

THE DETECTION OF COPPER-BASED PIGMENT DARKENING BY BIURET-REACTION IN MURAL PAINTINGS BY SEM-EDX, MICRO-XRF AND MICRO-RAMAN SPECTROSCOPY

Robert LINKE^{1*}, Martin A. ZIEMANN²

¹⁾ Austrian Federal Office for the Care of Monuments, Department for Conservation and Restoration, Arsenal, Objekt 15/4, 1030 Vienna, Austria

²⁾ Potsdam University, Institute of Earth and Environmental Science, Karl-Liebknecht-Str. 24/27, 14476 Potsdam, Germany

Abstract

Micro-Raman spectroscopy, micro-XRF and SEM-EDX were applied to samples of baroque mural paintings in order to investigate a quite uncommon pigment alteration from azurite to tenorite by forming a biuret-complex which gives the painting a violet color. The detection of this discoloration gives the proof for the presence of protein and indicates the application of casein.

Keywords: *Micro-Raman-Spectroscopy; SEM-EDX; Micro-XRF; Azurite; Malachite; Tenorite; Pigment alteration; Biuret reaction*

Introduction

Among the big variety of inorganic pigments used in mural paintings, copper- and lead-based minerals are mostly affected from alterations yielding to colour changes or darkening. Therefore, a number of investigations, which have been carried out in the past [1], reveal a wide range of environmental influences such as humidity [2, 3], temperature (heat damage caused by fire respectively) [4, 5], harmful gases [6], microbiological activity [7], alkaline materials [8], salts [9], organic binding media additives [10] or catalytic effects caused by trace elements to being responsible for these alterations. Due to the amount of parameters, a complete understanding of the alteration mechanisms has not been achieved so far and is still object of investigation. Frequently, a combination of these parameters is held to be responsible for these degradation processes mentioned above [11]. This survey describes a very rare pigment alteration of basic copper pigments like azurite or malachite into tenorite by forming a violet-coloured complex in the presence of protein.

* Corresponding author: robert.linke@bda.at, +43-1-7982146-35

During of the preservation and restoration of a Baroque garner in the county of Salzburg in Austria (address: Zankwarn 8, 5571 - Mariapfarr), called “Galli-Kasten” (Fig. 1), preliminary investigations, which were carried out in 2009 for conservation purposes, revealed this uncommon alteration [12].



Fig. 1. Front side of the garner with ornamental mural painting which derives from the 18th century.

The house was built at the beginning of the 18th century and it was initially used for the storage of grain. According to the archives [13], the façade was painted twice in the 18th century; the second painting is reported from 1758. Around 1900, the murals were covered with a white lime wash, which was removed anew in 1967, when the painting was recovered again and restored. Additionally, some fragments of a thin hydraulic plaster could be observed which apparently was applied in 1967. Today, the façade is covered with a white lime wash for conservation reasons, showing a reconstruction of the initial paintings. Below this lime wash, the façade contains fragments of ornamental mural paintings, coloured with red and black pigments, which derive from the time of construction as well as ornaments from 1758, painted with red and yellow ochre, smalt, malachite and carbon black (Fig. 2). A closer look on the surface of the black painted areas of the first original paint work shows a bright violet shade of the lime wash layer which apparently derives from an alteration or degradation process and which attracted the authors attention (Fig. 3). Referring to the literature, these violet shadings have occasionally been reported to be found on mural paintings, but they were usually connected to fluorspar (CaF_2) [14] or organic dyes [15].



Fig. 2. Detail of the original wall painting with black and red pigments

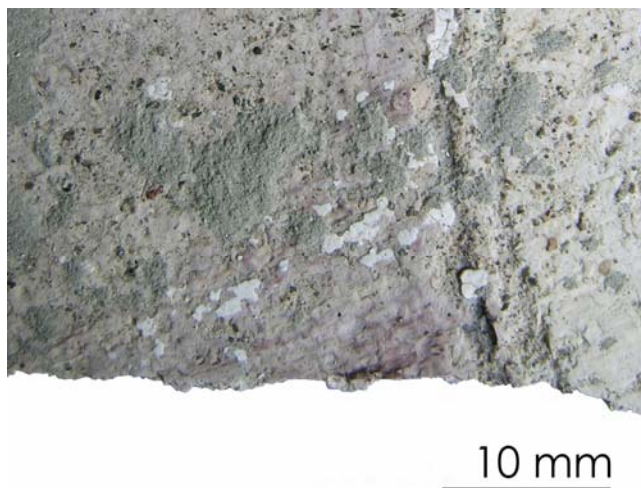


Fig. 3. A closer look at the black painted areas reveals a violet shading of the lime wash.

Experimental

In order to find the source of this quite uncommon alteration, studies were made on cross-sections of the painting layer. Therefore, samples of the plaster were embedded in epoxy, ground and polished in order to enable an investigation of the stratigraphy. Analyses were carried out by Light Optical Microscopy (LOM), using a Zeiss AxioScope A1, Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy (SEM-EDX), Micro-X-ray Fluorescence analysis (micro-XRF) and micro-Raman spectroscopy.

SEM-EDX

SEM-EDX was applied in order to characterize the elemental composition of the layers with a special focus on the black pigments. Analyses were performed by means of a Zeiss EVO MA 15, equipped with a tungsten filament at 20kV. A Si(Li) EDX detector (INCA dry cool) from Oxford Instruments was used to detect the X-ray from the sample. The sample was coated with carbon as conductive layer.

Micro-XRF

In order to increase the detection limits for low concentration elements micro-XRF was also used, combined with SEM. The X-ray source “iMoxs SEM” is produced by IFG - Institute for Scientific Instruments GmbH, (Berlin), using a polycapillary lens system which enables a beam diameter on the sample surface of approx. 100 μ m, mainly depending on the tube voltage. Qualitative analyses were carried out at 50kV using an Rh-anode.

Micro-Raman spectroscopy

Raman spectroscopy is a powerful method to identify pigments and their alteration products [1, 16]. In our study, Raman spectra were obtained in backscattering geometry using a confocal micro-Raman system LabRam HR800 (Horiba Jobin Yvon) equipped with a BX-41 microscope (Olympus), a motorized x-y stage and a Nd:YAG laser ($\lambda = 532$ nm) for excitation. The spatial resolution of the analyses is 1–2 μ m using a micro objective 100X/0.90 (Olympus). The laser power was reduced by a neutral density filter (D2) to about 0.3mW at sample surface to avoid any local heating and effects of laser-induced degradation of the studied substances. The measurement time was set to 180s. Silicon was used to check the system and calibrate scale for wavenumber position.

If Raman spectra are superimposed by intense broad bands of luminescence, the baseline correction routine of the LabSpec software of the spectrometer was used to subtract the background. The pigment identification is based on the comparison between the baseline-corrected spectra of the sample and Raman spectra collected on commercial pigments or standard minerals as well as spectra from literature [3, 16-18]. Because of discrepancies between reported Raman band positions for tenorite [17] and our first results of measurements (see below) it was decided to record own standard spectra of tenorite on a small sample provided by the Museum für Naturkunde, Berlin (tenorite; location: Vesuvio, Italy; inventory-no. 1998-5178 (part of)). As the reported spectra [17] were obtained with the 633nm excitation, a second laser, a He-Ne laser ($\lambda = 633$ nm, D2, 0.2mW at sample surface, measurement time 360s), was used for comparison reasons as well. The other instrumental parameters kept unchanged.

Results and discussion

Analyses, carried out by Optical Microscopy (Fig. 4) and by SEM (Fig. 5), show a final plaster, composed of a mixture of lime and silicate sand with a grain size diameter of up to

0.3mm. The plaster is covered by a 0.5mm layer of lime wash (number (1) in Figs. 4 and 5), containing some silicate grains with sizes up to 0.2mm. Remarkably, this lime wash shows an intensive violet colour which increases to the surface as can be seen in Fig. 4. This lime wash is followed by traces of black pigments (2), which apparently derive from pigment alteration. The detection of a layer of vitrification or densification respectively reveals that the pigment was applied by al-secco technique. The binding medium contains lime, however any further (organic) components cannot be concluded by SEM-EDX analysis. The painting is covered with a lime wash of approx. 100 μ m thickness, which was applied in two layers (3). According to the archives, mentioned in the introduction, this lime wash is assumed to be applied around 1900. The SEM image in figure 5 shows fragments of a thin hydraulic plaster (4), containing granulated cinder on the surface which apparently was applied in 1967. Analyses, carried out by SEM-EDX reveal that no traces of sulphation or any other salts could be observed in the complete sample.

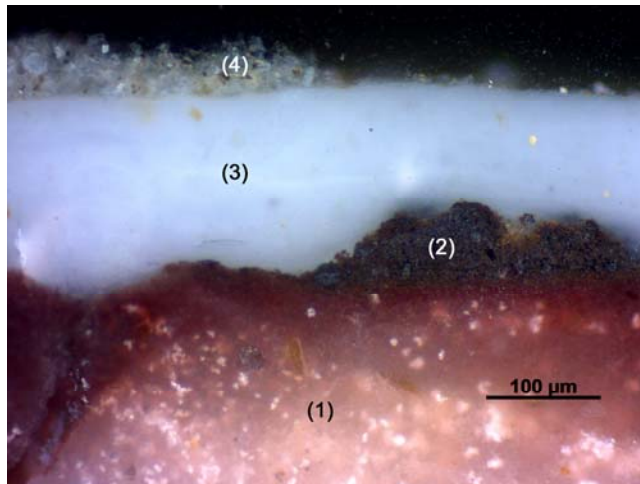


Fig. 4. Cross-section of the surface paint layers under the Light Optical Microscope.

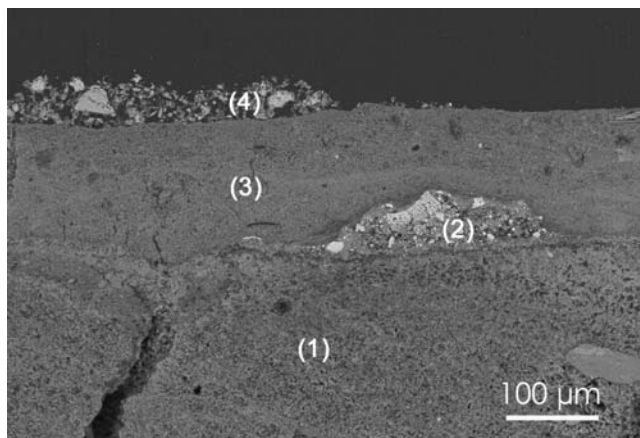
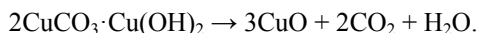


Fig. 5. Cross-section of the surface paint layers in the SEM in BSE mode.

Analyses of the black pigments, carried out by SEM-EDX, just show signals for copper and oxygen. No S, Cl or Si could be detected in significant amounts, which excludes the presence of basic copper-sulphates (e.g. brochantite or antlerite), basic copper-chlorides (e.g. paratacamite) or hydrated copper-silicates (e.g. chrysocolla). As can be seen in figure 5, the morphology of the black pigments shows a very porous structure, which is uncommon for copper minerals such as azurite or malachite, and it should be related with decomposing reactions of the original mineral pigments. Due to the better detection limits of EDX, when using X-ray excitation, compared to electron excitation in the SEM, micro-XRF was applied in order to detect the presence of any other elements in low concentration. Comparisons of the EDX-spectra, obtained by X-ray and electron-excitation do not show any significant difference.

From this follows that no minor elements were involved in this alteration phenomena. The results, carried out so far, lead to the assumption that copper (II) oxide, namely tenorite was formed from azurite or malachite. Additionally, traces of sulphur could be detected in the violet layer of lime wash by EDX, indicating in this context the presence of an organic additive, namely casein. Analyses of Austrian blue mural paintings from baroque times usually contain small; therefore it is striking that (more expensive) copper pigments were used for this ornamental painting.

According to the literature [19], the transformation from azurite ($\text{Cu}_3(\text{CO}_3)_2(\text{OH})_2$) into tenorite (CuO) occurs at temperatures above 400°C and/or at high pH. As the roof shows its original construction and even the main door can be dated to the beginning of the 18th century, a thermal decomposition of the pigments caused by fire can be excluded. Additionally, no traces of soot could be detected in-between the layers. Following the paper published by Petushkova and Lyalikova [7], who describe the transformation from lead white into lead oxide by means of bacteria, a degradation caused by microbiological activity could also be possible in a similar reaction. A dissolution of $\text{Ca}(\text{OH})_2$ has a pH ranging from approx. 10 to 12.5 depending on its concentration, which seems to be sufficient for an alteration from azurite to tenorite following the reaction equation:



As the conversion from azurite into tenorite is well known and has already been published so far [20], attention has to be drawn to the violet shading of the lime wash underneath the pigment layer. A prerequisite for this observed coloration is an alkaline reaction medium, which enables the reduction of the redox potential. Therefore, it can be derived that the copper pigments were applied on the alkaline lime wash before carbonation and that staining stopped when carbonation was completed. It seems to be implausibly that this colouration occurred when the second lime wash was applied on the pigmented surface by capillary moisture which shifted the colour complex into the sucking layer underneath as a sharp borderline can be observed between layers (2) and (3) in figure 4.

A referring chemical test in a test tube shows that violet colour shading occurs within a few hours when (synthetic) azurite was added to freshly slaked lime with approx. 1% casein (pH measured at 12.4), imitating a fresh plaster, which mainly consists of $\text{Ca}(\text{OH})_2$ and casein. Additionally, the large azurite crystals break down into a black powder of tenorite of fine grain size, similar to the pigments observed in the cross-section by SEM (Fig. 5). The presence of tenorite was proved by Raman spectroscopy. Figure 6 shows the Raman spectra of tenorite of the sample obtained with two different lasers for excitation at 532nm (graph a) and 633nm (graph c) as well as the spectra of the tenorite standard (graph b, 532nm laser) and (graph d, 633nm laser) in comparison. The peak positions of the Raman bands at 298, 346 and 632cm^{-1} (as listed in table 1) are identical for both of the standard spectra and in good agreement for the sample spectra in comparison to the standard spectra. Thus, tenorite is confirmed to form the black parts of the sample. Any Raman signal of azurite could not be registered in the sample of the paint layer.

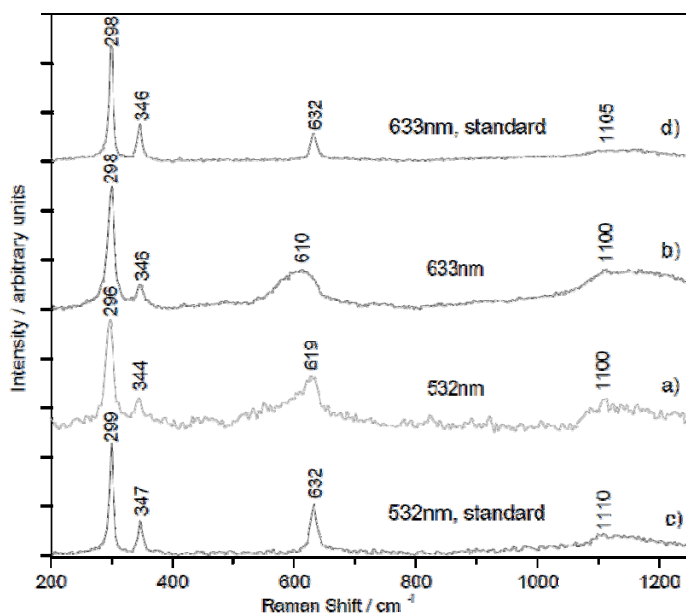


Fig. 6. Raman spectra of tenorite in the paint layer of the cross-section: a) with excitation at $\lambda = 532\text{nm}$, 0.3mW , $6 \times 30\text{s}$; c) with excitation at $\lambda = 633\text{nm}$, 0.2mW , $6 \times 60\text{s}$; b) and d) standard spectra of tenorite (see text) with $\lambda = 532\text{nm}$ and 633nm excitation, respectively.

Table 1. Raman peak positions obtained from the spectra of the sample and the tenorite standard compared to the results measured by Mattei et al. [17] with line intensities and shapes as mentioned (s = strong, m = medium, w = weak, br = broad)

Sample, This study		Tenorite standard, This study		Tenorite, Mattei et al. [17]			
laser 532nm	laser 633nm	laser 532nm	laser 633nm	standard commercial Fluka AG 633nm	Band intensity 633nm	formed due to laser-induced degradation 633nm	formed in the black zone of chemical altered sample 633nm
296	298	299	298	294	s	~ 294	294
344	346	347	346	338	m	---	338
619(br)	610(br)	632	632	611	w(br)	---	611
1100(br)	1100(br)	1110(br)	1105(br)	1100	w(br)	---	---

The Raman band positions at 298 and 346cm^{-1} in our study differ to the reported values (*cf.* Table 1) by Mattei et al. [17] at 294 and 338cm^{-1} , respectively. The shift to lower wavenumbers in [17] is most likely a result of sample heating due to high laser power during their experiments. The authors used a 633nm laser with 0.88mW at sample surface, i.e. an about 5 times higher laser power than in our study was applied. This difference can be important for the band positions of a black and hence strong absorbing substance like tenorite.

The Raman band at 632cm^{-1} is a narrow band in both of the standard spectra (Fig. 6 spectra b and d). However, this band appears in the sample spectra with both of the laser excitations (Fig. 6 spectra a and c) considerably broader and the peak position is shifted to a lower wavenumber at 610cm^{-1} and 624cm^{-1} , respectively. This could be an effect of the very small grain size of the tenorite particles formed by the degradation reaction of the former pigment.

Unsurprisingly, this reaction also occurs, when malachite ($\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$) was mixed with slaked lime and casein. Therefore it cannot be derived whether azurite or malachite were originally used for painting the murals at the garner. Even the absence of blue layers, painted with smalt, gives no evidence for an argumentum or contrarius of green malachite. A reference test, carried out with tenorite, which was mixed with freshly slaked lime and casein, showed no violet shading, which proves, that the initial use of black tenorite can be excluded from this point of view.

One can assume that the majority of the copper-pigment is being decomposed into tenorite, while some copper ions get dissolved by forming a violet copper-casein-complex. However, investigations carried out by SEM-EDX in the violet lime wash show no copper to be present, which results on one hand from the detection limits of SEM-EDX and XRF and on the other hand from the high intensity of colour of the complex. A high pH value seems to be an essential prerequisite for the reactions observed. Contrary to the results obtained so far, a retry of this experiment with a dissolution of diluted slaked lime (pH approx. 11), nearly shows no shading, which explains the rarity of the of this phenomenon.

Investigations of the sample, described above, show a complete conversion reaction, disabling the detection of the initial pigment (azurite). According to the big number of analyses of Baroque mural paintings, carried out within the past decades, no single evidence is given that tenorite has initially been used as black pigment. In that time black or grey lime paintings were usually produced by using carbon black or bone black. Apart from that, copper-based pigments were disproportional pricy compared to carbon black [20] which gives no reason for using tenorite as black pigment.

The formation of a violet coloured copper-casein complex itself was first observed in 1833 by F. Rose [21] and is known as microchemical detection of the presence of protein in solution for approx. 100 years [22, 23]. The so called “biuret test” is a chemical reaction used for indicating peptide bonds. In the presence of peptides, a $\text{Cu}(\text{II})$ ion forms a violet-coloured coordination complex in an alkaline solution (Fig. 7). Notably, despite its name, the reagent does not contain biuret ($(\text{H}_2\text{N}-\text{CO}-)_2\text{NH}$), the name is given as peptide-like bonds in the biuret molecule also give a positive reaction.

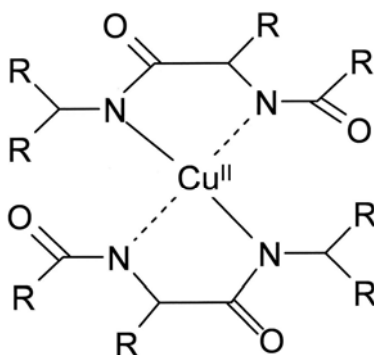


Fig. 7. Violet coloured coordination complex of the copper-biuret molecule.

Although, the combination of lime, casein and azurite is quite common in mural paintings, a violet discolouration could hardly be observed so far. Therefore a high pH seems to be an essential prerequisite for the biuret reaction.

Among thousands of samples from mural paintings, which have been subject of investigation within the past 35 years in the scientific laboratory of the Austrian Federal Office for the Care of Monuments, the phenomenon, described in this survey has been observed very rarely, namely at a mural painting from the 13th/14th cent. from St. Stephens Cathedral in

Vienna and at the one described in this article. Following the literature, purple shades connected to a conversion from azurite into tenorite have recently been described by Sotiropoulou *et al.* [4] and is interpreted as thermal conversion; however, no supplementary investigations concerning the presence of casein have been carried out in that paper.

Conclusions

This study shows how basic copper carbonate pigments were transformed into copper oxide by the effect of a highly alkaline lime and casein. Copper ions, which get dissolved during this conversion, form a complex with casein giving the lime wash a violet shade. The phenomenon, described in this survey, has been observed very rarely hitherto at mural paintings. Observations of violet shadings, which were reported in the literature, were interpreted as effect of thermal decomposition. Contrary to these conclusions, the results obtained in this survey reveal that the formation of the copper-peptide complex is independent from variation of temperature and exclusively a function of the pH.

Acknowledgements

R.T. Schmitt, curator of the mineral collection at the Museum für Naturkunde, Berlin is thanked for kindly delivering the sample of the tenorite standard. Heinz Michael, conservator of the building described in this article, is acknowledged for providing historic facts on this house as well as Figs. 1 and 2.

References

- [1] D. Karg, Brandenburgisches Landesamt für Denkmalpflege und Archäologisches Landesmuseum (Editor), *Umweltbedingte Pigmentveränderungen an mittelalterlichen Wandmalereien, Arbeitshefte des Brandenburgischen Landesamtes für Denkmalpflege und Archäologischen Landesmuseums*, **24**, Worms, 2009.
- [2] D. Saunders, J. Kirby, *The effect of relative humidity on artists pigments*, **National Gallery Technical Bulletin**, **25**, 2004, pp. 62-72.
- [3] S. Aze, J.-M. Vallet, M. Pomey, A. Baronnet, O. Grauby, *Red lead darkening in wall paintings: natural ageing of experimental wall paintings versus artificial ageing tests*, **European Journal of Mineralogy**, **19**(6), 2007, pp. 883-890.
- [4] S. Sotiropoulou, Sister Daniila, C. Miliani, F. Rosi, L. Cartechini, D. Papanikola-Bakirtzis, *Microanalytical investigation of degradation issues in Byzantine wall paintings*, **Applied Physics A**, **92**, 2008, pp. 143-150.
- [5] R.M. Gasol Fargas, *Study of the original technique of the wall paintings of the chapter house of Santa Maria de Sigena, Spain (1190 – 1200)*, **Preprints of the ICOM-CC 12th Triennial Meeting**, Lyon, II, London, 1999, pp. 467-472.
- [6] G.D. Smith, R.J.H. Clark, *The role of H₂S in pigment blackening*, **Journal of Cultural Heritage**, **3**(2), 2002, pp. 101-105.
- [7] J. Petushkova, N.N. Lyalikova, *Microbiological degradation of lead-containing pigments in mural paintings*, **Studies in Conservation**, **31**(2), 1986, pp. 65-69.
- [8] G. Cavallo, *Alteration of azurite into paratacamite at the St. Alessandro church (Lasnigo, Italy)*, **Conservar património**, **9**, 2009, pp. 5-11.
- [9] L. Dei, A. Ahle, P. Baglioni, D. Dini, E. Ferroni, *Green degradation products of azurite in wall paintings: identification and conservation treatment*, **Studies in Conservation**, **43**(2), 1998, pp. 80-88.
- [10] S. Giovannoni, M. Matteini, A. Moles, *Studies and developments concerning the problem of altered lead pigments in wall painting*, **Studies in Conservation**, **35**(1), 1990, pp. 21-25.

- [11] M. Pérez-Alonso, K. Castro, J.M. Madariaga, *Investigation of degradation mechanisms by portable Raman spectroscopy and thermodynamic speciation: the wall painting of Santa María de Lemoniz (Basque Country, north of Spain)*, **Analytica Chimica Acta**, **571**(1), 2006, pp. 121-128.
- [12] R. Linke, *Der Nachweis einer ungewöhnlichen Pigmentveränderung an Wandmalereien in Österreich*, **Umweltbedingte Pigmentveränderungen an mittelalterlichen Wandmalereien** (Editor D. Karg), Arbeitshefte des Brandenburgischen Landesamtes für Denkmalpflege und Archäologischen Landesmuseums, **24**, Worms, 2009, pp. 105-107.
- [13] * * *, Archives of the Austrian Federal Office for the Care of Monuments, ref. no.: 47.319/1/2006.
- [14] M. Koller, *Die Wandmalereien des Gerichtsgebäudes von Schwaz in Tirol, Original, Kopie, Fragment*, **Österreichische Zeitschrift für Kunst und Denkmalpflege**, **54**(2-3), 2000, pp. 359-366.
- [15] A. Zoppi, I. Osticioli, C. Lofrumento, E.M. Castellucci, *Raman investigation of a "Pompeian violet" specimen*, **8th International Conference on "Non-destructive investigations and microanalysis for the diagnostics and conservation of the cultural and environmental heritage"** (Editors: C. Parisi, G. Buzzanca and A. Paradisi), Lecce, Italy, 2005, pp. 9-12.
- [16] D. Bersani, P.P. Lottici, G. Antonioli, E. Campani, A. Casoli, C. Violante, *Pigments and binders in the wall paintings of Santa Maria della Steccata in Parma, Italy: The ultimate technique of Parmigianino*, **Journal of Raman Spectroscopy**, **35**, 2004, pp. 694-703.
- [17] E. Mattei, G. de Vivo, A. De Santis, C. Gaetani, C. Pelosi, U. Santamaria, *Raman spectroscopic analysis of azurite blackening*, **Journal of Raman Spectroscopy**, **39**, 2008, pp. 302-306.
- [18] A. Colombini, *Characterisation of a blue paint degradation process using Raman microscopy*, **8th International Conference on „Non-destructive investigations and microanalysis for the diagnostics and conservation of cultural and environmental heritage"** (Editors: C. Parisi, G. Buzzanca and A. Paradisi), Lecce, Italy, 2005, pp. 82-83.
- [19] D. Gutscher, B. Mühlethaler, A. Portmann, A. Reller, *Conversion of azurite into tenorite*, **Studies in Conservation**, **34**, 1989, pp. 117-122.
- [20] A. Burmester, Ch. Krekel, *The relationship between Albrecht Dürer's palette and fifteenth/sixteenth-century pharmacy price lists: The use of azurite and ultramarine*, **IIC, Painting Techniques History, Materials and Studio Practice**, Contributions to the Dublin Congress, 7-11 Sept. 1998, pp. 101-105.
- [21] F. Rose, *Über die Verbindungen des Eiweiss mit Metalloxyden*, **Poggendorffs Annalen der Physik und Chemie**, **28**, 1833, pp.132-142.
- [22] H. Hetterich, *Über mikrochemische Bilduntersuchung*, **Mikrochemie**, **10**, 1931, pp. 27-44.
- [23] V. Daniels, Y. Shashoua, *Evaluation of reagents for the determination of protein in solution*, **Conservation Science in the UK: Preprints of the Meeting Held in Glasgow** (Editor: N. Tennent), 1993, pp. 99-102.
-

Received: September, 11, 2013

Accepted: May, 03, 2014