

Multi analytical study on Khol residues from the ancient Egyptian city of Assiut

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Abstract—Multi-analytical chemical approaches are nowadays considered an important source of complementary information in archaeological functional studies. Here we report the results of an investigation carried out by means of liquid chromatography-mass-spectrometry (LC-MS), pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) and Raman micro-spectroscopy (μ Raman) applied onto residues sampled from ceramic vessels, found in the ancient cities of Assiut (Egypt, Middle Kingdom). These items, characterized by the typical conical shape of Kohl containers, represented an extraordinary opportunity to study, at the same time, well preserved archaeological records and the related contents. Thanks to the combined analysis, it was possible to hypothesize the original recipes adopted to prepare the ancient cosmetic. The profile of the fatty ingredients resulted congruent with the information collected by the archaeologist from the excavation site, regarding the culture and the ancient Egyptian practice of eye-painting. The analysis of the sedimentary material confirmed the provenance of the archaeological record

I. INTRODUCTION

In Ancient Egypt cosmetics were widely used for both decorative and therapeutic purposes. They included scented oils, ointments, face- and eye-paints [1]. Eye-painting was a common practice, not only for aesthetics, but also to prevent eye-diseases. Its origins date back to prehistoric times. Due to the importance in social and religious life, and considering Egyptian conception of afterlife, related containers frequently found in burial assemblage. Recent studies indicate that two main formulations were adopted: a green one, made of malachite, and a black one, called nowadays by the Arabic word kohl, containing galena. Other inorganic ingredients were added in small amounts and then

grounded into a fine powder to modify the texture of the product. These texturizing ingredients were mainly lead compounds such as cerussite, laurionite and phosgenite. Mixing operations were carried out in carbonate-free water using rudimental equipment such as pestles, palettes, and bowls. These colloidal solutions were then dispersed into carefully selected oils and fats mixtures to obtain creamy substances. Some authors suggest that this practice may be viewed as an example of the first “wet chemistry” procedures adopted in the humankind history, together with the renowned blue pigment production [2]. For sure, it looks extraordinarily close to very recent application of the nano science into cosmetics production,

In the frame of the National Italian Project (PRIN) Colors and balms in antiquity: from the chemical study to the knowledge of technologies in cosmetics, painting and medicine." granted by MIUR in 2008, we examined for the first time after their discovery, the residues of Khol vessels unearthed from the ancient cities of Assiut and Gebelein (Egypt, Middle Kingdom) by Ernesto Schiapparelli during an excavation campaign between 1911 and 1913 and preserved at the Museo Egizio di Torino (Museum of Egyptian Antiquity) of Turin.



Figure 1. Khol vessel from the Ancient Assiut.

The aim of this specific study was to test the performance of different analytical techniques in reconstructing the original formulation and enlarge the body of knowledge regarding the eye painting practice, in its relationship with the cultural aspects of the life in the Ancient Egypt

II. MATERIAL AND METHODS

A. Samples

A total of 12 residues were sampled from different conical vessels conserved in the Museum of Egyptian Antiquity of Turin, and submitted to the multi-analytical characterization by means of LC-MS, Py-GC-MS and μ Raman



Figure 2. Sampling of sample 4b .

Samples were obtained in replicates and included materials with different morphological appearance *i.e.* sticky residues, powders and encrusted materials. More details are available in Table 1.

Table 1. List of the studied samples

ID	DESCRIPTION	Catalogue number	Picture
1R	Black fragment, bright portion	P3870-1	206
1	Black fragment, dark portion	P3870-1	
2	Black fragment	P3870-2	207
3	Inner vessel material	P895	209-211-213
3B	Scalpel residues recovered with acetone	P895	
3C	Black material from the upper inner section of the vessel	P895	
3D	Black material from the middle inner section of the vessel	P895	214-215
4	Material from the mummy vessel	-	
4B	Material from the mummy vessel	-	finito
4C	Material from the mummy vessel	-	216-218-219-220
5	Material from vessel	P258	221-222-223-224-225
5B	Material from vessel, grey	P258	226
6	Material from vessel	P3872	227
7	Powder from vessel (gravity recovered)	S13074	228
7B	Encrusted powder (removed by scalpel)	S13074	
8	Inner material from little vessel	S13075	230
9	Inner material from little vessel	S13072	231
10	Inner material from vessel	vasoL	233-235
11	Inner material from alabastr vessel	VasoD	236-238
12	Inner material	-	246-247
12B	Acetone extract from inner canvas enclosed vessel	-	
12C	Inner material from the canvas enclosed vessel	-	

A. LC-MS

LC-MS was employed for the determination of triacylglycerols (TAGs) profile present in the lipid fraction. This technique offers the possibility of exploiting the TAGs intact, without the application of derivatization procedure that are instead required in the analysis of the related fatty acids by gas chromatographic. This approach is widely used in the study of vegetable oil, specifically in the identification of possible food products adulteration, but still poorly explored in archaeometry. The strength point is the possibility to characterize fats and oils avoiding the oversimplification of the information retained in the biochemical record, and therefore, in several cases, very precise identification of the original material. Two different soft ionization techniques coupled with two different detection system were applied: LC-ESI-qTOF and LC-APCI-MS. For the former, a 1200 Infinity HPLC coupled to a Jet Stream ESI interface with 6530 Infinity Q-TOF (Agilent Technologies, USA) spectrometer was employed. Collision energy (CE) was set at 50 V. Mass spectra of $[M+Na]^+$ ions of TAG were collected from 750 to 1450 m/z. The related tandem mass spectra generated by using auto MS-MS selection algorithm were analyzed from 200 to 750 m/z. Chromatographic separation was obtained by an Agilent Poroshell 120 EC-C18 3.0 x 50mm 2.6 μ m. Methanol and isopropanol were used to elute at 0.6 mL/min flow rate with gradient from 10% to 80% in 30 minutes. For the latter, a Shimadzu 2010A system equipped with a Rheodyne 7725 injector was employed. The column was an Ascentis C18 (15 cm x 4.6 mm x 5 μ m, Supelco, Bellefonte, USA). Isopropanol and methanol were used for eluting at 0.8 mL/min flow rate, applying a linear gradient. The operating conditions for the ion source were positive discharge, 2.5 ml/min nitrogen flow, 1.5 kV; detector voltage, 4.5 kV interface voltage; 400 °C interface temperature, 200 °C CDL temperature. Mass acquisition was in full scan from 300 to 1000 amu with a scan speed of 50 amu/s.

B. Py-GC-MS

Organic residues were analyzed by Py-GC-MS with the Thermally Assisted Hydrolysis and Methylation method (THM) using TMAH in aqueous solution at 25% w/w (Sigma-Aldrich-MI-Italy). Portions of the samples were loaded in a quartz tube and closed with quartz wool, then 5 μ L of TMAH in aqueous solution was added. Pyrolysis was performed at 600 °C with CDS Pyroprobe 1000 (Analytical Inc., USA) filament pyrolyzer directly connected to GC/MS system. Pyrolysis chamber was fluxed with helium. A 6890N Network GC System (Agilent Technologies, USA) equipped with a methyl-phenyl-polysiloxane cross-linked 5% phenyl methyl silicone (30 m, 0.25 mm

i.d., 0.25 μm film thickness) capillary column was used for the analysis. The temperature program was: 50 $^{\circ}\text{C}$ for 2 min, then a temperature ramp to 300 $^{\circ}\text{C}$ (heating rate 10 $^{\circ}\text{C}/\text{min}$ to 130 $^{\circ}\text{C}$, 5 $^{\circ}\text{C}/\text{min}$ to 180 $^{\circ}\text{C}/\text{min}$ then 15 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$, held for 5 min). The temperature of the injector and of the Py-GC interface was kept at 280 $^{\circ}\text{C}$. The carrier gas was helium (1.0 mL/min), and the split ratio was 1/20 of the total flow. The mass spectrometer was a 5973 Network MASS Selective Detector (Agilent Technologies, USA). Mass spectra were recorded under electron impact at 70 eV, scan range 40–600 m/z. The interface was kept at 280 $^{\circ}\text{C}$, ion source at 230 $^{\circ}\text{C}$, and quadrupole mass analyzer at 150 $^{\circ}\text{C}$.

C. μRaman

Raman spectroscopy combined with optical observation under the polarizing microscope was used to determine the composition of the inorganic particles present inside the organic residues. Specifically, we investigated the mineralogical composition of silt to sand-sized sediments extracted from the organic residues. The samples were spread gently on a glass slide, avoiding disaggregation, and accurate mineralogical data were collected from each single grain by Raman spectroscopy. Photograph of the grains were taken to enable description of their color and shape. Raman spectra were obtained by a confocal micro-Raman HR evolution spectrometer, with the 633 nm polarized radiation of a Helium-Neon laser beam, grating 1800 lines/mm in back-scattering configuration and with 50 mW as light source. The laser beam was focused through an Olympus objective 50x. The spectrometer was equipped with a Peltier-cooled Charge Coupled Device (CCD) detector and a motorized XY stage. Spectra were calibrated using the silicon Raman signal from a silicon wafer at 520.7 cm^{-1} as reference. All spectra were collected in the range 150–1200 cm^{-1} (HeNe), where diagnostic phonon modes are found, with a resolution of $\pm 1 \text{ cm}^{-1}$ and acquisition times of 1–2 minutes. Raman spectra were assigned by comparison with literature data, mineralogical databases, and in-house standards (7).

III. RESULTS

A. Mass Spectrometry

Results showed amounts of TAGs at $\mu\text{g}/\text{mg}$ concentration level in all the examined samples. This finding was unexpected due to the age of the samples. This extraordinary preservation of the lipid fraction can be explained by considering the anoxic and dark environment in which the residues were preserved, that probably inhibited enzymatic and autoxidation processes. The recognition of specific TAGs was carried out by comparison with commercial standard,

whereas identification of the original material was obtained by comparing the detected profiles with the profile obtained by the analysis of fresh and artificially aged reference materials.

Specifically, sample 3 was characterized by high levels of OOO (triolein) and traces of polyunsaturated TAGs that are supposed to be easily depleted during burial and therefore considered rare in archaeological findings. The recorded profile suggested that a vegetable oil rich in polyunsaturated acids was used, i.e. olive, almond, argan, or sunflower. No TAGs referable to animal adipose fats were detected instead.

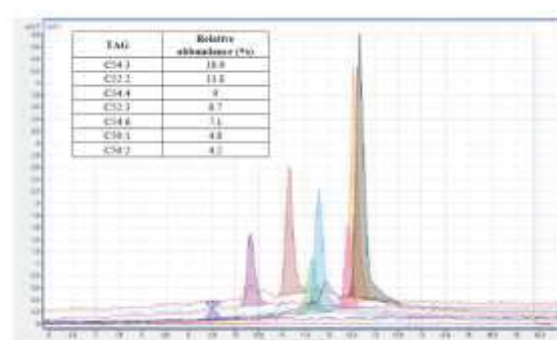


Figure 3. LC-MS chromatogram from sample 3.

Confirmation of the use of vegetable oils was also obtained by structural analysis of the acyl constituents of selected TAGs. In fact, a characteristic feature of plants TAGs is that they do not show palmitic acid in the sn-2 position. For instance, the fragments ratio $[\text{PP}]^+ / [\text{PO}]^+$ recorded from the TAG C50:1 resulted close to the reference value of the sn-POP standard. Similar stereochemical features were noticed in the other TAGs detected in this sample.

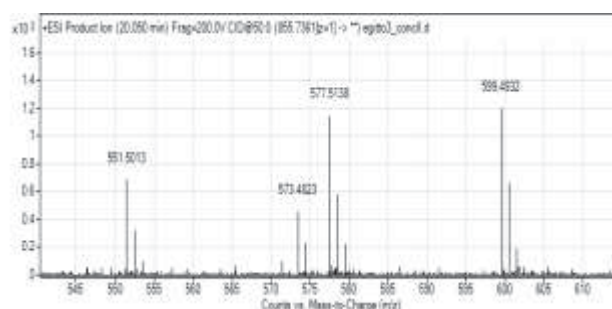


Figure 4. Recognition sn-POP positional isomer in the Egyptian sample number three to the identification of the related $[\text{PO}]^+ / [\text{PP}]^+$ fragment ratio

Also in sample 5, the recorded TAG profile indicated the presence of a vegetable oil. The LC-APCI-MS chromatogram showed in fact a TAG/TIC ratio surprisingly close to the values found in fresh reference olive oil, with high level of OOO. However, also PPP (tripalmitin) and TAGs that are typical of animal fats were detected (POS and SSS were the most abundant). A similar profile was highlighted with LC-ESI-QToF. Overall, the TAGs distribution indicated that a very elaborated formulation including animal fats and vegetable oil was employed. Due to the detection TAGs containing odd chain lengths fatty acids, it was hypothesized. That the detected fat was extracted from a ruminant animal.

Sample 7 displayed high levels of monounsaturated and polyunsaturated TAGs. Therefore, in this case for the preparation of the lipid base of the eye-paint a vegetable oil was used. The presence of tripalmitin and OOO rules out olive oil. Some TAGs typical of animal fats were also detected (POS, POL and POP), along with odd chain length TAGs suggesting the presence of a fatty substance of bovine origin. The presence of animal fat was also confirmed by structural analysis of TAG containing palmitic acid.

In sample 8 high levels of OOO and LLL were detected. Since these TAGs were found also in sample 3 and 8, and were also displaying comparable relative abundance, we inferred that most probably the same vegetable oil in the same relative concentration was used for the formulation of these cosmetics.

Sample 11d displayed high relative abundance of OOO and monounsaturated TAGs together with some saturated TAGs rich in stearic acid that are mostly linkable to a ruminant fat origin (Figure 5).

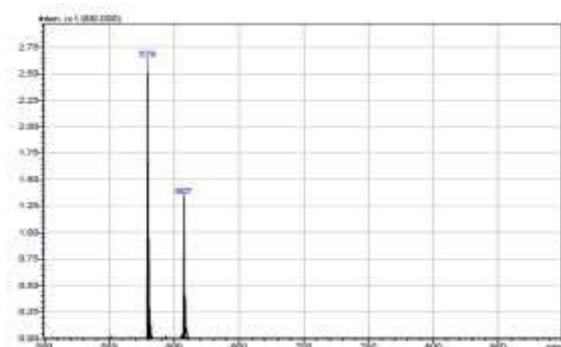


Figure: 5 Detection of PSS fragments HPLC-APCI-MS from the Egyptian sample 11.

Residues 12 was sampled from a sealed container after the opening. Since the related contents must be considered free from external contamination, the sample was used as reference in the interpretation of the profiles collected from the other residues (as an help in the recognition of contaminants TAGs). The

analysis carried out with LC-ESI-qTOF showed that the TAG distribution was compatible with the presence of animal fat since only saturated TAGs (C48:0, C50:0 and C52:0) were recognized. Moreover, we detected APS and PPO that are TAGs specifically found in porcine adipose fats. Odd TAGs that are characteristic of ruminants were not found.

B. Raman spectroscopy

Thanks to μ Raman bulk-mineralogy of sediments were recognized in four samples.

In sample 7B, made of the material encrusting the inner bottom of the related jar, we detected 8 grains, including transparent minerals of detrital origin (5 quartz, 1 calcite, 1 aggregate of organic matter and 2 altered volcanic rock fragments). In sample 8 Bis, a sticky residue from the inner bottom of the jar was analyzed and we observed 9 grains of transparent minerals (Fig.6) of detrital origin (6 quartz, 2 augite, 1 unidentified). In sample 11, all the observed grains consist of aggregates of small particles with very low Raman scattering, not identified.

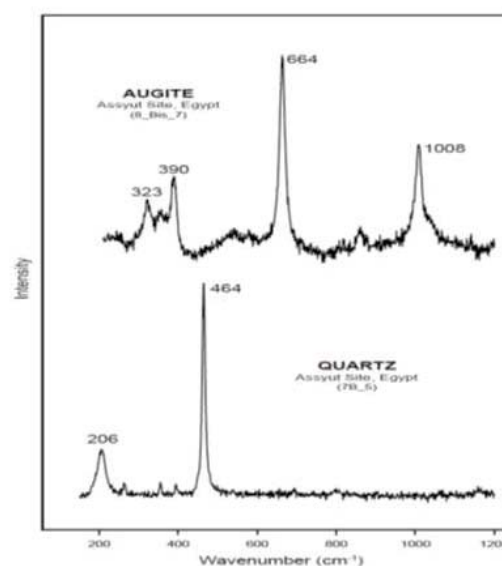


Figure 6 Analysis of sample 8 by Raman

Detritus carried by the Nile River is chiefly derived from Ethiopian volcanic highlands via the Blue Nile, carrying more K-feldspar and hornblende eroded from amphibolite-facies basement rocks, and the Atbara River, which carries more volcanic rock fragments, augite and olivine from volcanic basaltic rocks. An additional amount of almost pure quartz-rich sediment is supplied by the rest of the Nile catchment, representing the stable residue which survived extreme subequatorial weathering in southern Sudan swamps (White Nile and Sobat sands) or fluvial and eolian recycling of ancient quartzarenites (Nubian

sandstones) in hyperarid Saharan climate, (Garzanti et al 2006). Surface textures observed during counting by optical microscope provide valuable information for paleoclimatic or diagenetic interpretation (Andò et al. 2012). Quartz grains appear subrounded and partially corroded, whereas pyroxenes are deeply corroded. Such features are common in modern Nile River sands (Garzanti et al 2006) and are largely related to mechanical abrasion in hyperarid tropical eolian environments as well as to chemical weathering in upstream reaches characterized by humid equatorial to subequatorial climate. In summary, both the spectroscopic and morphological analyses of the preserved minerals confirmed the provenance and source area of the organic residues.

IV. CONCLUSION

The residues surveyed in the presented work were sampled from vessels found in the same archaeological site and belonging to the same class, that archaeologist indicates with no doubts to be related to the ancient Egyptian eye-painting practice. Thus, the possibility to study these findings by a multi analytical approach represented an extraordinary opportunity both to test the potential of the different analytical methods and to collect new information about the knowledge in ancient Egypt of the chemical formulation of cosmetics. Results indicated that mixtures of oil and animal fats were employed to prepare the lipid base of the eye-paintings. Unfortunately, we were not able to detect a specific exotic suite of minerals admixed to the organic base. However, the analysis in micro spectroscopy enabled the recognition of the local sedimentary provenance of the inorganic fraction

Lipid analysis by mass spectrometry techniques indicated that only one sample was formulated by using exclusively vegetable oil, and most probably safflower oil that several authors, on iconographic basis, indicate in use for daily cosmetic practices since the Eleventh Dynasty. In four samples, the presence of saturated TAG and highly unsaturated TAGs indicated that the lipid base was obtained by mixing vegetable oil with an adipose fat with a possible ruminant animal origin. Finally, the distribution found in one of the samples, clearly indicated that porcine fat was used. This information is interesting because there is a divided speculation among archaeologist about pork breeding in the ancient Egyptian society. For instance, Greek

Herodotus reports that “pig was accounted by the Egyptians as an abominable animal”; on the other hand, evidences that it was bred and used since the Predynastic times were also collected. Under this respect, the change of attitude of Egyptian to pork farming should be still accurately investigated and dated, and our study may be accounted in doing that.

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REFERENCES

- [1] S. Lucas, A. Cosmetics, Perfumes and Incense in Ancient Egypt, 1930, JEA 16, pp. 41-44.
- [2] Walter, P.; Welcomme, E.; Hallebot, P.; Nestor, J.; Deeb, Z.; Castaing, J.; Veysièrè, P.; Breniaux, R.; Leveque, J.L.; Tsoucaris, G. Nano Lett. 2006, 6 (10), 2215-2219.
- [3] M.P. Colombini, F. Modugno, M. C. Gamberini, M. Rocchi, C. Baraldi, T. Deviese, R.J. Stacey, M. Orlandi, F. Saliu, C. Riedo, O. Chiantore, G. Sciutto, E. Catelli, L. Brambilla, L. Toniolo, C. Miliani, P. Rocchi, J. Bleton, U. Baumer, P. Dietemann, G. Pojana, S. Marras, “A round robin exercise in archaeometry: analysis of a blind sample reproducing a seventeenth century pharmaceutical ointment” Anal. Bioanal. Chem. vol 401, no.6, October 2011 pp, 1847-1860
- [4] F. Saliu, F. Modugno, M. Orlandi, M.P. Colombini “HPLC-APCI-MS analysis of triacylglycerols (TAGs) in historical pharmaceutical ointments from the eighteenth century” Anal. Bioanal. Chem. vol.401, no.6, October 2011, pp. 1785-800.
- [5] C. Riedo, D. Scalarone, O. Chiantore, O. “Pyrolysis-GC/MS for the identification of macromolecular components in historical recipes” Anal. Bioanal. Chem., 2011, 401, 1761-1769.
- [6] S. Andò, E. Garzanti, M. Padoan, M. Limonta . “Corrosion of heavy minerals during weathering and diagenesis: A catalogue for optical analysis” Sedimentary Geology, ,vol, 280, 2012, pp.165-178