Direct Analysis in Real Time Mass Spectrometry for the Nondestructive Investigation of Conservation Treatments of Cultural Heritage

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The Dead Sea Scrolls (DSS) are a collection of about 1000 manuscripts; few are relatively well preserved, and the majority comprise thousands of fragments. These fragile manuscripts, which include the oldest existing copies of the Hebrew Bible, were preserved for two thousand years by the hot, dry desert climate and the darkness of the Judean Desert caves where they were hidden.

In the first years after they were discovered there was no awareness of their conservation needs: irreversible damage was caused by using adhesive tape for joining fragments; castor oil was lavishly spread on the fragments to enhance their reading; glycerol, BMLD (British Museum Leather Dressing, which is anhydrous lanolin, beeswax, and cedar
Figure 1: Color images of some Dead Sea Scrolls fragments: a well-preserved fragment of 4Q Deuteronomy (a), two fragments with darkening problems due to delamination—11Q Paleo Leviticus (b), and gelatinization—4Q Pesher Psalms B (c).
showing similar or opposite behavior, and the loadings (the weights of the original variables on each PC), giving insight on the reasons of the differences pointed out in the objects. PCA has already been applied to separate systematic information from experimental noise and to describe the conservation treatments in a compact and efficient way by using the more significant PCs [18].

LDA is a Bayesian classification method providing the classification of the objects considering the multivariate structure of the data [19]. In Bayesian methods each class is usually described by a Gaussian multivariate probability distribution and each object is assigned to a particular class if the so-called discriminant score is minimum; it is calculated using the more significant PCs [18].

The classification performance of the LDA models can be evaluated by the calculation of the nonerror rate (NER%) that represents the percentage of overall correct assignments. The prediction performance of the models can be evaluated by cross-validation techniques. The variables to be included in the LDA model can be chosen by a stepwise algorithm that selects the most discriminating variables. The procedure adopted here exploits a forward search (FS) algorithm: at each iteration a variable is added to the model according to the highest NER% in cross-validation. In this study, due to the large number of variables present, FS-LDA was applied to principal components rather than to the original variables: PCA was therefore used as dimensional reduction tool providing a set of descriptors (PCs) summing up systematic information in a restricted set of PCs.

The validation of the LDA model was performed as follows:

1. The samples were divided into training set and test set, including in the test set the 20% of the samples of each class.
2. PCA was applied to the samples belonging to the training set and samples from the test set were reprojected in the space given by the PCs calculated.
3. FS-LDA was applied to the training set selecting at each iteration the PC to be included in the model according to the highest increase in the NER% calculated in cross-validation. Cross-validation was applied by bootstrapping with 1000 iterations excluding from the training set the 20% of the samples.
4. The test set was used to evaluate the prediction performance of the LDA model.

Steps from (1) to (4) were repeated 100 times selecting each time a different test set by random sampling. Here, we present the average results obtained on the 100 random samplings performed; the final model presented represents the overall best solution.

3. Experimental

3.1. DART-MS. The analyses were conducted with a JEOL AccuTOF mass spectrometer (JEOL USA, Peabody, MA) equipped with a DART ion source (Ionsense, Saugus, MA) in positive ion mode, with helium being the ionization gas under the following conditions: flow rate of 2.5 L/min, gas temperature at 23°C, 45°C, and 90°C, and grid voltage of +350 V. Orifice 1 was held at 120°C and 30 V, orifice 2 was at 5 V, and the ring lens voltage was set to 5 V. The peaks voltage was held at 1500 V and the instrument was calibrated with a solution of PEG 600 in methanol. The mass spectrum recording interval was 1.00 s and the m/z values were from 50 Da to 800 Da. The mass resolution was approximately 6000. The ceramic outlet of the ion source was positioned 15 mm away from the pinhole orifice that leaks into the mass analyzer. All equipment in contact with the parchment samples was cleaned using rinses of purified water and acetone.

3.2. Parchment Samples. Parchment is a semitanned skin used as a writing surface. Of all the components that constitute the living skin, only insoluble proteins and water remain when this is transformed into a parchment.

Vegetable adhesives like flour paste, synthetic polymers (polyvinyl acetate (PVA) solutions), and acrylic resin solutions were widely used in the past in order to support the parchments. Sometimes parchments were lubricated with organic materials like glycerol, petroleum jelly, lanolin, oils, or other very different substances in a misguided attempt to “condition” the parchments and to enhance their readability. The use of lubricants caused alteration of the surface texture, increase of transparency, attraction of surface dirt, and darkening the skin. The use of unknown organic solvents caused damage to the collagen structure.

Glycerol, which is a simple polyol compound, and castor oil, a vegetable oil obtained from the castor bean, were often used in the past for the lubrication of horny parchments.

For this research we used a goat parchment made by an Orthodox Rabbi from Jerusalem in 2009. The parchment was prepared in compliance with rabbinical rules. Castor oil and glycerol parchment treatments were investigated in order to evaluate two different intervention processes that are suspected to have been applied to the Dead Sea Scrolls in the past and to be the cause of some of their evident deteriorations. Two separate parchment samples were spread with commercial castor oil (CVS, Woonsocket, Rhode Island, USA) and glycerol (Sigma-Aldrich, St. Louis, Missouri, United States). The samples were left for 48 hours at room temperature and were then analyzed by DART-MS.

3.3. Software. The statistical analysis, the spectral alignment, and the graphical representations were performed by The Unscrambler X (CAMO PROCESS, AS, Oslo, Norway), home-made routines built in MATLAB R2014a environment.
4. Results and Discussion

The aim of the research was the development of a nondestructive methodology for the analysis of unknown interventions of parchment by using DART-MS.

The temperature of helium significantly impacts the information obtained by DART-MS: the substances detected by the instrument strongly depend on the temperature of the carrier gas in the ion source.

Parchment is an organic material mainly made of collagen and water: temperature is one of its worst enemies. We performed the analysis using the flux of helium at room temperature in order to avoid causing any damage to the parchment surface.

We analyzed an untreated parchment sample, a parchment sample treated with castor oil, a parchment sample treated with glycerol, and castor oil and glycerol standards as shown by the total ion chromatogram (TIC) (Figure 2).

Although the carrier gas in the ion source temperature was set at 23°C, low-mass products (≈80 to 400 μ), generated by the impact of the carrier gas on the surface of the samples, from untreated and treated parchment and from castor oil and glycerol, are clearly identifiable. Figures 3(a), 3(b), 3(c), 3(d), and 3(e) show, respectively, the mass spectrum of untreated parchment, parchment treated with castor oil, and parchment treated with glycerol, castor oil standard, and glycerol standard.

The m/z of fragments of untreated parchment and parchment treated with castor oil and castor oil are not clearly assignable because of the complexity of the matrix and of the low working temperature. Moreover the three mass spectra are very similar to each other because both parchment and castor oil contain lipid groups.

The mass spectra of parchment treated with glycerol and of standard glycerol (Figures 3(c) and 3(e)) show that m/z 93.049 (M+H+) and 185.063 (M+M+H+) are major signals and correspond, respectively, to protonated glycerol and protonated glycerol clusters: the dimeric signal is probably due to the higher analytes concentration. The m/z 110 corresponds to the glycerol water adduct (M+H2O). The glycerol treatment on parchment is recognizable by looking at the mass spectrum: the characteristic m/z 93, 110, and 185 of glycerol are clearly present. While looking at the mass spectra of parchment treated with castor oil, it is not possible to identify the treatment because parchment and castor oil have a similar mass spectrum profile. This is due to the low temperature of the ion source (T = 23°C) employed for the analysis: the ionization and the formation of product ions are more difficult for castor oil and parchment. Several studies showed that the heater temperature affects the fragmentation and the signal of the analytes. In order to identify unknown parchment treatment without interpreting mass spectrum, especially when the interpretation is not easy to perform because of the complexity of the spectra, we performed a multivariate statistical analysis on the data collected by DART-MS.

4.1. Statistical Analysis and Variable Selection

4.1.1. Principal Component Analysis on Overall Dataset. The mass spectra of the different samples matched each other and the results were arranged in a 106 × 6346 matrix: 106 being the number of samples (19 parchment samples, 28 parchment samples treated with castor oil, 21 parchment samples treated with glycerol, 16 samples of castor oil, and 22 samples of glycerol) and 6346 being the m/z intensity count for each sample. Samples were first-rowwise range scaled between 0 and 1 to eliminate possible effects due to differences in the amount of sample used to record each spectrum. Then the dataset was columnwise range scaled between 0 and 1 before performing PCA. The amount of explained variance is distributed along several PCs showing a relatively low correlated data structure (PC1 = 6.9%, PC2 = 5.9%, PC3 = 3.5%, PC4 = 2.4%, and PC5 = 1.7%). The low correlation is explained by significant sample heterogeneity. In the score plot of PC1 versus PC2 versus PC3 (Figure 4(a)) the samples are well separated along the three PCs in five
Figure 3: (a) Mass spectrum of untreated parchment, (b) mass spectrum of parchment treated with castor oil, (c) mass spectrum of parchment treated with glycerol with the signal at m/z 93.049 (protonated: M+H+) and 185.063 (dimeric ions: M+M+H+), (d) mass spectrum of castor oil standard, and (e) mass spectrum of glycerol standard.
clusters that represent the untreated parchment sample (red),
the parchment sample treated with castor oil (blue), the
parchment sample treated with glycerol (green), the castor oil
standard (yellow), and the glycerol standard (pink).

The first component explains the differences between
untreated parchment (red) and the treated samples (blue,
green, yellow, and pink); the second component explains the
differences between the parchment samples (red, blue, and
green) and the standard samples (yellow, pink) as is shown in
Figure 4(b).

4.1.2. Principal Component Analysis on Parchment Samples.
Since the main target was the identification of unknown
parchment treatments, the analysis was restricted to parch-
ment samples in order to find the most significant PCs useful
to discriminate the conservation treatments.

PCA was then applied to the restricted set of data: the
spectra were arranged in a \(68 \times 3987\) matrix: 68 being the
number of samples (19 parchment samples, 28 parchment
samples treated with castor oil, and 21 parchment samples
with glycerol) and 3987 being the \(m/z\) intensity count
for each sample. PCA was carried out after rowwise range
scaling between 0 and 1 followed by columnwise range scaling
between 0 and 1. The amount of explained variance is quite
distributed along several PCs (PC1 = 15.99%, PC2 = 6.07, PC3
= 3.50%, PC4 = 3.38%, and PC5 = 3.27%). In the score plot of
PC1 versus PC2 versus PC3 (Figure 5(a)) the samples are well
separated along the three PCs in three clusters that represent
the untreated parchment sample (red), the parchment sample
with castor oil (blue), and the parchment sample
with glycerol (green). The first component explains the
differences between untreated parchment (red) and the
conservation treatment (blue and green) and the second
component explains the differences between the two different
conservation treatments: castor oil (blue) and glycerol (green)
as is shown in Figure 5(b).

PCA allows reducing the variable dimensionality: the
latest PCs, mainly accounting for noise and experimental
error, are not considered further in this study. The score plot
shows that the parchment samples treated with glycerol are
characterized by a small dispersion; untreated parchment and
parchment treated with castor oil are characterized by a quite
large dispersion; this can be explained by the complexity of
the analyzed matrix of the samples that is difficult to ionize
using the helium stream at room temperature.

Regarding the separation between classes, the score plot
shows that the samples are quite well separated: this is
particularly true for the samples treated with glycerol.

Instead, some untreated parchment samples overlapped
with samples treated with castor oil and vice versa: this
can be explained by the low temperature of analysis that limits
the ionization products of the two samples.

4.1.3. Linear Discriminant Analysis. The identification of pos-
sible markers, which are able to discriminate the conservation
treatment, was then achieved by linear discriminant analysis
applied to the first 20 PCs calculated: the use of the PCs
instead of the original variables allows the dimensionality
reduction and the elimination of the experimental noise. The
data were divided into training and test sets: the test set
included the 20% of the samples of each class (14 samples: 4
samples from untreated and glycerol treated parchment and 6
samples from castor oil treated parchment). PCA was applied
to the samples belonging to the training set and samples from
the test set were reprojected in the space given by the first
20 PCs calculated. FS-LDA was then applied selecting the
significant PCs by cross-validation (bootstrapping with 1000
iterations). The overall procedure was repeated 100 times and

Figure 4: Score plot of PC1, PC2, and PC3 (a). The samples are separated along the three PCs in five clusters: untreated parchment sample
(red), the parchment sample treated with castor oil (blue), the parchment sample treated with glycerol (green), the castor oil standard (yellow),
and the glycerol standard (pink). (b) represents the score plot of PC1 and PC2.
Figure 5: Score plot of PC1, PC2, and PC3 (a). The samples are separated along the three PCs in three clusters: untreated parchment sample (red), the parchment sample treated with castor oil (blue) and the parchment sample treated with glycerol (green). (b) represents the score plot of PC1 and PC2.

Table 1: Results of LDA on the training and test sets and in cross-validation calculated on the 100 test sets.

| Number of PCs included | NER% training set | NER% cross-validation | NER% test set |
|------------------------|-------------------|-----------------------|--------------|
|                        | Min   | Max   | Min   | Max   | Min   | Max   |
| 2                      | 96.30 | 100   | 93.48 | 99.52 | 85.71 | 100   |

the samples in each different test set were randomly selected from the overall dataset, as described in the theory section.

Table 1 reports the results obtained for the 100 test sets used: the models contained from a minimum of 2 PCs to a maximum of 8 PCs. All the models showed very good NER% ranges both on the training set (from 96.30% to 100%), in cross-validation (from 93.48 to 99.52%), or on the test set (from 85.71% to 100%).

Figure 6 represents the results obtained for the 100 models calculated representing on the x-axis the NER% on the training set, on the y-axis the NER% in cross-validation, and on the z-axis the NER% on the test set. The figure also reports the best theoretical model corresponding to a NER equal to 100% for all the three sets of samples (indicated in red in the figure); the overall best calculated model was selected as the closest to the best theoretical one. Figure 6 shows that the 100 models calculated show very good results, providing good performances not only on the training set but also in prediction, proving the robustness of the results obtained.

The overall best model provided very good results in calibration and prediction: all samples from the training and test sets were correctly assigned (NER and specificity of 100% for all classes) and no overlap was detected (selectivity of 100% for all classes). The results are slightly worse in cross-validation, with a NER% equal to 98.68%. The model includes 6 PCs: PC1, PC2, PC9, PC10, PC12, and PC15. Table 2 reports the coefficients of each PC included in the LDA classification model on the first two canonical roots calculated by canonical analysis. This result is graphically represented in Figure 7(a), reporting the samples along the two discriminant roots calculated by canonical analysis: circles correspond to samples belonging to the training set and crosses represent samples from the test set, while color indicates the class. The first root separates glycerol treated samples (at negative values) from the other two classes (at positive values), while the
second root separates untreated samples (at positive values) from castor oil treated samples (at negative values). The figure clearly shows the perfect classification of the samples from both training and test sets, since the three groups of samples are well separated.

Since the PCs are linear combinations of the original variables, it is possible to calculate the weight of each original variable (m/z signal) on the final model and to identify the variables characterized by the most significant contribution (i.e., markers). The results are reported in

Figures 7(b) and 7(c) separately for the two canonical roots identified: the original variables are represented on the x-axis and the weight on the corresponding root is reported instead on the y-axis; only the variables characterized by a significant weight were represented (the significant weights being identified by normal probability plots [17]). Negative weights on root 1 correspond to signals characterized by a large intensity in glycerol treated samples and a small signal in the other two classes, while positive weights correspond to signals with an opposite behavior. Positive weights on root 2 correspond instead to signals more intense in untreated samples (and in a minor way in glycerol treated samples) and characterized by a small intensity in castor oil treated samples, while negative weights show an opposite behavior. The complete list of the calculated coefficients is reported in Supplementary Information 1 of the Supplementary Material available online at http://dx.doi.org/10.1155/2016/6853591.

4.1.4. Effect of the Working Temperature. The effect of the ion source temperature on the mass spectra has been investigated by analyzing the treated parchment samples and the natural parchment samples (untreated) using several source (carrier gas) temperatures: 23°C, 45°C, and 90°C.
The mass spectra of the different samples matched each other and the results were arranged in a $232 \times 5323$ matrix: 232 being the number of samples (69 samples analyzed at $23^\circ C$, 78 samples analyzed at $45^\circ C$, and 85 samples analyzed at $90^\circ C$) and 5323 being the $m/z$ intensity count for each sample. PCA was carried out after range scaling between −1 and 1. The amount of explained variance is distributed along several PCs showing a relatively low correlated data structure (PC1 = 10.1%, PC2 = 7.4%, PC3 = 6.5%, PC4 = 3.5%, and PC5 = 1.7%).

For each working temperature it is possible to identify groups corresponding to the different types of treatment: natural parchment (star), parchment treated with glycerol (circle), and parchment treated with castor oil (diamond). The position of the clusters changes with temperature, with the exception of the samples treated with glycerol that remains approximately in the same position at all temperatures.

At lower working temperature (Figures 8(a) and 8(b)) the groups of samples are separated but are closer to each other.
and more homogeneous within the same group: increasing the temperature causes an increase of the distance between the 3 groups of samples in the PC reference system and a spread of the samples within each group. In particular the scores of natural parchment samples (star) and parchment samples treated with castor oil (diamond) move towards larger values.

In the score plot of PC1 versus PC3 (Figure 8(c)) the first principal component explains mainly the effect of the temperature at 90°C: at high scores there are the natural parchment samples (Figure 8(c), star) and the parchment samples treated with castor oil (Figure 8(c), diamond) analyzed at 90°C. Parchment samples treated with glycerol (circle) at 23°C, 45°C, and 90°C are characterized by a smaller dispersion: the mass spectra of these samples do not significantly change working at different temperature, probably since glycerol is volatile enough at room temperature to obtain good mass spectra also in the least extreme conditions.

PC3 separates the samples into three clusters: untreated parchment samples (star), parchment samples treated with castor oil (diamond), and parchment samples treated with glycerol (circle), all analyzed at 90°C (Figure 8(c)); all the parchment samples treated with glycerol (circle) analyzed at 23°C (Figure 8(a)), 45°C (Figure 8(b)), and 90°C (Figure 8(c)) are grouped together and have a low dispersion natural parchment samples (star) and parchment samples treated with castor oil (diamond) at 23°C (Figure 8(a)) and at 45°C (Figure 8(b)) have a similar pattern and are not well separated: increasing the temperature causes an increase of the distance between the 3 groups of samples in the PC reference system.

The third principal component mainly explains the differences between the natural parchment samples (Figure 8(c), star) and parchment samples treated with castor oil (Figure 8(c), diamond) at 90°C.

Increasing the carrier temperature the identification of different parchment treatment is easier because the gas ionizes also the less volatile molecules. Unfortunately this condition is not feasible for the analysis of the original Dead Sea scrolls, as using high temperature makes the analysis destructive.

5. Conclusions

In this study we developed a new noninvasive method for the identification of unknown conservation treatments of cultural heritage, in particular for parchment by using DART-MS and statistics.

Many traditional interventions can result in irreversible alteration of an artifact; because of its animal origin, parchment might respond to treatments in unpredictable ways. The conservator’s approach to the treatment of parchment must be extremely cautious and, due to the simplicity and no sample preparation requirement, the proposed analytical tool could help in the challenging analysis of unknown treatments in cultural heritage.

Castor oil and glycerol parchment treatments were investigated using the DART carrier gas in the ion source at room temperature in order not to cause any damage to parchment samples: this is very important while working with any cultural heritage and even more with the Dead Sea Scrolls which are extremely fragile and precious.

The method was able to identify both treatments: FS-LDA performed on principal components revealed to be a robust tool that could be employed for the classification of unknown treatments, overall in presence of samples with similar mass spectrum profiles.

While this study looked at a small number of conservation treatments, there is additional work that could be completed. A DART-MS library of treatments may be created and extended including also the degradation products that may appear after the ageing of the object. The technique may also be applied other cultural heritage objects, such as paintings and other artifacts. Moreover, the investigation of the working temperature of the ion source could help in the identification of unknown substances because at higher temperature the ionization of molecules is easier, even if the analysis could become destructive.

Several studies published the possibility of using different geometry/configuration of the DART [21]: the 45° configuration can be used to analyze or scan surfaces. This configuration allows the analysis of bigger samples like parchment fragments and manuscripts of the Dead Sea Sea Scrolls: our study confirmed that the method can be employed in a non-invasive manner and the next step will be the analysis of the scrolls using this configuration.

In conclusion, using chemometrics, we are able to identify unknown parchment treatment without using high temperature, preserving the integrity and the state of conservation of the samples.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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