

TIBETAN WALL PAINTING: INVESTIGATION OF MATERIALS AND TECHNIQUES AND DNA ANALYSIS OF PROTEINACEOUS BINDING MEDIUM

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Abstract

*The present study concerns the technology of Tibetan wall painting i.e. the pigments, binding medium and stratigraphic build-up. Original Tibetan materials were brought to the School of Conservation in Denmark by two Tibetan artists. Pigments and binding medium were analysed by Attenuated Total Reflection - Fourier-transformed Infrared Spectroscopy (ATR-FTIR), Micro-Raman Spectroscopy (MRS), X-ray powder Diffraction (XRD), Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy (SEM-EDX), High Performance Liquid Chromatography with Diode-Array Detector (HPLC-DAD) and thermally assisted Hydrolysis and Methylation Gas Chromatography-Mass Spectrometry (THM-GC-MS). The proteinaceous binding medium which was expected to be Yak ox (*Bos grunniens*) glue was identified by DNA analysis. The painting techniques of the Tibetan artist were followed during their painting of a traditional wall painting. The results show that several pigments were adulterated compared to the artists' belief and that the supposed Yak ox glue derived from Indian cow (*Bos indicus*).*

Keywords: *Tibetan wall painting, Pigments and dyes, Proteinaceous binding medium, DNA analysis, MRS, XRD, THM-GC-MS, HPLC*

Introduction

Aspects of the study of Tibetan painting techniques have been described by a few authors since mid-19th century through 1970 when Huntington [1] reviewed the existing literature and produced an overview of painting techniques with emphasis on painting on cloth (thangka paintings). Similar in 1970, V.R. Mehra [2] published a detailed account of the composition of the pigments used in thangka paintings. Since 1970 Tibetan paintings on cloth and to a much lesser degree wall paintings have been studied by a number of authors to determine material composition, paintings techniques, deterioration and possible conservation. A major account was given by D. Jackson and J. Jackson [3] where detailed descriptions are given on painting materials and painting techniques. Their descriptions and documentation were the result of numerous journeys to Tibet and the study of practicing Tibetan thangka artists and literature. In recent years a few publications have emerged, dealing with the techniques of Asian wall paintings and clay sculptures in temple complexes in areas close to Tibet [4-9].

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While the study and analysis of painting techniques and materials used in European school paintings is well established based on numerous papers and books [10-16], the study of Tibetan paintings is in an introductory phase. In order to eventually determine appropriate conservation measures for ancient Tibetan wall paintings, this article aims to combine the study of Tibetan wall painting techniques with advanced analytical approaches, based on the assumption that the materials used in modern-day Tibet still represent a continued tradition barely changed during centuries.

In collaboration with the Norwegian University of Science and Technology, the School of Conservation in Denmark invited two artists/lecturers from Tibet University in Lhasa, (Tibetan Fine Arts Teaching and Research Section, School of Art) to carry out a replica of a large Tibetan wall painting. Both lecturers, Kalsang Tseten and Penba Wangdu, are specialized in traditional *thangka* and wall painting techniques. The purpose of having the replica produced in Copenhagen was to document in minute details the traditional Tibetan methods in terms of the preparation, mixing and application of the colours/paint and the gilding technique, as well as analyzing the materials and the overall *stratigraphic build-up techniques*. All materials i.e. pigments, gilding metals, binding medium, brushes etc. were brought from Tibet by the two lectures. Pigments and binding medium were prepared at The Colour Factory at Tibet University and are supposed to be traditional manufactured materials.

Due to the need for transport the wall painting 205×152cm was made on canvas glued on aluminium honeycomb board and the canvas was grounded in traditional manner before painting. A small part of the wall painting of an area of 10×15cm was replicated on traditional clay ground and analyzed with respect to the stratigraphical build-up. The final wall painting and the areas analyzed on traditional clay ground are shown in figure 1.



Fig. 1. Left: The final wall painting. Right: The smaller parts of the wall painting on traditional clay ground. The numbers indicate areas analyzed with respect to the stratigraphical build-up.

Experimental

Cross sections/PLM

Paint samples were imbedded in spofacryl resin between two PMMA blocks and wet polished until the sample was reached, then dry polished using micro mesh (granularity 2400-12000). The cross sections were studied with an optical microscope Axioplan (Carl Zeiss) under incident white light and ultraviolet radiation (excitation bandpass filter from 390-420nm) with magnifications up to 1000X.

XRD

A Stoe Stadi P powder diffractometer was used to obtain diffraction data. The diagrams from samples were recorded in transmission mode using Cu K α radiation selected by a curved germanium monochromator. A position-sensitive detector covering 7° in 2 θ was used in 2 θ step scan mode with steps of 0.01° and data were collected from 5 to 90°. The software STOE WinXPOW (version 1.05, 1999) supplied with the instrument was used for data treatment.

ATR-FTIR

ATR-FTIR spectra were recorded on a Perkin Elmer Spectrum One FTIR-spectrometer, fitted with a Universal ATR sampling accessory having a one bounce composite zinc selenide and diamond crystal. Spectra of the samples were recorded over the range 4000-650cm⁻¹ with a resolution of 4cm⁻¹ and 4 accumulations.

SEM-EDX

SEM analyses were carried out on gold coated cross sections and bulk samples using a JEOL JSM6300 scanning electron microscope with an energy dispersive X-ray spectrometer (SEM-EDX). The SEM-EDX was equipped with X-ray detectors Pentafet Si(Li) and BSE (Tetra Link), both from Oxford Instruments. The EDX-analyses were run at an acceleration voltage at 15kV.

MRS

MRS analyses were carried out with a Renishaw inVia dispersive micro-Raman spectrometer equipped with a Peltier-cooled (203K) CCD detector. The laser used operated at 785nm (Toptica Photonics XTRA) in combination with a grating of 1200L/mm. Analysis was carried out directly on the cross sections under a direct-coupled Leica DMLM microscope with enclosure using the 50 \times objective. The laser power used was kept low at values between 0.1-1.0mW by neutral density filters to avoid any thermal degradation. Acquisition time was 10-80 seconds and accumulations from 1-5, depending on the circumstances, were used to obtain a spectrum with a significant signal-to-noise ratio. The spectra were subsequently baseline corrected with Raman software Wire 2.

THM-GC-MS

Prior to THM-GC-MS analyses, samples were crushed in a glass vial and put into suspension with 10 μ L of a 2.5% solution of tetramethylammonium hydroxide (TMAH) in methanol. A droplet of 3 μ L of the suspension was applied to the metal wire (Curie point 625°C) and dried in the MS oven at 50°C. The wire was inserted into the glass tube and placed in the pyrolysis chamber (625°C). The pyrolysis unit was a GSG Pyromat Curie point system. The chamber was mounted onto the injection port of a Trace GC Ultra gas chromatograph, coupled to a Thermo PolarisQ ion trap MS detector. The GC column was a fused silica capillary column (20m, 0.18mm i.d., 0.18 μ m film thickness). Helium was used as the carrier gas at a flow rate of 1.3mL \cdot min⁻¹. The temperature program adopted was an initial temperature of 50°C held for 1 minute and ramped at 10°C \cdot min⁻¹ to 320°C and held for another five minutes. The temperature of the left SSL was kept at 250°C with split Flow 10 mL/min and split ratio 8. The MS transfer line was kept at 290°C.

The PolarisQ mass spectrometer used Electron Impact (EI) ionization (70eV). The mass spectrometer was scanned from m/z 35 to 500 every 0.5 second (positive ions). The ion source temperature was 220°C.

HPLC-DAD

HPLC analysis was carried out after mild HF extraction (2M aqueous HF/dimethylformamide/acetonitrile = 2/1/1) [17]. The HPLC-DAD system used for the dye analyses was a Spetra-SYSTEM from ThermoScientific, controlled by ChromQuest 4 software. It consisted of a P 1000 XR pump, an AS 3000 autosampler equipped with a 20 μ L loop, and a UV6000 UV-VIS DAD detector equipped with an 50mm detector cell. The slit width was 5nm for scan-data and 11nm for discrete channels, 255, 350 and 495nm. The data from the detector were collected between 200-700nm. The eluent was on-line degassed by vacuum. The analytical column was an Alltima RP C18, 5 μ m, 250 \times 4.6mm (Alltech, Lokeren, Belgium), and the guard column a Sentra Nova Pak C18, 4 μ m, 3.9 \times 10mm (Waters, Brussels, Belgium). For on-line column-switching (when residual fluoride anions were eliminated by on-line clean-up), an automated valve (Valco Instruments, Schenkon, Switzerland) was inserted between the guard column and the analytical column. The eluents were (A) methanol, (B) 5% (v/v) acetonitrile in water, (C) 0.1% (v/v) trifluoroacetic acid in 1:1 (v/v) methanol:water, (D) acetonitrile. The flow rate was 1 mL \cdot min⁻¹. The elution program was without column switching with the gradient: 0-5 min: isocratic, 5A, 80 B, 10C, 5D; 5-55min: linear gradient to 45A, 0B, 10C, 45D; 55-65min: isocratic, 45A, 0B, 10C, 45 D. When on-line column-switching was used, the elution program was the same as above, but with the valve switched towards waste line for the first 5min.

DNA-analysis

Glue original identified as coming from Yak ox (*Bos grunniens*) by the artists were sequenced for a variable segment of the bovine mtDNA control region, to confirm the identity of the species used to produce the glue. Likewise, hair from a Yak ox belonging to the Zoological Garden in Copenhagen, Denmark was also sequenced using the same methods as below to compare the DNA from the glue with the nucleotides of a confirmed yak ox. All the glue samples are stored at The Royal Danish Academy of Fine Arts, School of Conservation until a Danish gene bank will be ready to store the samples. DNA Extraction, PCR Amplification, and Sequencing: The DNA segment used in this investigation has been identified through surveys in a range of cattle populations [18-21]. DNA was extracted from the sample using QIAmp Tissue Kit from QIAGEN following the supplier's tissue protocol. Subsequently, a standard double-stranded (ds) 50 μ L PCR was carried out using 1 μ L of the extracted DNA as template (see table 2 for variation in use of template). The region analyzed was a highly variable region of the mtDNA control region between bases 16.022 and 16,334 (including both primers) [18, 20-22]. Primers used for amplification of the fragment were AN2-forward: 5'- CCC CAT GCA TAT AAG CAA G - 3' and AN3-reverse: 5' - CGA GAT GTC TTA TTT AAG AGG - 3' [21, 22]. PCR-Conditions for the fragment were: One initial cycle of denaturation (94 $^{\circ}$ C for 3 minutes), followed by 35 cycles (94 $^{\circ}$ C for 40sec, 55 $^{\circ}$ C for 40sec, 72 $^{\circ}$ C for 4sec) and finally 4 min extension step at 72 $^{\circ}$ C [modified from 20]. The dsPCR was carried out on an ESCO Swift MiniProThermal Cycler. The PCR products were visualized on an agarose gel containing SYBR Safe DNA gel stain SYBR Green. One extraction control together with a PCR amplification control was following each set of sample. The dsPCR products were then cleaned using QIAquick Purification Kit (Cat. No. 28106). The purified dsPCR products were used as templates for a 12 μ L cyclic sequencing reaction using ABI prismTM BigDye Terminator v1.1 Cycle Sequencing kit. For the cyclic sequencing reaction the same primers as for the initial dsPCR reaction were used. Cyclic sequencing PCR conditions for the fragment were the same as for the initial dsPCR reaction. The cyclic sequencing PCR was carried out on a gradient Cyler, BIO-RAD, DNAEngive, Peltier Thermal Cycler. The purified sequencing products were run on a 3130xl Genetic Analyser Applied Biosystems HITACHI and the attached computer with Genetic Analysis Program. The sequences were assembled using the computer program "CLC Main Workbench 5" and submitted to NCBI nucleotide collection for comparison of the sequences with sequences hold at NCBL. These results were used to identify the sequences to taxa.

Results and discussion

An overview of the materials, analyses and results together with the Tibetan names and the English names based on information from the artist is seen in Table 1.

Table 1. Results of analyzed pigments, metals and binders. Minor amounts are given in parentheses. Analytical methods; 1: XRD, 2: MRS, 3: SEM-EDX, 4: ATR-FTIR, 5: HPLC-DAD, 6: DNA, 7: THM-GC-MS.

Colour	Tibetan name	English name	Result	Method
Inorganic pigments				
Red	Mtshal	Cinnabar	HgS	1, 2
Orange	Ldong ros	Realgar	As ₄ S ₄ + Pb ₃ O ₄	1, 2, 3
"	Li Khri	Minium	Pb ₃ O ₄	1, 2, 5
Yellow	Ba bla	Orpiment	As ₂ S ₃	1, 2, 5
"	Sa ngang pa	Ochre	SiO ₂ (quartz) + amorphous iron oxide	1, 3, 4
Green	Spang chen	Malachite coarse	CuCO ₃ ·Cu(OH) ₂ (azurite) + quartz	1, 2, 4
"	Spang ma	Malachite medium	CuCO ₃ ·Cu(OH) ₂ (azurite) + quartz	1, 2, 4
"	Spang skya	Malachite fine	CuCO ₃ ·Cu(OH) ₂ (azurite) + quartz	1, 2, 4
Blue	Mthing chen	Azurite coarse	2CuCO ₃ ·Cu(OH) ₂ (malachite) + quartz	1, 2, 4
"	Mthing	Azurite medium	2CuCO ₃ ·Cu(OH) ₂ (malachite) + quartz	1, 2, 4
"	Sngo sangs	Azurite fine	2CuCO ₃ ·Cu(OH) ₂ (malachite) + quartz	1, 2, 4
White	Rinpung Ka rag	Magnesite	MgCO ₃ (magnezite)	1, 2, 4
"	Ka rag	Calcite	CaCO ₃ (calcite)	1, 2, 4
"	Kyo bur sa	Dolomite	CaCO ₃ ·MgCO ₃ (dolomite) + quartz	1, 2, 4
Black	Snag tsha	Carbon soot	Fe ₃ O ₄ (magnetite) + graphite	2, 3
Organic pigments				
Red	Rgya tshos	Lac dye	Laccic acid A, B and D	5
Yellow	Zhu mkhan	Unknown, dye	Unidentified	5
Yellow	Zhu mkhan	Unknown, leaves	Unidentified	5
Blue	Rams	Indigo	Indigo	2, 4
Metals				
Yellow	Grang gser	Gold powder	Pure unalloyed Au	3
"	Gser shog	Gold leaves	Pure unalloyed Au	3
Binders				
-	Ko spyin	Hide glue	<i>Bos taurus indicus</i>	6
-	Ching Chi	Linseed oil	Drying oil, heated, based on linseed oil	7

Pigments and dyes

The inorganic pigments consisted of seven different colors: red, orange, yellow, green, blue, white and black. The inorganic pigments were analyzed by XRD, MRS, ATR-FTIR and SEM-EDX on samples taken from stock containers. The organic dyes consisted of three colors (red, yellow and blue) in the form of small pellets together with leaves from which a yellow dye could be extracted. The leaves were told to be used for the preparation of the red dye Rgya tshos. The yellow dye and the plant leaves were both called Zhu mkhan, however no further characterization could be given. In addition to the pigment and dyes gold powder named Grang gser and gold leaves named Gser shog, and an oily binder Ching Chi used in connection with gilding, was also present.

The analyses of the pigments were in general straight forward and the spectra fits the well known references from available databases and published papers [23-26]. The presence of minor amount of azurite in the malachite and vice versa, and the presence of quartz in most samples indicates that all mineral pigments were of natural origin and made by traditional powdering technique. The XRD spectrum of cinnabar shows a very pure compound of HgS. This could either be due to the artificial version vermilion or a pure sample of HgS. Back scattered image (BSE) by SEM (not shown) shows both small agglomerated particles (1-10µm) which could be vermilion and larger particles around 50µm which could be mineral cinnabar. The orpiment showed in the XRD pattern (not shown) lack of As(III) oxide which often present in the synthetic version [27] and based on this and the typical foliated structure of the pigment it

is most likely to be the mineral version.

XRD pattern of the orange pigment Ldong ros are shown in figure 2, left. As seen from the pattern the pigment is not pure realgar, but a mixture of realgar and minium. The spiking of the realgar was not known to the Tibetan artists and the spiking could have been done by the pigment supplier in order to manufacture a cheaper version of realgar.

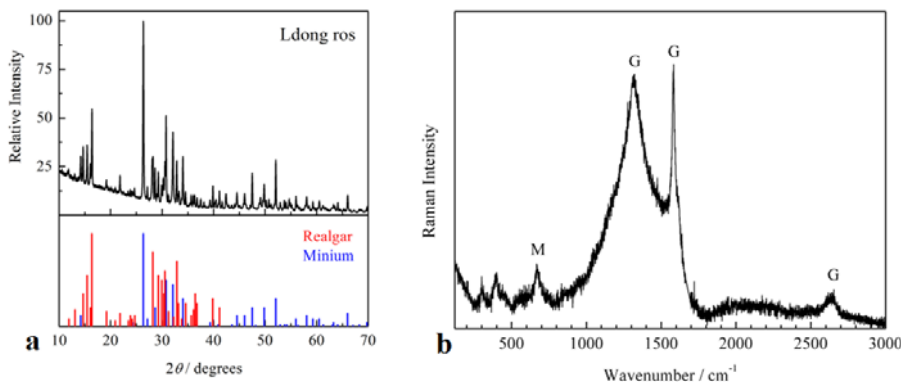


Fig. 2. Left: X-ray powder diffraction patterns of the orange pigment Ldong ros showing a mixture of realgar and minium. Right: Raman spectrum of black ink Snag tsha showing a mixture of magnetite (M) and graphite (G).

A similar change in the composition is also seen for the black ink Snag tsha which were supposed to consist of carbon soot and glue binder [3]. However, the ink was magnetic and as seen in figure 2, right the Raman spectrum showed presence of magnetite and graphite [28, 29].

The pigment Sa ngang pa was due to the presence of iron, quartz and various other minerals such as kaolinite and calcite identified to be yellow ochre by XRD and SEM-EDX. XRD could not identify the iron compound which may be due an amorphous structure.

The three white pigments Rinpung Ka rag, Ka rag and Kyo bur sa were identified by XRD, Raman and FTIR analysis to be magnesite, calcite and dolomite, respectively. The gold powder and the gold leaves showed by SEM-EDX to be pure gold without any alloy materials such as copper or silver.

Organic dyes

The binding medium in the three dye pellets were animal glue, but no further identification of species were made. The blue dye Rams was identified to be indigo as expected, however whether the indigo was of natural or synthetic origin is unknown. HPLC-DAD analysis of the red dye Rgya tshos showed laccaic acids A, B and D which are known constituents in Indian lac from scale insects like *Kerria lacca* [30]. Unfortunately we were not able to identify the yellow vegetable dye Zhu mkhan nor the leaves also called Zhu mkhan. However aqueous extract from the leaves and the yellow dye showed different HPLC spectra indicating that the yellow dye was not prepared from the leaves.

D. Jackson and J. Jackson [3] are mentioning that Tibetan artists are adding a leaf or two of Zhu mkhan when preparing dye from Indian lac and that Zhu Mkhan apparently is from the tree of the genus *Symplocos sp.* though other species are also possible.

Analysis of Tibetan Binding Medium

DNA analysis of glue

The binding medium was told to be Yak glue and to confirm the origin of the glue DNA analysis was performed since species determination from DNA in art and artifacts can be used to identify the product to species level, and to determine whether two fragments belong to the same specimen, and in addition give biological data on the animal and its population [31].

No contamination was evident for either the DNA extraction control or the PCR amplification control. Using the primer AN2-forward and AN3-reverse it was possible to obtain

positive amplification products for the glue and the Yak hair samples. The glue showed some noise in the amplification product. However, blasting each sequence against NCBI nucleotide collection showed that the amplification product was identical with haplotypes identified as Indian cow (*Bos indicus*). Comparison with the amplification product of the hair from Yak ox (*Bos grunniens*) did also clearly show that the glue was not derived from Yak ox.

The results showed that it was possible to identify DNA from proteinaceous binding medium and the authenticity of these data was corroborated by the fact that the DNA-laboratory used was just started and it was the first time samples from *Bos sp.* was amplified in the laboratory.

The results indicate that it might be possible to identify ancient DNA from glue used in wall paintings despite earlier attempts on rock paintings were unsuccessful [32]. This could depend on the locality of the paintings resulting in various degradation of the DNA [33]. The degradation of DNA in Tibetan wall paintings is likely lesser than in other wall paintings due to lower temperature and humidity environments [33]. It is also important to investigate for later contamination used to consolidate object as those products have shown to influence the results [34].

GC-MS analysis of oil binder

The oily binder used in connection with gilding was analyzed by GC-MS from two samples of the replica on traditional clay ground where gilded with gold leaves. The results showed a drying oil with diterpene resins. The ratio between azelaic (Az) and palmetic (P) acids were found to be $Az/P = 1.2$, and the ratio between the saturated palmetic and stearic acids (S) was found to be ca. 1 ($P/S = 0.9$ based on area and $P/S = 1.36$ based on the peak heights). The ratio between the dicarboxylic acids azelaic and suberic (Su) acids was found to be $Az/Su = 4.1$ (based on area) and $Az/Su = 3.5$ (based on the peak heights). These results, if found in European paintings, would be interpreted as heated linseed oil [35]. The diterpene resins was identified as dehydroabietates i.e. degradation products of abietic acid which are the primary components of resin extruded by the coniferous trees in the genus *Pinus* in the family *Pinaceae* [36].

Analysis of the stratigraphic build-up

The replica on clay ground was analyzed on 13 different spots in order to identify the use of pigments, dyes, binding media and the stratigraphic build-up.

The overall stratigraphic build-up is seen in the cross section taken from the red area on spot no. 7 (Fig. 3).



Fig. 3. Cross section of spot no 7 shows the layer structure with all the ground layers.
1: sandy ground with large particles of quartz, 2: isolating ochre layer infiltrated into the sandy ground,
3: white ground composed of three layers of magnesite, 4: red paint layer of cinnabar.

The ground was composed of three levels: a sandy ground layer, an ochre layer and a white layer. The sandy ground contains very large particles, sometimes over 200 μm , consisting mainly of quartz with clay and feldspars siliceous minerals. This was followed by a layer of yellow ochre (Sa ngang pa) with clay character. The ochre layer was partially infiltrated into the sandy layer and the purpose of this layer most likely to isolate the sandy layer in order to prevent penetration of following layers. The thickness of the ochre layer is very irregular and varies from 5 to 50 μm and the particle size is around 10-20 μm with a particular flake like morphology. The final white ground level consists of magnesite (Rinpung Ka rag) which is applied in three layers. The red layer applied on the white ground on spot no. 7 was cinnabar.

The dark red area of spot no. 2 was almost similar to spot no. 7. However, the cinnabar had been given a red glaze with lac-dye in order to obtain the darker red color. HPLC-DAD analysis of the red glaze with the identification of laccic acids A, B and D is seen figure 4. A second sample containing lac-dye was identified in the violet area in spot no. 13, where it was applied directly on the magnesite ground to obtain a violet color. The white area (spot no. 3) contained the three layers of magnesite only.

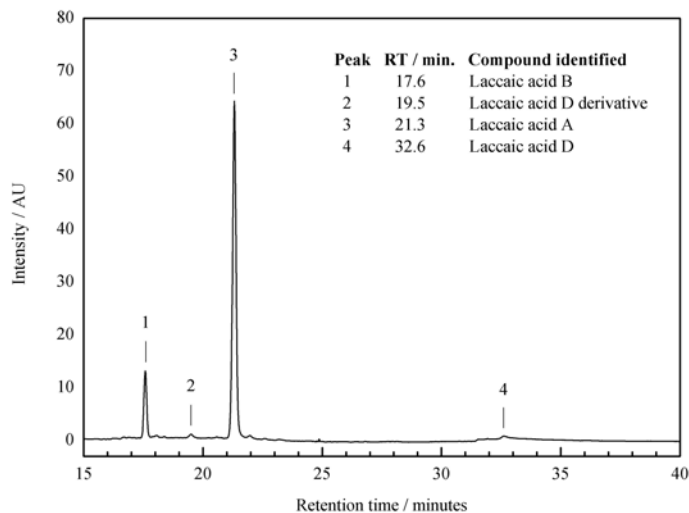


Fig. 4. HPLC chromatogram (500nm) of the dark red glazing of spot no. 2 from the clay replica. Identification of the peaks is inserted.

The four blue spots (nos. 4, 5, 9 and 12) with different shades of blue and described as dark blue, blue and middle blue and light blue consisted all of azurite of different particle size. The pigments were applied on the white magnesite layer and were pressed into the white ground with a polishing stone. This has most likely been done in order to minimize the amount of binder used and thereby maximize the effect of the color variations based on the different pigment size [37]. The dark blue color (spot no. 4) was obtained by giving the coarse azurite (Mthing chen) an indigo glaze, whereas the blue area in spot no. 5 consist coarse azurite (particle size up to 150 μm) only. In spot no. 9 the blue colours arise from the medium grinded azurite with particle size between 10-80 μm , but the surface was also smoother due to a better packing and polishing of the particles. The very light shade blue colors seen in spot no. 12 were

obtained by the use of very fine grinded azurite (5-12 μ m) incorporated into the magnesite ground. The green layer from spot no. 6 consists of a compact layer of coarse malachite particles and the pigment on yellow spot no. 8 showed to be orpiment.

The three spots nos. 1, 10 and 11 painted with gold were analyzed for the inorganic materials and the binders. Spot no. 1 showed to be a gold powder painted on an orange layer of minium seen in the area around the spot, and the spots nos. 10 and 11 were layers gilded with gold leaves with a thickness of 0.1 μ m. Both gold powder and gold leaves were pure unalloyed Au. The gilding technique in both spots was mordant gilding where the gold leaves were applied on an oil-containing adhesive layer. Stratigraphically spot no. 10 was a flat layer with the gold applied directly on the magnesite ground, whereas spot no. 11 was a relief part. The relief-forming matter was similar to the ochreous layer used for isolating the sandy ground before the magnesite layers are applied.

Conclusions

This study shows that the materials and techniques used by Tibetan artists at Tibetan Fine Arts Teaching and Research Section, School of Art at Tibet University in Lhasa, by an overall view, still are in accordance with the traditional manner. The pigments are primarily made from the natural minerals available i.e. azurite, malachite, orpiment, realgar and cinnabar and the lac-dye from insects traditional imported from India according to D. and J. Jackson [3]. Minium being a synthetic material is known to be imported from Nepal, China and India since ancient time and has been used in Asian art for centuries [3]. The mixing of realgar with minium is not described in books dealing with Tibetan arts and was unknown to the Tibetan painters and could have been done by the suppliers in order to manufacture a cheaper version of the pigment. Similar the presence of magnetite in the black ink Snag tsha is also a breach on the traditional manufacturing of the ink which is expected to be made of carbon black from burning of wood [3].

The proteinaceous binding medium believed to Yak ox (*Bos grunniens*) glue were shown by DNA analysis to be glue from the Indian cow (*Bos indicus*). According to D. and J. Jackson [3] Tibetan painters living in India and Nepal today mostly obtain size and binder from the local markets and in Nepal a common type is prepared from water buffalo skins.

The analyses of the replica on clay ground supports in general that the stratigraphic build-up is similar to the one observed on thangka paintings [3]. The support is grounded with a white pigment Ka rag which is either magnesite or calcite and the grounding is flattened and smoothed by a polish stone. The shading of colors is obtained by the different grain size of the same pigment and by glazes with organic dyes such as indigo and lac-dye.

Today's increased economic interaction between neighboring countries does often lead to changes in the traditional materials which can be difficult to observe unless advanced analytical techniques are used as done in this work. In addition to this we have introduced DNA analysis into conservation science, which gives a new perspective for future analysis of paintings with more accurate identifications of the proteinaceous binding media used.

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