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Unlocking the Information Potential of Lake Pigments through SERS and Chemometrics

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***Abstract* – Natural product residues found in objects of historical or artistic significance present a highly complex chemical matrix. With ongoing advances in analytical and instrumental techniques, the strategies for identifying these residues are evolving. The traditional marker-based approach, which focuses on specific target molecules, is increasingly being replaced by more holistic methods that consider the full chemical profile. In this context, multivariate data analysis plays a crucial role in extracting meaningful information from such complex datasets. This study employs untargeted Surface-Enhanced Raman Scattering Spectroscopy (SERS) to explore variables that distinguish dyes extracted from various lake pigments, prepared using distinct recipes and subjected to different extraction methods. The use of ANOVA Simultaneous Component Analysis (ASCA) enabled the assessment of how various factors influence the molecular pattern observed.**

I. INTRODUCTION

Research in the field of cultural heritage, whether driven by historical or conservation inquiries, frequently involves the investigation of materials characterized by a high degree of chemical and structural complexity. This complexity stems not only from the diverse range of substances used, but also from the ageing and degradation processes these objects have undergone over time. Environmental exposure, chemical transformations, and physical stressors all contribute to alterations in the original materials, making their analytical characterization particularly challenging.

Among the various materials encountered in such studies, natural dyes represent a distinct challenge due to their complex composition and susceptibility to degradation [1]. Derived from biological sources, these dyes typically consist of a heterogeneous mix of hundreds of molecules, often present in low concentrations and in a chemically altered state. Consequently, efforts to obtain detailed information, such as the dye origin, its preparation method, or its use in specific artistic contexts, require the ability to detect and identify trace analytes within highly complex matrices.

The traditional approach relies on the identification of specific markers, molecular species that are unequivocally associated with a particular source. However, this single-compound strategy is prone to interpretative errors, such as false positives due to contamination or false negatives resulting from issues in sample preparation. With recent advancements in multivariate analysis of large and complex datasets, a more holistic approach has become feasible. By analyzing the complete molecular pattern, this approach allows for the extraction of valuable information from complex data, revealing insights that were once deemed inaccessible [2].

To meet these analytical challenges, advanced techniques capable of delivering detailed molecular-level insights in an untargeted manner are indispensable. High-resolution mass spectrometry (HRMS) has proven to be a powerful tool in this domain, offering good molecular specificity and enabling the separation and identification of individual components within complex samples. However, despite its high selectivity and resolution, HRMS can sometimes fall short in sensitivity [3]. In such cases, SERS spectroscopy provides significant advantages, particularly when analyzing historical and artistic samples, where quantities are often limited due to degradation or sampling constraints. SERS enhances the Raman signals of molecules through the interaction with plasmonic nanoparticles, allowing for the detection of substances at very low concentrations. The effectiveness of SERS, however, is closely tied to the chemical affinity between the analytes and the nanoparticle surface, a factor that can vary considerably depending on the specific nature of the sample matrix [4]. This selectivity presents a unique analytical opportunity.

II. METHODS

A. SERS measurements optimization

To gain deeper insight into the dynamic interactions between various analytes and nanoparticles, real-time liquid-phase SERS mapping experiments were performed on dye standard solutions of increasing complexity. Certified standard solutions of alizarin, purpurin, and carminic acid were analyzed individually and in ternary mixtures. Aggregation conditions were optimized by comparing two pH levels and two types of aggregating salts at different concentrations (Fig. 1).

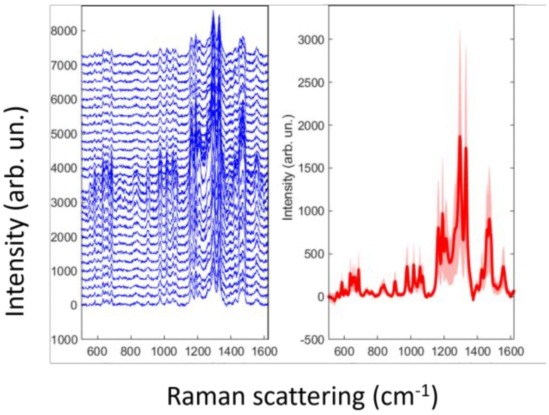

Fig. 1. On the left: Most intense spectra (1st quartile in the integrated intensity distribution, i. e. above the 75th percentile) from the real-time liquid-phase SERS map acquired on a standard solution of alizarin, purpurin, and carminic acid, at pH 7 and $MgSO_4$ 0.1 M.

On the right: mean and standard deviation of the spectra shown on the left.

Once the optimal conditions were established, real-time liquid-phase SERS mapping was carried out on dyes extracted from lake pigments. The lake pigments were prepared starting from a range of natural sources, including madder roots from two different plants, and kermes and cochineal insects. These sources were used to dye wool, and lake pigments were subsequently prepared both from the dyed wool and directly from the dye sources. For each lake pigment, two different extraction strategies were compared [5, 6].

All the analyses were carried out in liquid using a Jobin-Yvon HR Evolution micro-Raman spectrometer with 632.8 nm laser (Horiba, Japan) coupled with a microscope with a set of interchangeable objectives. Ag colloids were prepared following the protocol developed by Leopold e Lendl [7].

B. Data analysis

The 60 spectra from the real-time maps were subjected to Principal Component Analysis (PCA) to study intramap variability. Subsequently, the 60 spectra for each sample were averaged, and PCA and ASCA were performed on the samples to investigate the variance arising from different dye sources, as well as their preparation recipe and extraction methods for analysis [8].

III. RESULTS

Analyzing the principal components of the spectra obtained during real-time mapping provides insight into the evolving interaction between the solution-phase analytes and the nanoparticles at the analysis spot. As the mapping step progresses, the spectra exhibit a gradual trajectory within the principal component space. Examination of the loadings enables the identification of SERS peaks corresponding to this progression (Fig. 2 and Fig. 3).

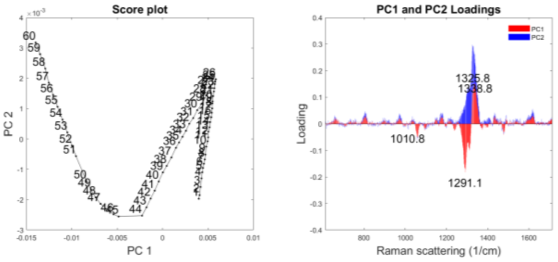

Fig. 2. On the left: Most intense spectra from the real-time liquid-phase SERS map plotted on their two first principal components.

On the right: loading plot for the first and second principal component.

The real-time liquid-phase SERS map was recorded on dyes extracted from a lake pigment prepared from madder roots using a mild basic solution.

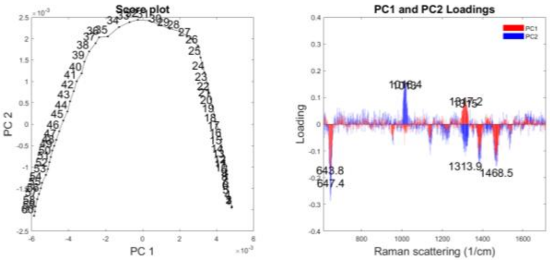

Fig. 3. On the left: Most intense spectra from the real-time liquid-phase SERS map plotted on their two first principal components.

On the right: loading plot for the first and second principal component.

The real-time liquid-phase SERS map was recorded on dyes extracted from a cochineal pigment prepared from wood shearings using a mild basic solution.

After averaging the 60 most intense spectra for each sample, the dataset underwent exploratory analysis and ASCA. The overarching goal was to assess whether the proposed approach could effectively give insights into the variance related to specific factors, such as preparation recipes and extraction solvents, despite the inherent samples' complexity.

While PCA alone was insufficient to achieve separation among the compared samples, and thus to allow a chemical interpretation of the distinguishing signals, ASCA highlighted the significance of the three factors under study: natural source (p-value 0.09%), preparation recipe (p-value 0.49%), and extraction method for analysis (p-value 1.19%) (Fig. 4).

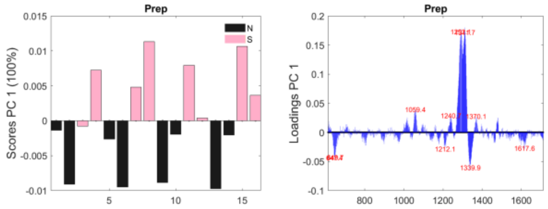

Fig. 4. On the left: PC1 score plot illustrating the effect of the preparation recipe factor (N is for natural source, S is from dyed shearings).

On the right: PC1 loading plot showing the contributions of variables associated with the preparation recipe factor.

IV. CONCLUSIONS

In conclusion, the application of ASCA allowed us to determine the significance of various factors affecting the SERS signals, including the recipe used to prepare the lake pigment, whether derived from dyed shearings or natural sources. This insight is particularly valuable for the dating and authentication of artworks, as different historical periods employed distinct recipes and techniques for lake pigment preparation [9].

The combination of untargeted SERS analysis with chemometric methods provides a powerful approach for exploring complex datasets, enabling chemical analyses to uncover patterns and address questions that had previously remained unconsidered.

REFERENCES

[1] M. Aceto, Pigments - the palette of organic colourants in wall paintings. Archaeol Anthropol Sci 13, 159, 2021

[2] D. Badillo Sanchez, M. Serrano Ruber, A. Davies Barrett, D. J. L. Jones, M. Hansen, S. Inskip "Metabolomics in archaeological science: A review of their advances and present requirements", Science Advances 9, 32, 2023

[3] E. Geddes da Filicaia, R.P. Evershed, D.A. Peggie "Review of recent advances on the use of mass spectrometry techniques for the study of organic materials in painted artworks", Analytical Chimica Acta 15, 340575, 2023

[4] F. Pozzi, S. Zaleski, F. Casadio, R.P. Van Duyne "SERS discrimination of closely related molecules: a systematic study of natural red dyes in binary mixtures", Journal of Physical Chemistry C 120, 37, 2016

[5] I. Serafini, L. Lombardi, G. Vannutelli, C. Montesano, F. Sciubba, M. Guiso, R. Curini, A. Bianco "How the extraction method could be crucial in the characterization of natural dyes from dyed yarns and lake pigments: the case of American and Armenian cochineal dyes, extracted through the new ammonia-EDTA method", Microchemical Journal 134, 2017

[6] K. Lech, E. Fornal "A mass spectrometry-based approach for characterization of red, blue, and purple natural dyes", Molecules 25, 2020

[7] N. Leopold, B. Lendl "A new method for fast preparation of highly surface-enhanced Raman scattering (SERS) active silver colloids at room temperature by reduction of silver nitrate with hydroxylamine hydrochloride", The Journal of Physical Chemistry B 107, 24, 2003

[8] A. K. Smilde, J. J. Jansen, H. C. J. Hoefsloot, R. J. A. N. Lamers, J. van der Greef, M. E. Timmerman, ANOVA-simultaneous component analysis (ASCA): a new tool for analyzing designed metabolomics data, Bioinformatics 21, 13, 3043-3048, 2005

[9] J. Kirby, M. Spring, C. Higgitt 'The Technology of Red Lake Pigment Manufacture: Study of the Dyestuff

Substrate', National Gallery Technical Bulletin 26, 2005