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Identifying the Unknown Content of an Ancient Egyptian Sealed Alabaster Vase from Kha and Merit's Tomb Using Multiple Techniques and Multicomponent Sample Analysis in an Interdisciplinary Applied Chemistry Course

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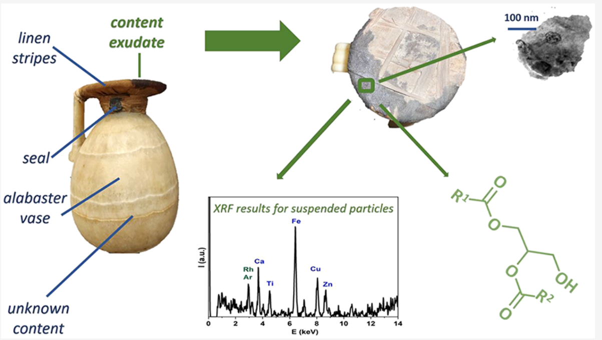

ABSTRACT: This article highlights the multianalytical study of exuded liquid from an ancient Egyptian sealed alabaster vase by Master's students in an applied chemistry for cultural heritage course. Master students are introduced to the field of Archaeometry that see the collaboration of experts in different areas of research such as conservators, curators of museums, physicists, chemists, etc. The sample is a residue exuded on the linen strip sealing an ancient Egyptian alabaster vase (inventory number S.8448) from the collection of the Museo Egizio in Turin (Italy). The students start to plan the noninvasive investigation by X-ray fluorescence (XRF), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS) for the inorganic compounds characterization, followed by the extraction of the organic components (such as oils, fats, and waxes) to be analyzed by high-resolution ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and 2D NMR correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and long-range heteronuclear correlation (HMBC) techniques and by gas chromatography coupled with mass spectrometry (GC/MS). Reference standards, spectral databases, and published data on similar artifacts served as the basis for the interpretation of the instrumental results. The approach was introduced in the course of Applied Chemistry for Cultural Heritage for Master students in Archaeology (University of Palermo, Italy), where the need is to know how to approach the scientific investigation together with the conservation scientists and how to manage with a very low amount of sample. Pedagogically, the approach introduces students to the main techniques currently used in the field of Archaeometry while reinforcing fundamental concepts in sample collecting and multicomponent microsample analysis. This interdisciplinary approach provides a unique experience that demonstrates chemistry's broad applicability outside of the traditional laboratory. Students are guided to identify the inorganic and organic components of the exudate liquid: the first one is ascribable to clay minerals iron oxides, which could impart the brown color to the sample; the second one is ascribed to triglycerides of various kinds, which probably comes from vegetable oil.

KEYWORDS: *Graduate Education/Research, Upper-Division Undergraduate, Analytical Chemistry, Interdisciplinary/Multidisciplinary, Collaborative/Cooperative Learning, Problem Solving/Decision Making, Applications of Chemistry, Instrumental Methods*

■ INTRODUCTION

The investigation of cultural heritage through a scientific approach is a topic of big interest for archeologists and conservators and often allows for gaining crucial information about our past. In the last decades, a huge increase of the methodologies and techniques in chemistry to support the studies of the archeologists was observed.[1−6] Cultural heritage objects can be unique and irreplaceable, and every time, the approach needs to be adapted to the specific case study. The best practices in this field should follow a Standard Operating Procedure (SOP),[7−11] which plan the use of the techniques of analysis as a function of the goal of the investigation. The SOP provides the necessary guidance because a multianalytical noninvasive and microinvasive approach to a work of art implies a number of tasks with a strict order of compilation, which is strictly related the kind of information that is possible to gather

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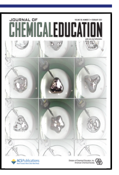

Figure 1. Photographs of the Egyptian alabaster vase from which we obtained the microsample (front view and upper view)—inventory number n. S.8448. Vase dimensions: height 20.5 cm, width 14.3 cm.

from each analysis. When we face a very small amount of sample, it is critical to have a good analysis plan to reach most of the information in the proper sequence to maximize the consumption of the sample itself. The proper sequence is to carry out the noninvasive investigations such as X-ray fluorescence (XRF) as first, followed by destructive methods such as gas chromatography coupled with mass spectrometry (GC/MS). The latter need to be preceded by a sample preparation through solubilization and extraction of the interesting compounds. In this perspective, the discipline of Archaeometry can be considered an ideal platform to introduce the students to chemistry, physics, archeology, and conservation science through the practical applications of the chemical and physical techniques.[12,13] Students can benefit from research-based experiments demonstrating the fundamentals of analysis techniques and their application to cultural heritage. The results of every technique are used to plan the following experiments and reach the final goal of the investigation. Moreover, students are educated to analyze a small amount of sample to obtain as much information as possible.

The first step is introducing the students to the archeological questions and the nature of the studied sample. The skills required for participating students are based on knowledge of instrumental chemistry. The paper introduces the student to the main uses of these techniques in the cultural heritage field while reinforcing the fundamental concepts of microanalysis and nondestructive techniques.

Students were introduced to the archeological context through gathering background information on the grave goods of Kha and Merit and ancient Egyptian culture.[14−21]

On February 15, 1906, in a valley next to the village of Deir el-Medina (Luxor, west bank), the Missione Archeologica Italiana (MAI), guided by the archeologist Ernesto Schiaparelli, discovered the shaft of an underground tomb, which had been sealed by a landslide:[14−21] the intact burial assemblage of two upper-class individuals, 'the director of works' Kha and his wife Merit. The complete grave goods are now preserved at Museo Egizio in Turin (Italy). They include coffins, tunics, beds, pottery and alabaster vases, cosmetics, and prestigious luxury objects inscribed with royal names, which allow us to date his career between the reigns of Amenhotep II, Thutmosis IV, and Amenhotep III (1425−1353 BCE).

Some of these objects have been investigated in the framework of the ARchaeology of the invisible: unveiling the grave goods of KHA (ARKHA) Project[17] through noninvasive chemical and physical techniques, using light and neutron probes (ISIS - UK) to provide detailed morphological reconstructions of the inner parts of a lot of objects together with their phase composition.[17−19] The results of this project are part of the temporary exhibition "*Archeologia invisibile*" at the Museo Egizio in Turin.[20] Among the investigated artifacts and objects, there is a set of seven alabaster vases containing unidentified semiliquid substances, and they are suspected to be or to represent the seven sacred oils used by ancient Egyptians during the embalming procedures.[21] To address the function of the sealed vases, we focused our attention on one alabaster vase (inventory number n. S.8448) whose content is unknown (Figure 1). The vase is closed by a stopper and a seal impression with the name of Thutmosis IV.[14−16] Though stoppered, some of the vase's contents appear to have leaked and collected on the linen strips used to secure the stopper. The students were introduced to the sampling procedures and to the best practice to collect any information from a small amount of sample obtained in an accidental and unexpected way together with the best practice for the application of experimental techniques, data analysis, and integrated interpretation of the results.

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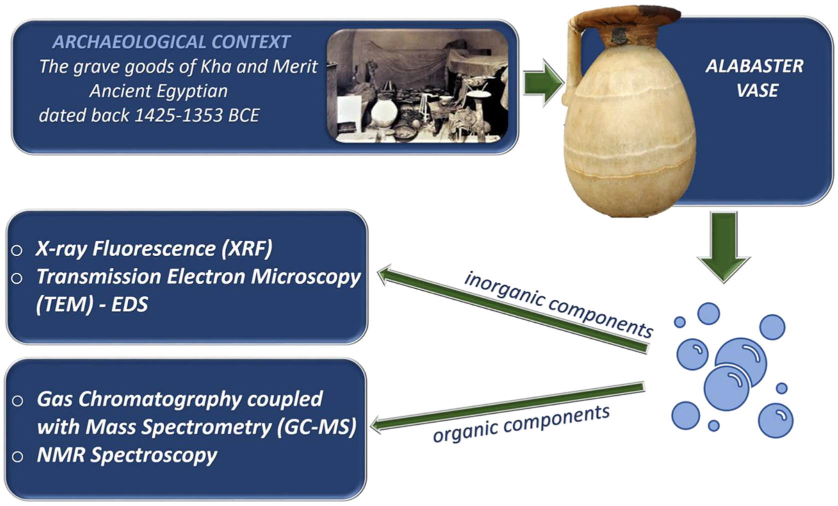

Figure 2. Concept of the integrated approach.

EXPERIMENTAL OVERVIEW

The exuded liquid was collected (a few milligrams) by pressing a polyethylene film onto the linen strips in the stopper region. The substance stuck to the film, and it was covered by a second film to avoid contamination. The sample appeared as a dense and homogeneous liquid/gel of a brownish color and, following the hypothesis of the Egyptologists, could be related to a liquid colored with some pigments or to an oxidized oil.[21] The students should note this observation in their notebook, and it can be introduced into the investigation plan. The analysis of the collected oil began first with XRF, transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS), followed by destructive methods such as GC/MS and nuclear magnetic resonance (NMR) spectroscopy. NMR can be considered a nondestructive method as one simply needs to evaporate the deuterated solvent to recollect the sample due to a low probability of solvent−sample reactions.

XRF and TEM/EDS were used to investigate the inorganic components, while GC/MS and NMR were used for the organic compounds.

The experimental activities are designed to be completed in six lessons of 1 h, with the first one to introduce the case study and the overall approach (Figure 2), four lessons to introduce each technique, and the latest one for the discussion of the results. For each technique, students were introduced to basic concepts into the fundamental theory and then to the problems about the sample collection and its treating (solubilization or extraction procedure) as well as to the spectra interpretation. The approach introduced in the course is based mainly on the applications of techniques in the archeological field; for the students, the fundamental theory of each technique is necessary in order to understand the results and the choice of one analytical technique in a real case.

Details about the used instruments and methods are reported in the Supporting Information.

HAZARD AND SAFETY

Students followed all the standard laboratory safety procedures adopted in a chemistry laboratory such as wearing lab coats, eye goggles, masks, and nitrile gloves when they handled chemicals. Before the experiment, detailed information about what chemicals to use was furnished and they were asked to examine the safety sheets of all chemicals. Moreover, the students followed a preliminary lesson on the sample handling in the archaeometric field with the aim of avoiding contamination due to natural skin oils. During the experiments, the students should wear nitrile gloves, and after any experiment, hands should be accurately washed. At the end of the experiments, all waste materials should be collected in a special waste container following the chemistry laboratory rules. The XRF equipment uses an X-ray source, and students were informed about the radioprotection prescriptions that regulate the use of this instrument.

RESULTS AND DISCUSSION

Inorganic Components

In order to shed new light on the nature of possible inorganic compounds, as particles suspended in the exuded liquid/gel, XRF and TEM/EDX were used. The XRF spectra of the sample, acquired by using a Tracer III SD Bruker AXS instrument directly on the liquid/gel without and with a red filter, are reported in Figure 3. The red filter was used to enhance the heavier elements signals. Data were analyzed by using the software ARTAX 7. An XRF spectrum of the polyethylene film was also acquired in order to exclude signals due to contaminants.

In all XRF spectra, the signals of Ar and Rh are due to the atmosphere and the source of the XRF spectrometer, respectively. The students can be introduced to the identification of the signals, taking into account the interferences due to the parts of the instruments and due to matrix effects. The following elements were identified in the sample: aluminum (Al), silicon (Si), calcium (Ca), titanium (Ti), iron (Fe), copper (Cu), and zinc (Zn). TEM micrographs, acquired at different magnifications by using a JEOL 2010 electron microscope on the liquid/gel dispersed in ethanol, are reported in Figure 4. The EDS spectra were collected in several area of the samples.

Particles of various shapes and sizes were observed in the TEM micrographs. Among the particles of size around 100 nm, some dark cubic particles of 40 nm were observed. The EDS spectra acquired on the particles showed a different elemental composition (Figure 4) of the observed objects. In both spectra, the signal of copper (Cu) in the EDS spectra was related to the grid used as a support. The signals of sulfur (S), oxygen (O), calcium (Ca), sodium (Na), potassium (K), and zinc (Zn) were

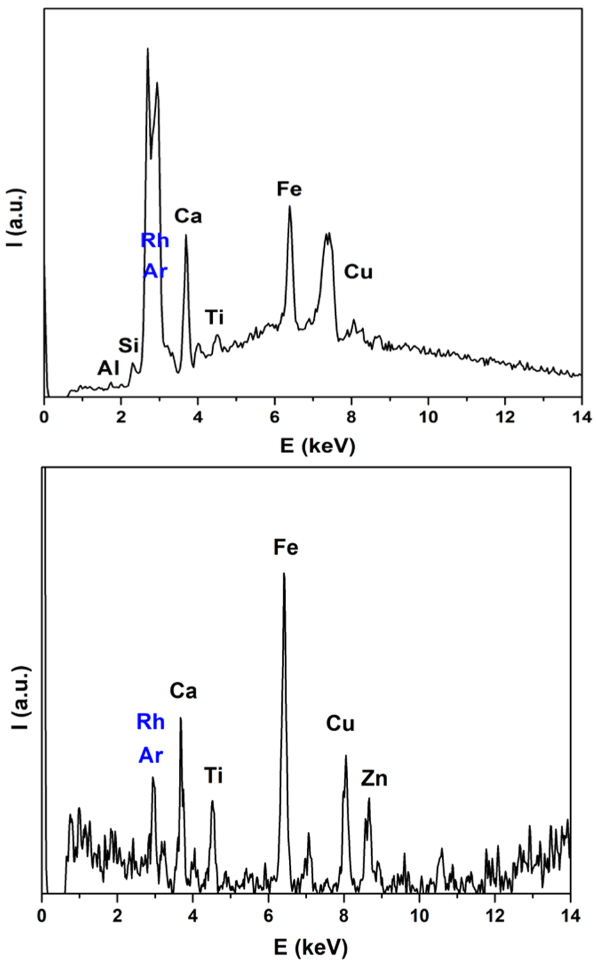

Figure 3. XRF spectra acquired without (above) and with (below) a red filter on the S.8448 sample. Signals of Argon (Ar) and Rhodium (Rh) due to the atmosphere and the X-ray source are reported in blue.

identified in the particles of 100 nm. Signals of silicon (Si), aluminum (Al), and iron (Fe) were identified in the cubic dark particles. The students can be introduced to the data interpretation combining the XRF and the EDS data, taking into account the analogies and differences between the techniques in terms of investigated area, sensitivity, and source difference. Through looking for the data interpretation, some of the elements (S, Ca, Na, K, and Si) can be ascribed to the alabaster vase. The presence of Al and Fe were interpreted as inorganic pigments like iron-red pigments from natural clay minerals[22] or, considering that the vase was exposed to the museum room atmosphere, could be due to possible airborne particulate matter deposited on the vase surface during the sampling. For this reason, even if the hypothesis of the presence of a red pigment is reasonable, the investigation of the other alabasters is in progress [23, unpublished data].

Organic Components

GC/MS and NMR spectroscopy techniques were used to investigate the nature of the organic component (oils, fats, and waxes). In both cases, a procedure of extraction was applied. GC/MS techniques applied to the study of organic residues as biomarkers to identify the residue materials are well-established techniques in archeological science.[24] The GC/MS analysis of the sample S.8448 aimed to identify the characteristic compounds, while the lipid extraction technique was performed to check for the presence of free fatty acids (FFA) related to vegetable oils.[25] FFA contained in dry lipid extracts were derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) to increase their volatility and proceed with their identification and quantification in GC/MS. In particular, we got two extracts that contained fatty acid methyl esters (FAMEs) and trimethylsilyl (TMS) fatty acid esters, respectively. Further details are available in the Supporting Information.

The trans-esterification with methanol leading to the formal methylation of the FA is the most employed, in which TAG reacts with methanol in the presence of an acid or alkaline catalyst to form fatty acid methyl esters (FAMEs). Alkaline catalysts, in this case KOH and its respective methoxide CH_3KO, are preferred due to higher reaction yields associated with low costs and a lower reaction.

Acid catalysis is more time consuming and uses higher reaction temperatures until 100 °C, thus being less effective in terms of total reaction yields. The catalysis with an alkaline catalyst is preferred to avoid subjecting the unknown archeological sample to high temperatures. Atmospheric pressure chemical ionization (APCI) has been shown to be an effective technique for the mass spectrometric analysis of triacylglycerols, since it allows for the identification of constituent fatty acyl moieties and also gives valuable

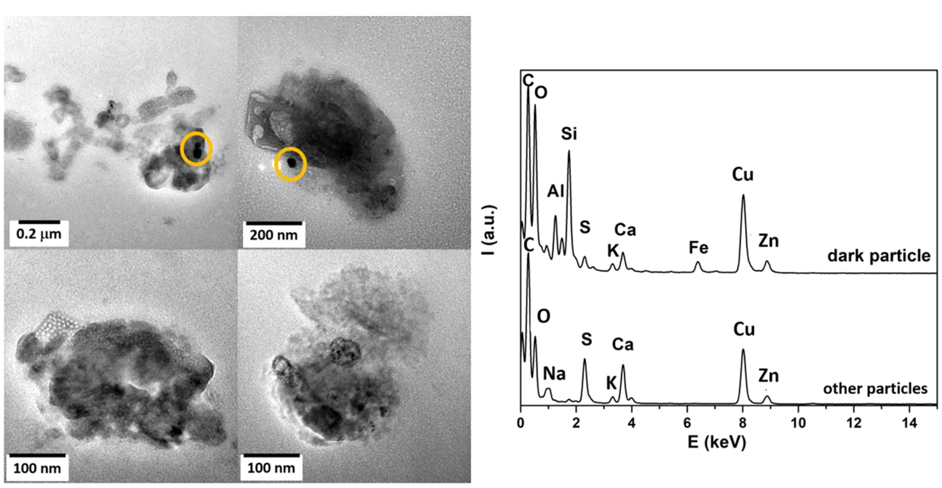

Figure 4. (left) TEM micrographs of the investigated sample. (right) EDS spectra of the dark particles (highlighted in yellow) and of other particles.

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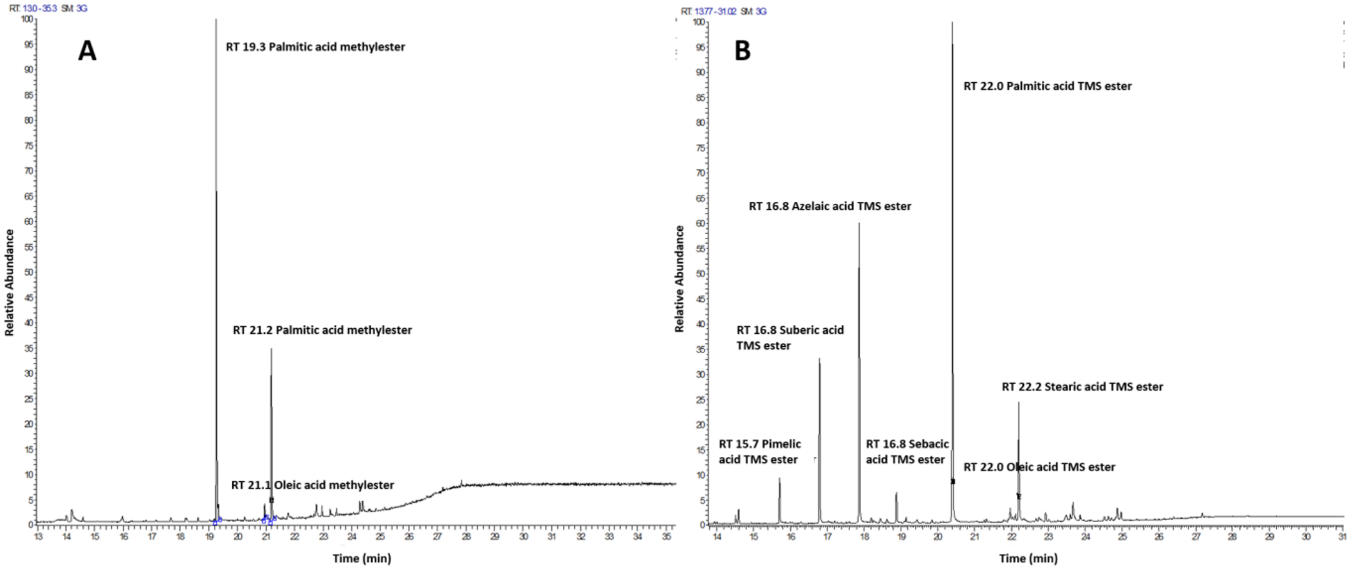

Figure 5. Total ion chromatograms of (A) fatty acid methyl esters (FAMEs) and (B) of trimethylsilyl (TMS) fatty acid extract from the sample S.8448.

Table 1. Chemical Shift Assignment and Main Signals in the ^{1}H NMR Spectrum

Sample	δ, ppm[a]	Functional Groups	Identification	Integrals[b]
1	4.99/4.86	CHCOR	1,2-diglycerides	0.18
2	4.30	CH_2OCOR	1,2-diglycerides	0.04
3	4.18/4.17	CH_2OCOR	1,3-diglycerides	0.17
4	4.06	CHOH	1,3-diglycerides	0.06
5	3.70	CHOH	1-monoglyceride	0.04
6	3.63	CH_2OH	1,2-diglycerides	0.03
7	3.59	CH_2OH	1-monoglycerides	0.07
8	2.35	CH_2COOR	Esterified acids and diacids	0.59
9	2.33	CH_2COOH	Not esterified acids and diacids	0.63
10	1.64/1.62/1.5	CH_2CH_2COO-	All acids and diacids	2.08
11	1.26	$(CH_2)_x$	All acids and diacids	5.78
12	0.88	CH_3	All acids	1.00

[a]Signal identification was performed by acquiring bidimensional spectra (see Figure 6 and Figures S2 and S3 in the Supporting Information) and using the literature values. See ref 28. [b]The reported integrals were normalized with respect to the signal at 0.88 ppm.

information on the position at which these fatty acids are esterified to the glycerol backbone of the TAG.

GC/MS analysis allowed for the identification of several fatty acids, among other compounds. The identification of marker compounds was achieved by a comparison of retention times (RT) and mass spectra against authentic standards as well as a comparison against the NIST11 mass spectral database.[26] Typical chromatograms of fatty acid methyl esters (FAMEs) and trimethylsilyl (TMS) fatty acid esters extracts are reported in Figure 5.

Tables S1 and S2 reported in the Supporting Information show the retention times of the identified components, together with their percentage relative areas. In order of abundance, the main constituents of the FAME extract were palmitic, stearic, and oleic acids. Several dicarboxylic acids were also found in the TMS extract. In order of abundance, the main constituents were azelaic, suberic, pimelic, and sebacic acids. The students can learn the reason for the differences found between the two derivatized extracts. It is due to a strong silylation that allowed them to derivatize also carboxylic groups of dicarboxylic acids.

In general, the presence of fatty acids might indicate an oil component in the sample. In this case, the percentage of oleic acid is low compared to those of palmitic and stearic acid. On the contrary, the presence of fatty acids having short chains can be due to the degradation of medium- and long-chain acids, even if the shorter chain acids are much more water-soluble and volatile than the medium- and long-chain fatty acids. The dicarboxylic acids are an indication of the oxidation process of an oil. However, the identification of aged oils is still not fully clear because the ratio between the components can be influenced not only by the aging time but also by the presence of inorganic compounds like pigments.[27] The presence of these fatty acids together with other acids such as lauric, palmitoleic, linoleic, behenic, and lignoceric shows the presence of fat, but it does not allow us to ensure the animal or vegetable origin. Stigmastanol, present in significant amounts in the analyzed sherds, indicates the presence of vegetable fat. This compound originates from degradation processes that occurred over the years, after the reduction of the β-sitosterol, which is the precursor and the typical marker of vegetable oils. Dehydroabietic acid and its oxidation products are typical components of oxidized resin that exudes from plants of the Pinaceae family. Further confirmation was derived from the identification of heating markers such retene and 8-isopropyl-1,3-dimethyl phenantrene. The presence of the stigmastanol together with that of oleic acid, in all samples, suggests that vegetable fat residues are present in the sample.

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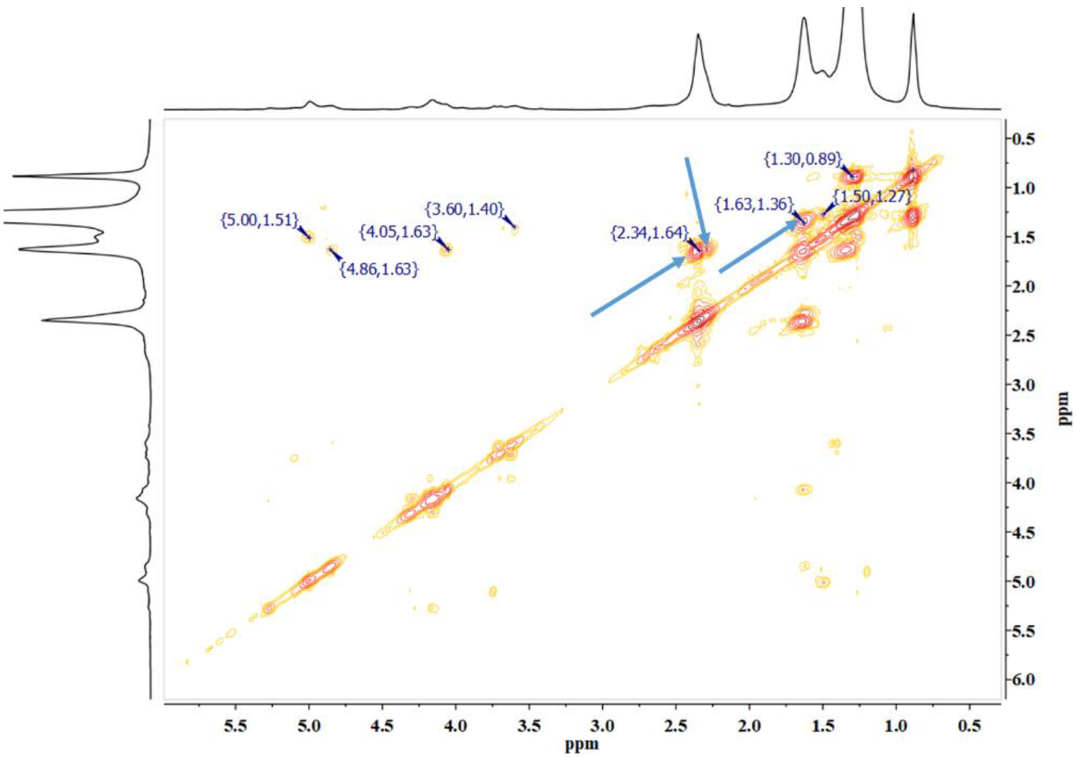

Figure 6. COSY spectrum of sample S.8448 together with the cross-peaks indication. Signals due to diacids are evidenced by the blue arrows.

Only the high level of stearic acid may suggest the presence of animal fats. However, the absence of cholesterol and other animal sterols indicates that it can be a contamination rather than an indication of the amphora content.[25]

This kind of consideration can give the student the possibility to deepen the techniques or the different results already published in the literature in order to know the limits of the techniques.

At the end of the learning process, the same substance, extracted by sonication using deuterated chloroform, was analyzed by high-resolution ^{1}H and ^{13}C NMR spectra and 2D NMR correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and long-range heteronuclear correlation (HMBC) techniques. A Bruker Avance II 400 spectrometer operating at 400.15 and 100.63 MHz for ^{1}H and ^{13}C, respectively, was used. The ^{1}H NMR spectrum of the S.8448 sample is reported in Figure S1 of the Supporting Information. The chemical shift assignments were performed according to the literature.[28] The chemical shift assignments and the peak integrals are reported in the Table 1.

The signal identification was performed by acquiring the bidimensional spectra (see Figure 6 and Figures S2 and S3 of the Supporting Information) by using the references in the literature.[28]

According to the GC/MS data, the sample consists of esterified fatty acids and diacids. The high value of the integral of the signal at 1.26 ppm also indicates that these fatty acids are mostly long chain (up to C16). The presence of diacids proves that the sample is oxidized, as expected, considering the age of the sample. No resonances at 5.3, 5.2, and 2.0 ppm related to fatty acid unsaturation were identified, indicating that a cross-linking due to aging occurred. The presence of these compounds shows the presence of fat but does not allow us to attribute an animal or vegetable origin. However, the absence of characteristic signals related to compounds present in animal fat (i.e., cholesterol) is an indication that the nature of the organic component in the sample could be due to a vegetable substance. Due to the oxidation of the oil, nothing can be claimed about the specific nature of the vegetable oil. A more precise consideration of the obtained data could be done considering the ratio between the components of the oil, as reported in the literature.[29,30] However, considering the very small amount of analyzed sample, that another sampling cannot be collected because the alabaster vase is closed, and the degradation of the sample due to its age, we believe that every other consideration could be affected by an error. The student is guided to all this consideration, taking into account the small quantity of samples and the limits of each technique.

■ SUMMARY AND CONCLUSIONS

This case study was introduced to Master students, showing the information on the best practices in the field of Archaeometry. Together with the methodological problems related to the research, this case study allowed students to understand how to reach the objective of the investigation when the amount of sample to be analyzed is very small. Due to the possible lack of analogous real sample, a replica could be used for teaching purposes. The replica could be prepared by mixing a vegetable oil with a pigment (ochre) and previously aging it in a climate chamber. The approach could be considered similar to the ones used in forensic chemistry.[31−34] However, its use has been extended in the field of Cultural Heritage give to students in Archaeology, Conservation Science, or Chemistry the possibility to know how a scientist could operate, even comparing with other approaches.[35−37] In fact, in this field, there is a different problematic need to choose the specific and proper approach for the problem solving.

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The case study includes an introduction to four analytical techniques, with practical experience in the acquisition of XRF spectra and on preparing the samples for GC/MS, NMR, and TEM. Since the acquisition of data, in these cases, is performed by a specialized technician, the students can only assist and discuss the data. Pedagogically, the case study introduces students to some of the techniques currently used in Archaeometry, reinforcing fundamental concepts in the methodology (best practices). The approach was already successfully used in the course of Chemistry applied to Cultural heritage for the Master degree in Archeology in 2018 (number of students was 15).

A similar approach with a high level of details can be used for Master students in Chemistry.

First, the content of a sealed alabaster vase (inventory number S.8448), dating back to the second half of 14th century BCE, was identified without damage to the unique object through integrated analytical techniques to collect clues about its use. The vase is part of the grave goods of Kha and his wife Merit, the richest and most complete nonroyal burial assemblage ever discovered in ancient Egypt, now preserved at the Museo Egizio in Turin (Italy). The multianalytical characterization of small milligrams of the sample was performed to give indications about the vase content. The inorganic components are ascribable to clay minerals iron oxides in the content of the vase, which explains the brown color of the sample. The organic component is ascribable to triglycerides of various kinds, which probably comes from vegetable oil. This information opens the way to the hypothesis that the seven sealed alabaster vases preserved with care in a wooden box could be the famous seven secret oils used in the embalming procedures in Ancient Egypt.

Upon completion of the cycle of six lessons, the students compile a general report on the case study that includes an introduction that clearly defines the archeological question to be answered and the archeological context, the used methodology, and some personal comments about the advantage and disadvantage of the use of a multianalytical approach. Answers to a series of guided questions on the used techniques are also included.

By the end of the lessons, students will be able to know

- how to approach the scientific investigation together with operators of different disciplines
- how to analyze a small amount of sample to obtain as much information as possible
- how to interpret the data in the light of different techniques and experimental approaches

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.0c00386.

Discussions about instruments, methods and methodology used for GC/MS, tables of retention times and percentages relative areas and chemical shift and main signals in the ^{1}H NMR spectrum, and figures of ^{1}H NMR, HSQC, and HMBC spectra (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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