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A study of the interaction between rabbit glue binder and blue copper pigment under UV radiation: A spectroscopic and PCA approach

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ABSTRACT

The effects of a UV-accelerated ageing test on model samples of azurite glue tempera, pure azurite and pure rabbit glue, all elaborated according to medieval recipes, were studied. The color changes and modifications in composition and texture for both pure and mixed samples are shown and discussed. Special attention is given to the physico-chemical interactions occurring when azurite and glue are combined. Ageing effects on the model samples after up to 3000 h of UV irradiation were periodically analyzed by scanning electron microscopy (SEM) with X-ray energy dispersive spectrometer (EDS) microanalysis, X-ray diffraction (XRD), reflectance spectrophotometry and Fourier-transform infrared spectroscopy (FT-IR). Once the ageing process ended, a chemometric study using principal component analysis (PCA) of the FT-IR data was carried out independently for every model sample and for all the azurite-laden samples. Loadings from the significant principal components were analyzed to identify the FT-IR frequency (cm⁻¹) involved in the degradation process. PCA proved capable of identifying significant changes in pure glue samples. Also this work showed the lack of photochemical effects of UV irradiation on both pure azurite samples and those mixed with glue, in agreement with the SEM-EDS, XRD and colorimetrics results. Nevertheless PCA revealed that the azurite FT-IR spectral variability decreased in the presence of glue, being more affected by exposure to the IR region between 2100 cm⁻¹ and 3600 cm⁻¹, where the azurite band is located. In addition, PCA managed to separate the azurite/glue mixture samples from pure azurite samples.

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1. Introduction

In heritage science considerable attention is given to model studies on the effects of environmental factors on artists' materials [1–4]. In the particular case of paint artworks, the main goal is to characterize painting components and interacting processes under diverse conditions to promote painting conservation. Ancient paintings are complex microlayered composite materials made by heterogeneous mixtures of organic and inorganic components [5–7]. Throughout a paint stratigraphy (from inside to out: ground layer, priming layer and coloring layers), micrometer-scale pigments are mixed with binders. Over time, historical paintings transform due to exposure to diverse environmental conditions which promote reactions between painting components [1–5]. Particularly important in affecting color of paintings are ultraviolet (UV) and visible (vis) irradiation [1,3–4,8–9]. Effects induced by radiation on paints include chemical changes and color modifica-

tions that cause aesthetic damage. This makes characterization of ancient paintings a challenging task.

Especially sensitive to alteration are organic components used as binders, such as linseed oil, egg, casein, animal glue or dammar resin. The stability of protein binders exposed to various atmospheric conditions is said to be exceptional as compared with the yellowing and brittleness of aged oils and resins. The high susceptibility of oils and resins to light-induced damage has promoted numerous studies concerning their ageing processes, whereas fewer studies have analyzed ageing of protein binders in paintings or just mixed with pigments [8,10–12].

Concerning stability of pigments, diverse authors have investigated the light-induced damage to a variety of pigments [13], including copper pigments [3]. Azurite is a basic copper carbonate of formula $Cu_3(CO_3)_2(OH)_2$ used for centuries as blue pigment, particularly during the Middle Ages and Renaissance. Azurite on its own is remarkably stable under ordinary conditions [14], but the stability of azurite combined with different binders is different under natural conditions. Thus it turns into green tones when mixed with oil and egg yolk [15], while proteinaceous binders seem to protect azurite from color degradation, as found in the Van Eyck

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brothers' painting [16]. Thermal (DSC and TGA) and spectroscopic (XPS) studies have revealed that azurite egg tempera is altered by thermal ageing and pollutants, while less changes occurred under exposure to light irradiation [17]. In fact, the latest direct laser irradiation tests at IR, vis and UV wavelengths on azurite-laden paint samples corroborate that azurite is more stable than the majority of ancient pigments, both alone and when mixed with binders. Among others techniques, colorimetry, vibrational spectroscopies, SEM-EDS and XRD have been used to discern ageing processes [18].

Fourier-transform infrared spectroscopy (FT-IR) is commonly used to characterize organic and inorganic paintings materials present in art works [19]. A main advantage of this technique is that it provides complex data sets represented by samples' spectra suitable for being treated with multivariate analysis techniques. In fact, in recent years chemometric techniques have been increasingly used in the field of Cultural Heritage since they can extract information from correlated data sets, such as spectroscopic sets [8,20–22].

Assessments of physico-chemical changes induced in painted models by UV–vis irradiation using SEM-EDS, XDR and colorimetrics techniques are routine but essential tools, principally in the field of laser interaction with polychromy. In fact variation in colorimetric characteristics is the major type of side-effect in light ageing processes of paintings, triggered mainly by chemical modifications but also through modification of pigment morphology and textural relations of pigment-binder systems [9,23].

Despite the above, numerous areas remain open for research, including colorimetric behavior and chemical and texture interactions when azurite is blended with glue binders, as well as the application of chemometric tools to the obtained data to detect minor ageing variations. The present work investigates modifications in terms of changes in color, composition, texture and chemical interactions occurring on model samples of azurite rabbit glue tempera, pure azurite and pure rabbit glue under accelerated UV irradiation. The aim is to assess the stability of both painting components when standing alone and also when combined to further clarify the interaction process between them. Analysis of samples was periodically performed by measuring colorimetrical changes and recording their FT-IR spectra before and throughout increased UV radiation doses. In addition composition and texture characteristics were examined by SEM-EDS and DRX. PCA was applied to FT-IR spectra to reduce its multidimensional space in order to extract information about the azurite and glue ageing process by studying similarities in the samples.

2. Materials and methods

2.1. Reagents

The azurite pigment was purchased from Kremer Pigments GmbH & Co. KG. Pigment reference is 10,200 natural azurite (CI: PB 30.77420) with particle size $0-120 \,\mu$ m. The standard protein binder used was rabbit skin glue (collagen) purchased from Sigma (Barcelona, Spain). This binder has been used since ancient times, mostly in traditional woodworking, gilding and paintings due to its high strength, viscosity and elasticity.

2.2. Model samples

Five painting model samples were prepared according to Old Master recipes to obtain standards similar to those used by medieval artists [24]. The first model comprises the pure azurite pigment and the second model the pure rabbit glue binder. The third, fourth and fifth model samples include azurite/rabbit glue mixtures in various ratios (w/v).

Preparation of the pure azurite model sample was as follows: 5 g of powder azurite was formed as a crater-shaped mass and six drops of pure water (total volume of 30 µl) were added to form a dense paste. Then, four layers of this azurite paste were sequentially spread in fine coats onto a glass slide with a paintbrush. Each layer dried thoroughly before the next was applied. This is the traditional way to obtain an opaque paint layer guaranteeing full luminosity of the color. The pure glue binder model sample was prepared as follows: 10 g of rabbit glue was diluted to 10% by w/v in deionized water (MilliQ-System Millipore, Bedford, MA) by gently adding the glue to the water during 24h, and stirring periodically. Next the obtained blend was gradually heated in a bain-marie water bath below 50 °C to obtain a homogeneous mixture. Then the glue was carefully spread on a glass slide with a paintbrush in six successive fine coats, each applied after the previous layer had dried to a constant weight using a gel air dryer system.

Azurite/rabbit glue mixture model samples were prepared by blending the azurite with the glue both components previously prepared as described above in various ratios (w/v): 5.2/1; 5.2/0.4and 5.2/3. Next, these mixtures were spread on glass slides in three fine coats. This procedure was adapted to emulate real paint layers with variable pigment concentrations as found in ancient paintings. In addition, this enables discrimination among contributions from each pure painting material, i.e. azurite and rabbit glue, in terms of the physico-chemical changes occurring between them when interacting under UV exposure. The different ratios examined were: (i) 5.2/1 (w/v) for the so-called "ideal" combination, following the old recipes; (ii) 5.2/0.4 (w/v) to obtain a mixture with less glue (1/3 less in volume) for a sample overly rich in azurite; and (iii) 5.2/3(w/v) to obtain a mixture with extra glue (1/3 more in volume).

2.3. UV-accelerated ageing test

The painting model samples on the glass slides were aged in a UV-accelerated test chamber. To avoid azurite transformation into malachite the temperature was maintained at 30 ± 5 °C and the relative humidity at $15 \pm 5\%$. Before UV irradiation exposure (time = 0) fresh samples of each type were removed and stored in glass vials in the dark to be further analyzed. These are the blank samples to be compared with the aged samples. All the azurite-laden samples were UV irradiated during 3000 h, when the test was judged to have finalized and no color changes had been observed. At the start of the test at 200 h, 500 h, 800 h, 1500 h, 2500 h and at 3000 h spectrocolorimetry analyses were performed. Throughout the test at the above mentioned times, aged micro-samples were removed from the slides in order to analyze their composition and morphology (DRX and SEM-EDS) and to measure their IR spectra. Regarding the pure glue binder model samples, due to the higher susceptibility of the binder to UV damage (compared to the azurite-laden samples) color changes were checked at earlier UV exposure times, i.e. 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 200 h and at 500 h. Colorimetric analysis at 500 h was not possible due to the need for an even surface to obtain suitable data, whereas 500 h of UV irradiation left the glue surface cracked, blistered and flaked. Micro-sampling and analyses similar to those performed for the azurite-laden samples were applied for the pure glue sample at analogous times, i.e. from the start of the test and at 200 h, 500 h, 800 h, 1500 h and 3000 h.

2.4. Instrumentation and software

A high-speed exposure unit SUNTEST CPS, Heraeus (Hanau, Germany), equipped with a Xenon lamp was used for the ageing test. A special UV glass filter was employed for limiting the radiation at wavelengths greater than 295 nm, corresponding to outdoor solar exposure. Irradiance was set at 765 W m⁻², and range of temperature on the samples was kept at 30 ± 5 °C and 15 ± 5 % relative

humidity measured by the thermohygrometer Station OREGON, mod. EMR812HGN (Portland, Oregon, USA).

The diffuse spectral reflectance of each model sample was measured with a Hunterlab Ultrascan spectrophotometer. Spectra were acquired in the visible range of 375–750 nm at 5 nm intervals, with diffuse illumination and 8° from specimen-normal viewing, 25 mm circular area. Once the spectral reflectance curves were drawn, the CIE-1931 chromaticity coordinates were calculated under illuminant D65. The CMC (2:1) version of the color difference formula [25] was used to colorimetrically compare the samples. In general terms, these differences will be visually perceptible when the values change by more than three units.

The FT-IR spectra were collected using a NICOLET spectrometer 20SXB, working in transmission mode. The instrument was connected to a Pentium 200 and the instrument software was OMNIC v 4.1, running under Windows 2000 Professional (Microsoft Corporation, USA). The FT-IR spectra were registered from 400 cm⁻¹ to 4000 cm⁻¹ with a resolution of 2 cm^{-1} and 200 scan. Each model sample on the glass slide was characterized by five FT-IR spectra at the start of the test (fresh samples) and at each of the selected times above mentioned (aged samples). These spectra were obtained from five KBr pellets that were prepared by homogeneously mixing ~50 µg of sample powder removed from five randomly places from the glass slide. In this way, FT-IR spectra were independent from the position where the powder was taken in the glass slide.

Powder XRD was used to determine the mineral composition of all azurite-laden samples at each check time. A Philips PW-1710 diffractometer was used with the Bragg-Brentano focusing geometry, a graphite secondary monochromator, Cu Kα radiation ($\lambda = 1.5405$) and automatic divergence 1° slit. Diffraction patterns were obtained using continuous scan mode exploring an area over 3–64° 2 θ , with 0.01° 2 θ s⁻¹ goniometer speed. The voltage was 40 kV and the tube current 40 mA. Samples were slightly milled in agate mortar to <40 µm particle size and then analyzed. Automatic acquisition, evaluation and identification of minerals were carried out with the Xpowder software [26].

An SEM-EDS technique was used to study the chemical composition and morphology of the models. An Inca 350 version 17 Oxford Instrument was used. Analyses were done in secondary electron mode (SE) and backscattered electron mode (BSE). Both operating modes are complementary, though SE-mode provides information on texture and structure and BSE-mode on elementary composition, based on the different grey range of regions, a consequence of the atomic numbers of the elements constituting the materials. The SEM-EDS working conditions were 1.3 nA beam current measured in Faraday cage, 20 ev/ch resolution and acquisition time of 50 s. Samples requiring no preparation were mounted on a tee and coated with carbon for microanalysis purposes (azurite-laden samples), and with gold to visualize morphology in the pure glue samples.

A system of Gel Air Dryer, BIO-RAD Laboratories S.A. (Madrid, Spain) was used for drying the painting layers. Analytical balance METTLER-TOLEDO model AE163, with range of weighed 0–30 and 0–160 g, and precision 0.01 and 0.1 mg, respectively was used for weighting the painting compounds.

Chemometric data treatments were performed using Statistical Product and Service Solutions program (SPSS, for Windows ver. 15, USA).

3. Results and discussion

3.1. Spectrocolorimetry analysis

For all azurite-laden model samples a slight difference in the reflectance values is seen throughout the UV exposure times, particularly in the range of c.a. 450–500 nm and mainly in the pure

Table 1

Colorimetric values calculated for UV-aged pure azurite samples (Az) and azurite/rabbit glue mixture samples (see text for explanation). IMixt = ideal azurite/glue mixture; Mixt-g = mixture with less glue; Mixt-G = mixture with extra glue.

	Color	Check	Time			
Samples	400	850	1400	2100	2600	3000
Az						
$\Delta E_{\rm cmc}$	0.84	1.29	1.52	1.75	2.33	2.10
ΔL^*	-0.23	-0.04	-1.12	-1.22	-1.30	-1.39
ΔC^*	-0.54	-0.81	-0.94	-1.00	-1.50	-1.33
ΔH^*	-0.60	-1.01	-0.41	-0.76	-1.22	-0.84
IMixt						
$\Delta E_{\rm cmc}$	2.17	1.92	1.67	1.71	1.53	1.43
ΔL^*	-0.84	-0.71	-0.53	-1.00	-1.12	-0.90
ΔC^*	1.16	0.93	0.70	0.75	0.58	0.70
ΔH^*	1.63	1.53	1.42	1.17	0.87	0.87
Mixt-g						
$\Delta E_{\rm cmc}$	1.00	2.10	0.52	0.82	1.15	0.66
ΔL^*	0.96	1.09	0.48	0.26	0.91	0.58
ΔC^*	0.28	1.28	-0.17	0.45	0.61	-0.10
ΔH^*	-0.07	1.26	-0.11	0.64	0.35	-0.30
Mixt-G						
$\Delta E_{\rm cmc}$	1.63	2.21	1.01	1.09	1.06	1.22
ΔL^*	-1.15	-0.65	-0.03	-0.03	0.63	1.10
ΔC^*	-0.09	0.43	-0.31	-0.05	-0.02	-0.38
ΔH^*	-1.15	2.07	0.96	1.09	0.85	0.36

azurite model samples. For the pure rabbit glue model samples a darkening seems to occur in the first 24 h of UV irradiation, principally beneath 550 nm. However, these changes in reflectance require a specific reworking of the data on the basis of the color coordinates (CIE-1976) to obtain via color difference formulae CMC (2:1): the color differences $\Delta E_{\rm cmc}$ (total color), ΔL^* (brightness), ΔC^* (chroma) and ΔH^* (hue). These values shown in Tables 1 and 2 indicate whether the apparent chromatic differences observed in diffuse reflectance spectra are due to perceptible visual changes, achieved only when $\Delta E_{\rm cmc}$ exceeds three units [27].

Table 1 shows the results of ΔE_{cmc} , ΔL^* , ΔC^* and ΔH^* in azuriteladen samples. The scattered data across the color-check times reveals the important role of the spreading of painting materials on the glass slides, where obtaining a homogeneous surface is key for spectrophotometric evaluation. Further differences can arise due to the spreading of painting materials on the glass slides, where obtaining a homogeneous surface is key for spectrophotometric evaluation to positioning errors. In fact, the difficulty in systematically recording the same area in a studied sample is well known. Nonetheless taking into account these results, some indications can be made regarding the chromatic behavior of these samples.

The UV radiation effect on all azurite-laden model samples is a variation in total color, however imperceptible in terms of visual perception due to the fact that $\Delta E_{\rm cmc}$ is less than three units, i.e. $\Delta E_{\rm cmc}$ equals 2.10 at t = 3000 h (Table 1). In the pure azurite model sample the decrease of ΔL^* and ΔC^* accounted for the $\Delta E_{\rm cmc}$ increases during the 3000 h of UV irradiation because changes of ΔH^* are too small to be significant. This result is in agreement with the findings of Price [16] who indicated that chroma is critical in

Table 2

Colorimetric values calculated for UV-aged pure rabbit glue samples.

Check time	$\Delta E_{\rm cmc}$	ΔL^*	ΔC^*	ΔH^*
24	1.72	0.36	-1.67	-0.16
48	2.11	0.53	-2.04	-0.07
72	2.37	0.62	-2.29	-0.16
96	2.53	0.67	-2.43	-0.16
120	2.67	0.69	-2.58	-0.12
144	2.79	0.64	-2.71	-0.14
200	3.15	0.70	-2.74	-0.17



Fig. 1. FT-IR spectra of fresh samples: (a) pure glue sample and pure azurite sample; (b) azurite/glue mixtures in the different ratios studied.

Table 3 PCA results.

Data matrix	FT-R region (cm ⁻¹)	РС	Variance account (%)	Variance accumulated (%)
Glue	2900-3600	PC1	93.9	93.9
		PC2	4.9	98.9
Azurite	2100-3600	PC1	43.4	43.4
		PC2	29.1	72.5
		PC3	11.4	83.9
		PC4	7.2	91.1
		PC5	4.4	95.6
Azurite/Glue	2100-3600	PC1	97.7	97.7
(less glue)		PC2	1.4	99.1
Azurite/Glue	2100-3600	PC1	95.0	95.0
(ideal mixture)		PC2	3.5	98.6
		PC3	1.1	99.7
Azurite/Glue	2100-3600	PC1	93.9	93.9
(extra glue)		PC2	4.5	98.4
		PC3	0.9	99.3
All azurite samples	2100-3600	PC1	93.5	93.5
(pure and mixed with glue)		PC2	3.8	97.3
		PC3	1.6	98.9

azurite color changes. In the azurite/glue mixture samples the differences in ΔE_{cmc} are even smaller than in the pure azurite sample after 3000 h of UV irradiation (Table 1).

Table 2 shows the results of $\Delta E_{\rm cmc}$, ΔL^* , ΔC^* and ΔH^* in the pure rabbit glue model samples. An increase in $\Delta E_{\rm cmc}$ and a decrease in ΔC^* values are seen, as in the pure azurite model sample, although by contrast a slight increase in ΔL^* is detected. Our results indicate that azurite is photostable after 3000 h of UV irradiation (no color changes detectable by human eye occurred). Nonetheless the results for the pure glue sample indicate that beyond 200 h of UV irradiation exposure a color change takes place. However when glue is mixed with azurite this tendency to darken is not observed, which suggests that the presence of azurite promotes photostabilization of the glue and thus permanency in the overall paint system. This result agrees with the preliminary findings of Price regarding the stability of azurite mixed with diverse protein binders under ordinary conditions [16].



Fig. 2. PCA performed on the IR region between 2900 cm^{-1} and 3600 cm^{-1} of the pure glue sample: (a) score plot of PC1 and PC2; (b) loading plot of PC1; (c) loading plot of PC2.

3.2. PCA analysis on FT-IR data. SEM-EDS and XRD results

The FT-IR spectra were registered from 400 cm^{-1} to 4000 cm^{-1} and they were formed by 3734 data points. At each check time studied, the five model samples were characterized by five FT-IR spectra. Fig. 1 shows the FT-IR spectra of pure glue sample (Fig. 1a), pure azurite sample (Fig. 1a), and the different azurite/glue mixture samples (Fig. 1b).

Several IR regions were selected to perform the PCA analysis in accordance with the sample composition. Fingerprint was selected in all samples by its unique absorption pattern ($600-1450 \text{ cm}^{-1}$). The IR region between 1500 cm^{-1} and 1750 cm^{-1} , representative of adsorption bands due to carbonyl stretching, and the IR region between 2900 cm^{-1} and 3600 cm^{-1} , that includes the amide bands A (around 3300 cm^{-1}) and B (around 3100 cm^{-1}) were tested in the PCA of the pure glue samples. The IR regions ($600-1450 \text{ cm}^{-1}$, $1455-1600 \text{ cm}^{-1}$ and $2100-3600 \text{ cm}^{-1}$) used to perform PCA in azurite-laden samples were selected from the visual analysis of the azurite FT-IR spectra. These regions include, in addition to the fingerprint, FT-IR regions between 1455 cm^{-1} and 1600 cm^{-1} (small absorption band) and between 2100 cm^{-1} and 3600 cm^{-1} (characteristic azurite absorption band).

The PCAs were performed on the data matrices of every model sample. These data matrices were constructed using the IR spectra of the model samples at the different UV irradiation check times and also including fresh samples. In this way, as samples were checked seven times and every check was characterized by five IR spectra, 35 IR spectra were represented in every data matrix (five data matrices, one for each studied model sample). The PCs were obtained using both the covariance data matrices (scaling



Fig. 3. SEM photographs of pure glue samples showing: (a) the smooth surface of the fresh glue sample; (b) the polygonal cracking developed on the surface of the glue sample aged by 1500 h of UV irradiation.

by mean-centered data) and the correlation data matrices (scaling by unit variance). The results were clearly better when PCA were performed on the correlation data matrices, so the results shown and discussed here correspond to autoscaled data. A simple data-centering procedure is often adopted for spectral data [28,29], because the applied autoscaling procedure assigns the same relevance to each IR spectral region. Thus spectral regions with small variation – no relevant IR bands – can acquire the same importance as large IR bands related to important functional groups. Therefore in this work, only IR bands containing the greatest variability were selected to apply PCA (not the whole IR spectrum). This may explain why results were more interpretable when data were autoscaled since no relevant bands were included in the PCA. The results of the PCA in terms of explained variance (%) and cumulative explained variance (%) for each model sample are shown in Table 3.

In accordance with previous work [8], PCA successfully detected changes associated with ageing processes in the glue model sample. In the score plots of PC1 and PC2 (Fig. 2a), accounting for 98.89% of the total spectral variance, there is a clear separation between aged and fresh samples. PC1 alone separated fresh samples from aged ones, so this PC accounts mainly for the separation of aged and fresh glue samples with a 93.94% of the total spectral variance.

ance. Fresh samples gave the highest scores on this PC. From the corresponding loading plots (Fig. 2b), it could be assumed that the IR region between 2900 cm⁻¹ and approximately 3333 cm⁻¹ suffered a general IR transmittance decreased when glue samples were aged because this IR region had the highest weight on the scores. The band at 3423 cm^{-1} on the loading plot was no discriminant on this PC1 since its weight was the smallest. Nevertheless, the information related with this band was explained on PC2, where this band showed larger weights (highest loading values). Scores for PC2 separated samples aged up to 500 h from samples aged more than 500 h; fresh samples gave zero scores or lightly negative score. From the analysis of the corresponding loading plots, it is clear that samples with negative scores had high IR transmittances in the band between 2900 cm⁻¹ and 3313 cm⁻¹, and samples with positive scores had high IR transmittances in the 3423.99 cm⁻¹ band. In fresh samples, the transmittance values of these two IR bands compensated each other. Nevertheless a combination of both components, PC1 and PC2, was needed to separate samples aged at different times. Again in agreement with previous work [8], these results show that the UV irradiation on glue samples causes changes mainly in the IR region between 2900 cm⁻¹ and 3600 cm⁻¹, where the amide bands A and B are included.



Fig. 4. Loading plots of azurite-laden samples for the IR region between 2100 cm⁻¹ and 3600 cm⁻¹: (a), (b) and (c) correspond to the PC1, PC2 and PC3 of the pure azurite sample respectively; and (e), (f) and (g) correspond to the PC1 of the less-, ideal- and extra-glue contents in the azurite-laden samples respectively.

The SEM study in SE-mode of the pure glue samples' microstructure at different UV exposure times revealed that damage processes occurred prior to 200 h of UV irradiation (first inspection time). Damage intensified with increased UV irradiation doses, leading to a polygonal crack pattern in the surface of the glue (Fig. 3).

PCA of the different azurite-laden sets gave no information related to the UV-ageing test in the IR regions studied. The distribution of the scores plots related to ageing was indistinct. For example, Fig. 4 shows the scores plot of azurite/glue ideal mixture when PCA was performed on the IR region between $2100 \,\mathrm{cm}^{-1}$ and 3600 cm⁻¹. Although glue was present in the three model of mixture samples, PCA could not detect changes associated with the UV test - contrary to what was obtained for pure glue samples. The lack of an interpretable pattern in azurite-laden model samples (both pure and mixture samples) suggests that the UV-ageing process did not introduce systematic chemical changes that could be detected by multivariate analysis of the IR spectrum. Therefore it seems that the pigment is UV-photostable either when alone or mixed with glue as already reported in the literature [16]. Moreover this finding suggests that azurite could prevent the glue UV-photodegradation when both components are blended, irrespective of their concentrations in the mixture, since the pure glue model samples degrade under UV irradiation, as mentioned above.

In fact, XRD revealed that azurite (ICDD card 11-682) was the main crystalline phase in all fresh samples, with quartz, SiO₂ (ICDD card 46-1045) being present only in minor amounts (c.a. 2%). Throughout the UV-ageing test no changes in mineral composition took place in any of the azurite-laden samples, neither in the pure samples nor in the azurite/glue mixture samples. The SEM-EDS study in BSE-mode provided elementary composition information. Results from fresh pure samples revealed the occurrence of grains made of Cu, O and Si (Fig. 5a) that were attributed to azurite and quartz respectively, in agreement with the XRD results. In addition grains containing Fe, Mg, Al, and Si apart from Cu and O were detected with this technique and ascribed to clay minerals, Fe-Mgrich aluminosilicates (Fig. 5b). SEM analyses performed at different UV irradiation times in both pure and mixed samples did not yield interpretable results in terms of mineral changes, since the same elements were analyzed.

Nevertheless differences in the PCA results were found in all azurite-laden samples mainly in the IR region $2100-3600 \,\mathrm{cm}^{-1}$, where the characteristic band of azurite is located, together with the amide bands A and B of the glue. Table 3 shows that the first five PCs accounted for 95% of the total spectral variance when the PCA was performed on the azurite pure data matrix, whereas the PC1s accounted for more than 90% of the spectral variance when the PCA was performed on the different kind of azurite/glue mixed data matrices. No interpretable results were found when analyzing the score plots and the loading plots of the corresponding PCA. Fig. 6 shows how the loadings of the PCs of the pure azurite model sample were characterized by great variability in their weights (Fig 6a-c). Instead the PC1s for azurite/glue mixtures were a weighted average of the intensities from 2100 cm⁻¹ to approximately 3300 cm⁻¹ (Fig. 6d-f), contributing all this variable (wavenumber) to the scores with similar weight. The PC2 in the three azurite/glue mixtures accounted for the information related to the band at 3518 cm⁻¹. This band corresponds to the characteristic band of azurite, and could be related to the different azurite contents in the KBr pellet. From these results it can be inferred that the presence of glue in azurite samples removed different sources of spectral variability.

As revealed by SEM analyses in ES-mode, which provide information on particle morphology (size and shape) and on overall sample micro-texture characteristics, when both fresh azurite and glue are mixed, glue wraps azurite particles but these remain independent of each other (Fig. 5a). With increasing UV-irradiation



Fig. 5. SEM photographs of azurite-laden samples: (a) glue wrapping azurite crystals leading to the formation of isolated azurite-glue clusters; (b) continuous and homogeneous azurite-glue paste; (c) glue film adsorbing azurite particles onto its surface; (d) irregular azurite particles covered with "dust" made of smaller azurite crystals and impurities.



Fig. 6. PCA performed on the IR region between 2900 cm^{-1} to 3600 cm^{-1} of all azurite-laden samples (pure and mixed): (a) score plot of PC1 and PC2; (b) loading plot of PC1; (c) loading plot of PC2.

doses, particles start to form clusters enlarging in size, until a homogeneous azurite/glue paste is formed at 1500 h of UV irradiation (Fig. 5b). Thus, higher UV-irradiation doses cause glue to behave as an adhesive tending to adsorb loose azurite particles onto its surface (Fig. 5c, to be compared with Fig. 3b). Similar trends were observed in the mixtures with less glue and with extra glue. This encasement of azurite particles in the layer of glue could justify why variations in the IR spectral data were not detected. Additionally it could be the cause of the increased stability of the glue when combined with azurite.

Instead, when azurite stands alone – pure azurite samples – SEM images show loose and irregular particles of uneven size ranging from <1 μ m to around 120 μ m. Additionally, particles are covered with "dust" (Fig. 5d) that according to Price [16] corresponds to impurities of smaller crystals normally associated with this natural pigment. In our case impurities of quartz and clays were confirmed by SEM-EDS. Both this "dust" and the variation in azurite particle size could be responsible for the different sources of variability in the IR spectral data, since both can vary the amount of light reflected, refracted and transmitted in the azurite single particles.

Finally, PCA was performed on a data matrix constructed using all the FT-IR spectra of all azurite-laden samples, both pure and mixture samples samples at (w/v) different ratios. The FT-IR regions $(600-1450 \text{ cm}^{-1}, 1455-1600 \text{ cm}^{-1}$ and $2100-3600 \text{ cm}^{-1})$ were used to perform PCA on mean-centered data and autoscaled data.

The most informative FT-IR region was again that between 2100 cm⁻¹ and 3600 cm⁻¹, but using mean-centered data. The results of this PCA are shown in Table 3 and the projection of the original data onto the plane of the first two PCs, explaining 97.3% of the total variance, is shown in Fig. 6a. Two clear groups can be distinguished, one containing all azurite/glue mixture samples and the other containing pure azurite samples, with both PCs necessary to separate the samples. Loading plots of PC1 (Fig. 6b) indicate that the IR region between 2100 cm⁻¹ and approximately 3250 cm⁻¹ dominates the distinction between azurite samples with or without glue. The presence of glue in the samples decreased the IR transmittances in this IR region, since samples without glue showed the highest score on PC1. No pattern distribution was identified into the groups. PC2 accounts for the information related to the azurite band at around 3430 cm⁻¹ (Fig. 6c). Although the FT-IR spectra were quite similar for all samples containing pigment, PCA was capable of distinguishing whether glue was present or not in the samples.

4. Conclusions

This work represents a study of artificial UV-ageing processes of a blue copper pigment-glue tempera painting technique, particularly on azurite and rabbit skin glue, focused mainly on component interactions, thus emulating real situations that can be found in historical paintings. The study shows the successful application of several analytical techniques to this aim. Spectrocolorimetry together with PCA on FT-IR data supplied valuable information in this regard; in addition to detecting physico-chemical changes occurring in painting model samples, these techniques were capable of providing insights into age-induced alterations. SEM-EDS and XRD analyses were essential to corroborate the obtained results.

Moreover, the results show that the glue binder alone suffers strong degradation after UV irradiation. By contrast, the presence of azurite seems to improve the photostabilisation of the glue against UV damage, thus promoting the permanency of the overall paint system, whatever the glue concentration used in the analyzed model samples. This finding suggests that azurite protects the glue binder against UV degradation.

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