The characterisation of paint binders in the polychromies and gildings of the Gandharan artworks

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Abstract

In a polychrome artefact, coloured paint layers are applied on architectural elements or sculptures. Paint layers are made up of the colour, which is most typically an inorganic pigment and, with the exception of frescos, an organic binder, which enables the pigment to be dispersed and applied with a brush. From an analytical point of view the characterisation of organic paint binders is very challenging: the organic matter represents a very small amount of the total weight of the sample which is very small and aged. In this paper we describe the analytical approach applied for the characterisation of samples collected from a selection Gandhara polychromies. The analytical strategies and techniques employed are described and examples of the results obtained are presented.

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Fig. 3
Painted clay Brahma head from Milan Museum (A.09.9421, sample 25) (photo Simona Pannuzi).

Introduction

In a polychrome artefact, coloured paint layers are applied on architectural elements, sculptures, etc (Harris, 1977). Paint layers are always made up of the same fundamental components: the colour, which is most typically an inorganic pigment — a fine powder of inorganic coloured material — and, with the exception of frescos, an organic binder, which enables the pigment to be dispersed and applied with a brush. The organic binder is a fluid material that, upon drying and curing, produces a solid and elastic film, which keeps the pigment particles together, and ensures the adhesion of the coloured layer on the support. In the course of the centuries, artists have always experimented with a variety of organic natural materials to be used as paint binders, alone or in mixture, which are all based on four main classes of natural occurring organic compounds - proteins, lipids, carbohydrates and terpenoids (Mills and White, 2012). In most cases artists used many layers of paint to produce the wanted aesthetical effects, making a polychromy a complex, highly heterogeneous, multi-material and a multi-layered structure.

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The chemical analysis of organic paint binders

From an analytical point of view the characterisation of organic paint binders is very challenging (Colombini et al., 2010). Several organic materials are often simultaneously present in the layered structure, mixed with inorganic materials. In these mixtures, the organic matter represents a very small amount of the total weight of the sample (a few percentage points in the overall weight, or even lower), and the sample is, for obvious reasons, very small (0.1-100 mg). Moreover, non-original compounds, which have formed as a result of curing and ageing, interaction with the environment as well as other materials simultaneously present, or which were introduced during past restoration treatments, are also present.

As a result of all this, analytical approaches must be specifically developed for the characterisation of organic materials in the field of cultural heritage, and much research has been devoted at this task by the scientific community (Colombini et al., 2010; Vinciguerra et al., 2016; Bonaduce et al., 2016; Cartechini et al., 2010; Dallongeville et al. 2015;, Calvano et al., 2016).

Among the paint binders used in ancient polychromies, saccharidic and proteinaceous materials are the most commonly found, from the Far East to the Mediterranean Basin (Bonaduce, Ribechini et al. 2016), as for example the polychromy of the Terracotta Army, Xi'an, China (3rd century BC (Bonaduce et al., 2008)), that of the lost Giant Buddhas of Bāmiyān, Aghanistan, (6th-7th century AD) (Lluveras-Tenorio et al., 2017), and the murals of the Palace of Nestor, Pylos, Greece (13th century BC (Brécoulaki et al., 2012)). Saccharidic and proteinaceous materials were both identified in the samples collected from a selection Gandhara polychromies, and in the following paragraphs their analysis is discussed and examples are presented.

Saccharide materials

Plant gums are the most commonly used saccharide materials as paint binders. These are naturally occurring polysaccharides, exuded from several species of plants or extracted from the endosperm of some seeds, in-

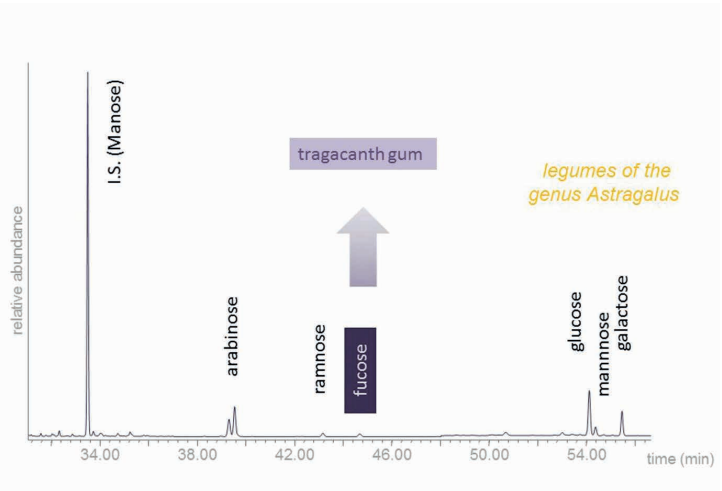

Fig. 1 Chromatogram in the SIM mode relative to the fraction containing the saccharide material of a polychrome sample coming from an excavation site in Afghanistan (Tapa Sardar, bulk sample), and dated to Late Gandharan period. Chromatographic peaks correspond to the different sugars identified. I.S. corresponds to internal standard of derivatisation (mannitol).

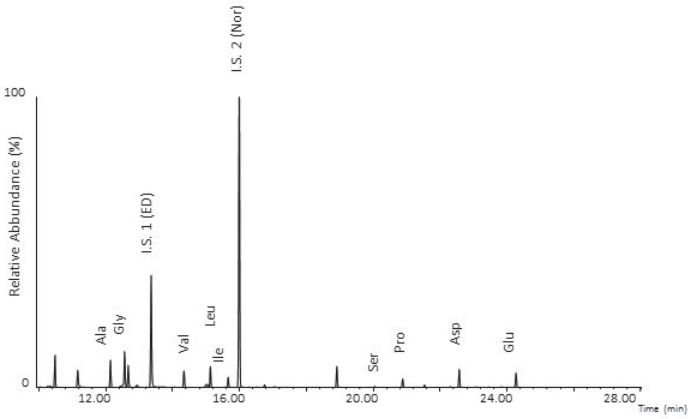

Fig. 2
Chromatogram in the SIM mode relative to the fraction containing the proteinaceous material of a polychrome sample coming from an excavation site in Pakistan, and dating back to the 3rd century AD (sample AKD14C, preparation layer). Chromatographic peaks correspond to: S.I.1 corresponds to internal standard of injection (Hexadecane), S.I.2 corresponds to internal standard of derivatization (norleucine).
Amino acids:
Ala=alanine; Gly=glycine; Val=valine; Leu=leucine; Ile=isoleucine; Prot=proline; Ser =serine; Phe=phenylalanine; Asp=aspartic acid; Glu=glutamic acid.

cluding arabic gum, exuded from *Acacia Senegal* and *Acacia Seyal* plants, tragacanth gum, exuded from *Astragalus genus* plants, and fruit tree gum, obtained mainly from cherry, apricot, peach and plum trees. Honey is also a saccharide material, which has been used as additive to increase the cohesion of paint layers in ancient polychromies (Lluveras-Tenorio et al., 2017). From the chemical point of view, plant gums are made up of aldoses (xylose, arabinose, fucose, rhamnose, mannose, galactose) and uronic acids (glucuronic and galacturonic acids) - polymerised thought the glycoside bond, while honey contains free ketose (fructose) and aldose (glucose). The most common approach for the analysis of saccharide materials aimed at their identification in a paint sample is based on the use of gas chromatography coupled to mass spectrometry (Colombini et al., 2010). The analysis of polysaccharides by gas chromatography requires an initial chemolysis step to free the sugars, that is a chemical reaction to decompose the original polymer into the constituting building blocks: the sugars (Andreotti et al., 2008). The choice of GC is driven by the fact that several sugars are isomers and thus the resolution and determination of the molecular profile is essential in order to identify the source of the saccharide material. The identification of the source of the saccharide material is then based on the evaluation of molecular profiles, and on the comparison between the chromatographic sugar profile of the sample with that of reference gums (Bonaduce et al., 2007). The presence of mixtures of saccharide materials, the effect of ageing, interaction with inorganic materials, biological and environmental contaminations can all affect the sugar profile of a sample from a paint or polychromy, seriously challenging the data interpretation (Lluveras-Tenorio et al., 2012).

A sample scraped from one of the paint layers of a polychrome fragment coming from an excavation site in Afghanistan (Tapa Sardar bulk sample), probably dated to Late Gandharan period, showed the presence of aldoses above the detection limit of the procedure used, indicating that they did not originate from environmental contamination (Lluveras et al., 2009).

The saccharide profile comprised arabinose, rhamnose, fucose, glucose, mannose and galactose (Figure 1). The evaluation of the saccharide profile, its comparison with a database of reference materials (Lluveras-Tenorio et al., 2012), and an evaluation of its level of contamination, suggests the possible identification of tragacanth gum as the polysaccharide binder used in this paint layer materials (Lluveras-Tenorio et al., 2012).

Proteinaceous materials

Among the proteinaceous materials, those that most commonly have been used as paint binders (Mills and White, 2012) are:
animal glue, obtained by boiling bones, hide or other cartilaginous parts of animals. It is made of (partially hydrolysed) collagen;
egg, which can be used whole, or using only one of its components: the yolk or the glair. Dry whole hen's egg contains 45% of proteins, 41% of fats, and 2% of cholesterol. Ovoalbumin (54%), conalbumin (12%), ovomucoid (11%), e lysozyme (3.4%) are the most abundant proteins;
milk. Milk is a water emulsion of proteins and lipids. Dry cow milk contains 26% of proteins (casein, lactalbumin, lactoglobulin), 26% lipids, and sugars.
From the chemical point of view, a protein is made up of amino acids. Twenty are the natural amino acids, which are biosynthesised in cells: alanine - ala; arginine - arg; asparagine - asn; aspartic acid - asp; cysteine - cys; glutamine - gln; glutamic acid - glu; glycine - gly; histidine - his; isoleucine - ile; leucine - leu; lysine - lys; methionine - met; phenylalanine - phe; proline - pro; serine - ser; threonine - thr; tryptophan - trp; tyrosine - tyr; valine - val. In proteins of our interest, another amino acid is very imprortant, hydroxyproline (hyp), which can be found in animal glue, and is produced in a post-translational modification. In a protein, amino acids are bonded one to the other through the peptide bond, constituting natural high molecular weight polymers. From the analytical point of view, several analytical approaches have been proposed, which can be used to identify and characterise proteins in samples from paintings and polychromies (Dallongeville et al., 2015). They can be based on spectroscopic, immunochemical or mass spectrometric approaches. Proteinaceous materials can be analysed by GC-MS after decomposition of the polymer into its constituent building-blocks, the amino acids (Colombini et al., 2010). The sequence of the amino acids (relative abundances and order) determines the characteristics of each protein. For this reason, a way of distinguishing a protein from the other after GC-MS analysis, is to compare relative amino acidic composition of the sample to a database of amino acid profiles of reference materials (Dallongeville et al., 2015).
A sample collected from a polychrome decoration on plaster coming from an excavation site in Pakistan, and dating back to the 3rd century AD (sample AKD14C, preparation layer) showed the presence of amino acids above the limit of detection, indicating that they were not originating from environmental contamination. The chromatogram is show in Figure 2.

Protein name	Matching peptides	Sequence coverage
collagen alpha-1(I)	11	10%
collagen alpha-2(I)	10	12%
collagen alpha-1(III)	5	5%

Table 1
Results of the proteomics analysis of a sample from an excavation site in Pakistan, and dating back to the 3rd century AD (sample AKD14C, paint layer).

Source	Sample codes	Results
Milan Museum (2-3rd A.D.)	A.09.10692	animal glue
Milan Museum (4-5rd A.D.)	A.988.02.1	animal glue
Milan Museum	A.09.9421	animal glue
Paris, Museum Guimet (7rd A.D.)	MG 18957	animal glue, milk, egg
	MG 18959	animal glue, milk, egg
Pakistan, Swat, Barikot (second half of 3rd A.D.)	BKG 1123A BKG 1123B	traces of proteins – source not identified
Afghanistan,Tapa Sardar (probably Late Gandhara)	no code	tragacanth gum traces of proteins – source not identified in the paint layer
Pakistan, Swat, Amluk-dara (late 3rd AD)	AKD14C	animal glue in the paint layer and egg in the preparation

Table 2
Results of all investigation performed so far using both GC-MS and MS proteomics approaches on samples from Gandhāra polychromies.

As amino acids were only present at the trace level (that is below the level of quantitation), quantitative comparisons between the sample amino acid profile, and the databases of profiles of reference materials was thus not possible. The absence of hydroxyproline, though allows us to asses that animal glue is absent, and possibly milk or egg were used.

One of the most promising analytical approaches to identify proteins currently available is that based on proteomics, and most commonly bottom-up proteomics. Proteomic was introduced in the field of cultural heritage about thirteen years ago (Tokarski et al., 2006), and was imported from the clinical and biological research. In a bottom-up proteomic approach, proteins are extracted from the sample and subsequently treated with an enzyme -trypsin in the vast majority of the cases - to obtain specific peptides. These peptides are then analysed by mass spectrometry (Dallongeville et al., 2015). Unlike GC-MS, this approach allows to retain information on the amino acid sequence in the peptide, resulting in the fact that, proteomics enables us to unequivocally identifying a protein, and, in some cases, even the biological source of the protein.

Proteomics was used to analyse also a selection of the polychrome samples from Gandhara (fig.3). As an example, the results of the proteomics analysis of another sample from an excavation site in Pakistan, and dating back to the 3rd century AD (sample AKD14C, paint layer), are reported in Table 1.

The identification of 26 peptides ascribable to collagen allows us to ascertain the presence of animal glue in the sample. Moreover, a comparison of the peptide sequences with the available databases, allowed us also to identify the biological source of the collagen: bovine.
Results of all investigation performed so far using both GC-MS and MS proteomics approaches to determine organic binders in samples from Gandhāra polychromies are summarised in Table 2.

Conclusions

Scientific investigations of organic materials in polychrome objects may help us to unravel the complex, structured mixtures of aged natural materials that constitute paint layers. Identifying the materials present in polychrome objects, organic binders and pigments, is also fundamental to reconstruct the original appearance of the artifact, and can also contribute to the selection of suitable preservation strategies to be put in place in the course of a conservation treatment. Moreover, such knowledge is of great use in improving our understanding of cultural traditions, technical know-how, knowledge circulation of a certain period of time in a specific geographical area, finally helping to recreate a more accurate picture of our past. It has to be kept in mind, though, that each analytical technique gave us information only on a specific chemical aspect of the investigated material; thus, complex problems may be solved by using a wide range of techniques and exploiting the synergy of complimentary data and knowledge. In this context it is important to stress that, when planning an analysis, the questions to be answered are the key in determining the analytical approaches to be used. This entails a detailed discussion of the problematics between the conservator, the archaeologist and the analytical chemist. Also, scientific investigation should take place before conservation treatments are undertaken, in order to avoid the loss and/or contamination of the residual polychromies remaining on the object, irredeemably loosing forever such inestimable traces of our past.

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