Art Conservators, taken from Appendices of Handouts, 2820 South Michigan Avenue, Chicago, IL 60616)

#### **Method**

1. Tweeze a few individual parchment fibers from the edge of the sheet of tear site.
2. Place the fibers on a microscope slide and add a drop of water and a coverslip.
3. Heat the slide on a hotdatge at a rate of 2-4 °C/ min.
4. Between room temperature and 70°C the fibers will quickly shrink to around a half of their original length.

McCrones Parchment shrinkage graph!!!!!!!



- The sample was then diluted with a drop of distilled water
- A crystal of potassium iodide was added to the solution and the formation of a bright lime green precipitate was observed. The production of a strong smelling gas was also noted. After a few seconds the green precipitate then turned yellow. The possible equation for the reaction is as follows:

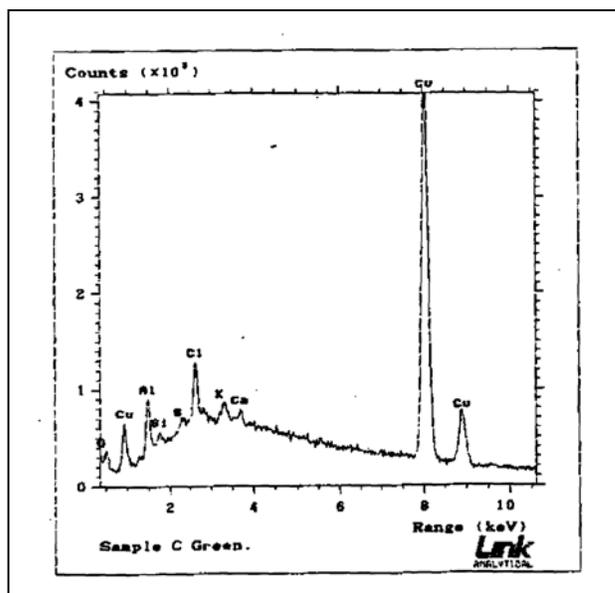
## Electron Microprobe Analysis

### Basic Principle

The basic principle of Electron Microprobe analysis is essentially the same as XRF. The difference is that the detector is fitted to a scanning electron microscope. The analysis is a part of Energy Dispersive X-ray Analysis (EDX). The technique can be used to analyse extremely small samples or to analyse specific parts of a sample, by utilising the resolving power of the SEM. In modern SEMs resolving power down to 4 nanometers is typical. The beam of electrons is focused on the spot required to study the composition of a single feature such as a pigment particle. Alternatively, it can be moved across the surface of the specimen for example to determine the variation in concentration of chemical elements in the layers of a cross-section.

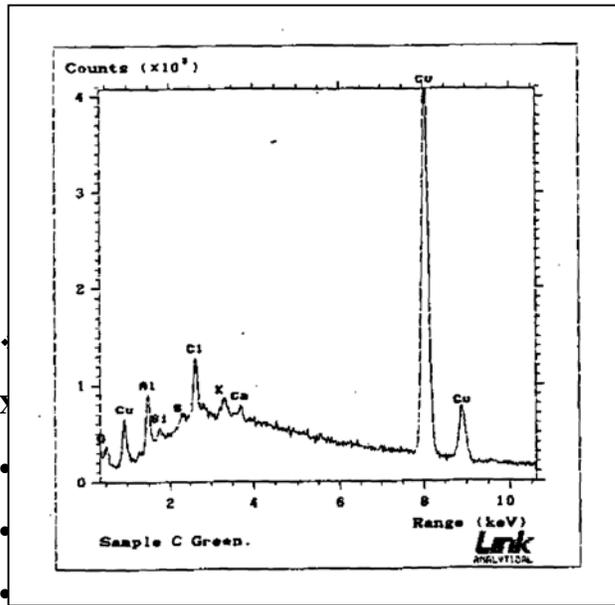
### Questions

**Sample C** was a green pigment from a polychrome sculpture. It contained particles which had a refractive index of less than 1.66 and appeared as coarse pleochroic rods, pale to deep bluish green and clearly anisotropic. There were also traces of colourless particles which had a refractive index of less than 1.66 and appeared as rounded hexagonal anisotropic plates and rods with higher birefringence.



The description of the sample above seems to correspond to the characteristics of Verdigris based on the findings in microscopic analysis of green pigments. The colourless particles mentioned could be a filler, clay, chalk or another inert white pigment. The EDX spectrum spectrum shows a large peak for copper. This would confirm Verdigris as the green pigment as it contains copper in its structure ;

**$\text{Cu}(\text{H}_3\text{C}_2\text{O}_2)_2 \cdot 2\text{Cu}(\text{OH})_2$** . The presence of aluminium and silicon strongly suggest that the colourless crystals belong to a clay. There also seems to be Calcium and sulphur present. This suggests the presence of gypsum (calcium sulphate) either in the ground or as an additive to the clay.



The description of the sample above seems to correspond to the characteristics of Verdigris based on the findings in microscopic analysis of green pigments. The colourless particles mentioned could be a filler, clay, chalk or another inert white pigment. The EDX spectrum spectrum shows a large peak for copper. This would confirm Verdigris as the green pigment as it contains copper in its structure ;



The presence of aluminium and silicon strongly suggest that the colourless crystals belong to a clay. There also seems to be Calcium and sulphur present. This suggests the presence of gypsum (calcium sulphate) either in the ground or as an additive to the clay

is connected to a computer. Simply the sample is placed under the beam of x-rays, this then refracts off the sample and the results read by the computer.

- The computer can then be set to match up our results to known samples from its database
- *The diffraction of an x-ray beam, passed through a crystal, in certain directions(determined by the >von Laue condition or > Braggs law) in which the path difference between the beams scattered by the adjacent atoms differs by a whole number of wavelengths. This allows both the structure and the lattice spacing to be determined. The necessary experiments can be carried out with a crystal of fixed orientation, or by observing the diffraction of an x-ray beam from a powdered sample which contains pieces of the crystal in all orientations (>powder photography). The latter techniwue determines the lattice spacing directly and allows the structure to be deduced.*
- Samples should ideally be no smaller than ½ cm. In the case of works of art on paper this is rather a large sample and it is unlikely that we could ever take such a large sample from an object. However smaller samples can be analysed but this increases the time of analysis by the instrument (often overnight) and as the samples get smaller then the accuracy of test results decreases. If a sample is too small the results may be inconclusive.
- Taking samples of pigments from works of art has to be done only if it is necessary to identify the pigment and no other 'in situ' analysis available has been effective.
- Also if an object is suitable enough to have a sample taken from it then it is likely that a large enough sample can be taken for instrumental analysis. The taking of tiny samples for a high chance of inconclusive instrumental results is hard to justify in the name of conservation.

### Explanation and Method Summarised

(for more extensive notes and a detailed practical method refer to Chemistry notes, Year 2, semester 1.)

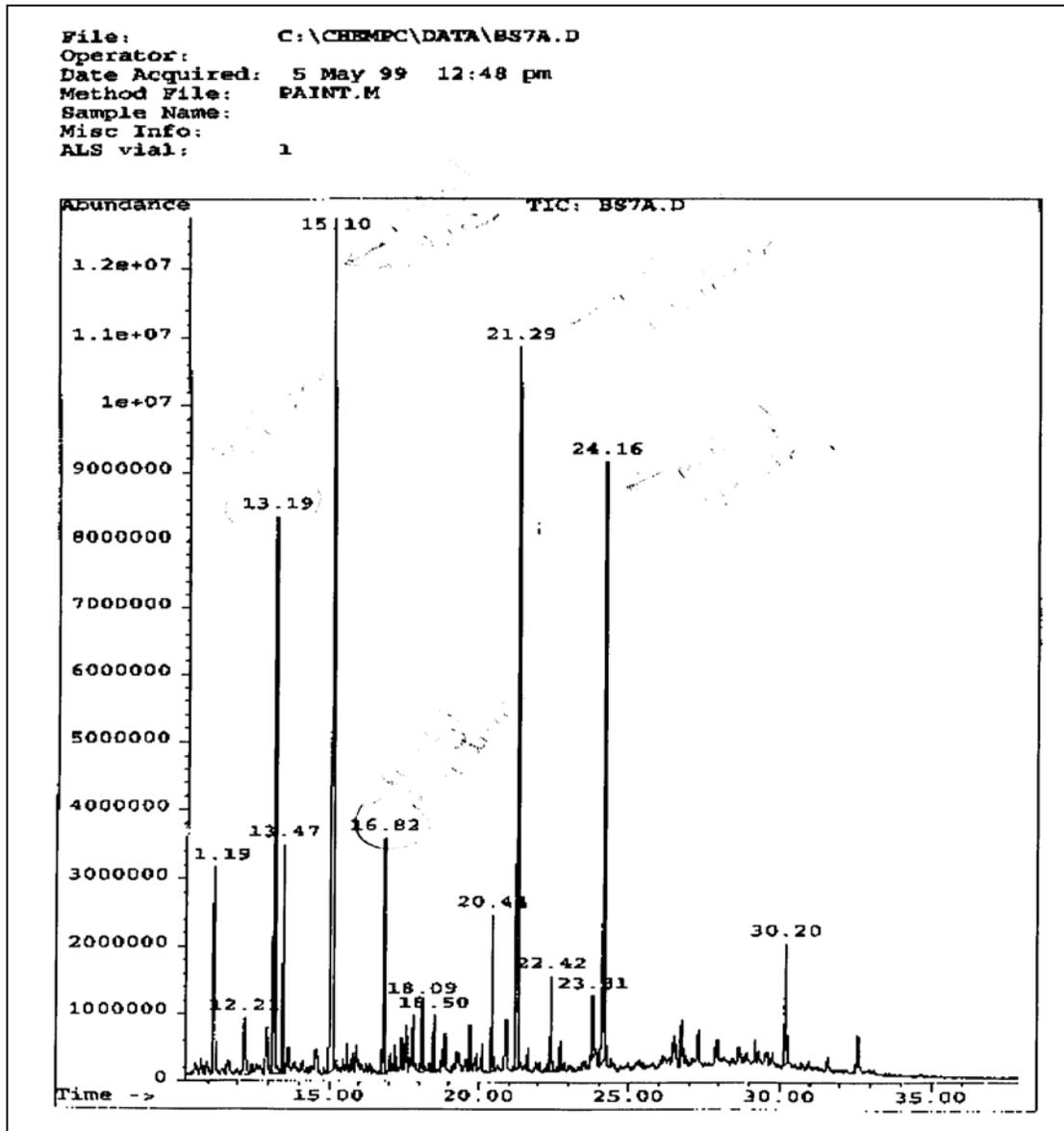
Gas chromatography (GC) and Liquid chromatography (LC) depend upon the equilibrium set up when a compound distributes itself between two phases; The **stationary** phase (contained within a column) and the **moving** or **mobile** phase. Different compounds distribute themselves between the two phases to different extents, and so move along the mobile phase at different speeds. The time a compound is held on a column under given conditions is characteristic of the compound and is called the **retention** time. A detector on the outlet tube monitors compounds coming off the column. The area under each peak depends on the amount of compound present. If the peaks are very sharp, their relative heights can also be used. Therefore compounds can be identified by their retention times and compared with known standards.

The mobile phase of the sample analysed in gas chromatography is injected into the stationary phase and is vapourised into a gas. The stationary phase is silicone oil or waxes either adhered to the surface of tiny particles of solid or as a coating on the inside of a very long (100m) thin glass or silica capillary tube. This tube is often coated on the outside with a polymer to give it strength. The components of the sample dissolve into and therefore absorbed by the stationary phase to form intermolecular bonds. These components are eluted through the stationary phase by a supply of an inert gas such as helium, argon or nitrogen. The component substances are separated both by polarity and volatility into bands or zones along the column. Those compounds that favour the mobile phase are carried along more quickly and the most volatile compounds usually emerge first.

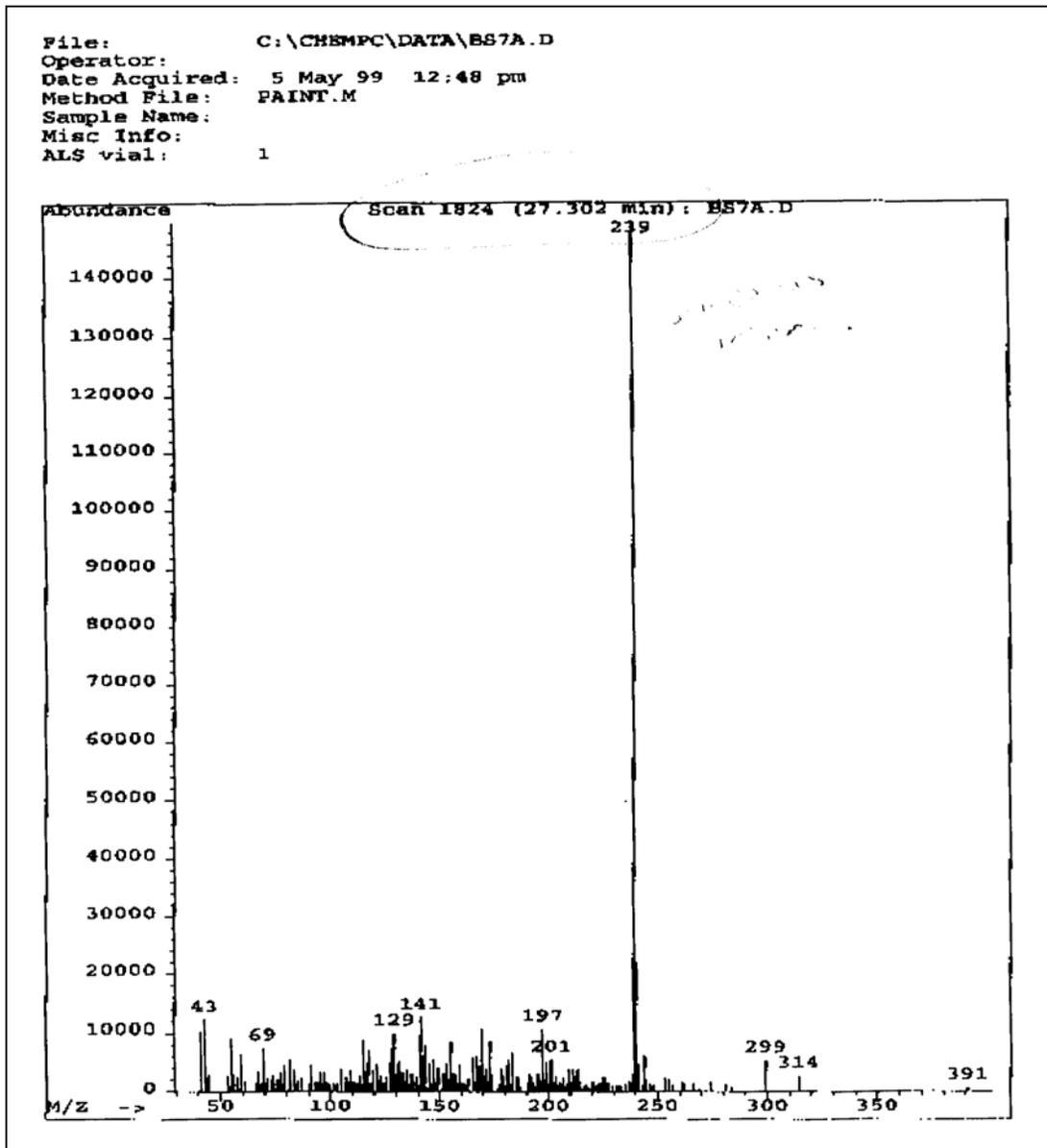
GC-MS (Gas Chromatography- Mass Spectrometry) is considered to be a more powerful tool than GC alone for the analysis of organic materials. With GC-MS, a mass spectrometer is attached to the end of the gas chromatography column where energy in the form of a stream of electrons is applied to the vapourised compound which becomes ionised. This molecular ion is usually unstable and undergoes partial breakdown into a pattern of fragment ions which, separated according to their masses and measured in intensity, form the mass spectrum. The resultant mass spectrum gives valuable clues to the structure of an unknown compound and can be also be used as a fingerprint for the identification of a compound whose spectra are already known. However there may be uncertainty in results which should always be considered. Experimental error may occur during hydrolysis and derivitisation and the natural variation in fatty acid content of drying oils may alter results.

Analysis of Results

Refer to the practical manual for treatment of results and chromatograms.



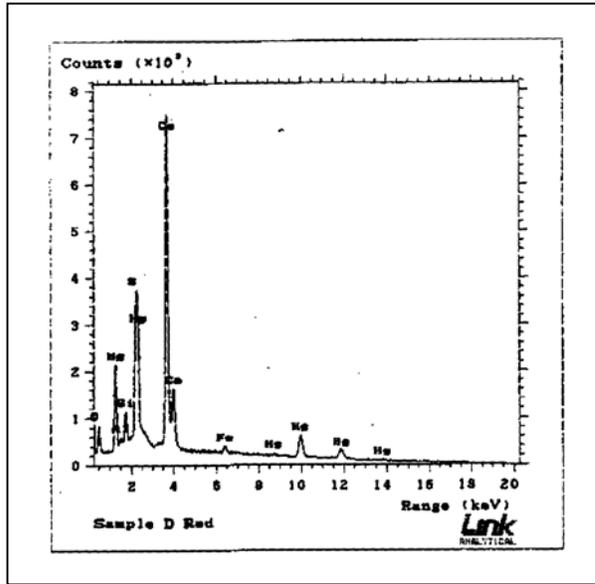
- The azelate to palmitate ratio is calculated by taking the azelate ratio percentage and dividing it by the percentage palmitate ratio. In this case  $100 \div 68.122 = 1.46$ . A ratio above indicates the presence of a drying oil in comparison to egg tempera which has a ratio of approximately 0.1.
- The palmitate to stearate ratio is 1.2 ( $68.122 \div 52.823$ ). This ratio indicates that it falls between the ratios known for linseed oil of 1.4 to 1.9
- The azelate/ suberate ratio (Az/Sub) is 1.85 and the azelate/ decanoate ratio (Az/Dec) ratio is 6.14. If the Az/Dec ratio is around 4 and the Az/Sub around two then this may indicate that the oil has been heat bodied. In this case the Az/Sub ratio matches that of a heat bodied oil but the Az/Dec ratio is a little higher than 4 although this may still indicate a heat bodied oil which were commonly used in the nineteenth century.



- The mass spectrums for paint sample BS7a (as seen above) show high base peaks. Scan 1824 shows the compound sample giving a base peak of 239 at 27.302 minutes which appears to relate to a database compound called methyl-7-oxohydroabietate M314. Scan 2129 shows base peaks of 253 at 30.213 minutes. This seems to relate to methyl oxohydroabietatae M328. In conclusion both these base peaks show that a coniferous resin is present

The brown paint sample therefore may be a heat bodied linseed oil mixed with a resinous material.

### Analysis of Proteinaceous Media and Adhesives by HPLC



The description of the sample appears to correspond to the pigment vermilion. As mentioned before the colourless particles could be clay. The EDX spectra shows the presence of Mercury and Sulphur from vermilion which is mercuric sulphide. Calcium, sulphur and oxygen could confirm the presence of gypsum ( $\text{Ca SO}_4 \cdot 2\text{H}_2\text{O}$ ). Again, the presence of aluminium and silicon confirms the presence of clay

## Summarised Explanation and Method

**High performance liquid chromatography** employs phenyl isothiocyanate as a derivitising agent for the amino acids obtained by hydrolysis of the media samples. It has been suggested that HPLC has an advantage over GC as a chromatographic method for the analysis of proteins because the amino acid ratios obtained are not affected by the volatility of the derivatives. However its disadvantage is that sample sizes need to be slightly larger than those for media analysis.

For the particular components used refer to the practical manual.

Experiments with standard samples of typical proteins found in paint media or conservation adhesives show the following typical results. As expected for a collagen, rabbit skin glue contained hydroxyproline and glycine peaks in a 1:3 ratio. Proteins in animal connective tissues contain approximately 30 percent glycine and 10 percent hydroxyproline. The identifying feature of animal and fish glues is the presence of hydroxyproline in considerable quantity. Hence we are not surprised to find that this amino acid in gelatine and isinglass. As expected only trace quantities of hydroxyproline are detected in egg-white or egg-yolk, the strongest peaks in the chromatogram for the former being due to proline and serine. In contrast, egg-yolk shows a smaller proline: serine ratio and higher ratios for alanine: serine and glutamic acid:serine than egg-white. Gluten is the protein present in wheat and therefore in flour paste, an adhesive often used in conservation. Analysis of gluten also shows only trace quantities of hydroxyproline and while the serine: glycine ratio is similar to that found in egg-yolk, the most distinguishing feature of the chromatogram is the much larger glutamic acid: serine and glutamic acid: glycine ratios as well as the smaller relative quantity of alanine.

### Problem

**Table 1 amino acid composition of a sample of an adhesive found on the back of a water-colour compared with common proteins.**

amino acid	sample :% of FMOC derivs. by peak area	animal glue	casein	gluten (wheat flour)
gly	26.2	24.7	1.7	4.0
ala	9.8	10.1	2.7	2.1
val	2.3	2.2	7.2	2.7
leu	3.5	3.7	9.0	?
iso leu	1.5	1.2	6.0	11.9
pro	12.5	13.0	13.2	13.6
phen	2.0	1.6	5.1	6.4
ser	3.7	4.0	4.0	4.9
meth	trace	1.4	2.3	1.7
asp	5.0	5.0	6.1	1.3
glu	9.8	9.7	20.2	45.7
hypro	7.5	7.4	0.0	0.0

The sample appears to correlate most closely to an animal glue