

Original article

Characterisation of the pigment “Jaune Peruvien” using in-situ SERS reveals murexide-Zn(II): From guano/snake faeces to pigment palette

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ABSTRACT

Accurately identifying natural, organic dyes and pigments is crucial for art conservation, preservation, and historical interpretation. While past studies showcase the sensitivity and selectivity of surface-enhanced Raman scattering (SERS) for red lake pigments using various treatments, investigations of yellow pigments and paints remain limited. This study addresses this gap by employing in-situ SERS spectroscopy, coupled with non-invasive XRF analysis, to characterise a yellow organic pigment from two historical French Lefranc & Cie catalogues. The analysis successfully identifies both samples as murexide, a pigment containing zinc as the mordant metal. This finding provides the first scientific evidence supporting Lefranc & Cie's claims of using snake excrement for pigment production. Because 19th-century literature suggests efficient extraction methods for murexide from snake excrement, definitively proving the source material in these samples remains a challenge. Beyond expanding our understanding of historical artistic practices, this discovery highlights the potential of in-situ SERS and XRF for unlocking the secrets of historical pigments. Further research is needed to refine and broaden the application of these techniques for various pigments while simultaneously considering the ethical implications of micro-invasive approaches.

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1. Introduction

1.1. History of murexide

Murexide or ammonium purpurate (Fig. 1) is an interesting red powder with the chemical formula ($C_8H_5N_5O_6 \cdot NH_3$) that holds a unique yet short-lived position in the history of dyes. Unlike its natural dye counterparts, murexide boasts the distinction of being the first synthetic mordant dye [8]. This meant it required a specific metal compound, known as a mordant, to give an insoluble pigment and bond effectively with fabric fibres. Though its time as a prominent dye was brief, murexide's journey from a scientific curiosity to a commercially produced colourant offers valuable insights into the early development and the constant evolution of synthetic dyeing techniques.

The story of murexide starts in the 18th century with the work of Carl Wilhelm Scheele. In 1776, while investigating the mysteries of bladder stones, Scheele stumbled upon this red substance. However, a practical method for producing murexide on a larger scale remained elusive for decades. This hurdle was overcome in 1818 by William Prout, who discovered that heating uric acid with nitric acid and then introducing ammonia yielded the fashionable

red-purple colour. Recognising its potential as a dye, particularly for wool and other animal fibres, Prout laid the groundwork for the future application of murexide [8,10].

Earlier attempts in the 1830's to obtain the necessary ingredients were prepared from an unconventional source: snake excrement. German scientists Justus von Liebig and Friedrich Wöhler explored this method, but the scarcity and impracticality of collecting snake droppings ultimately limited its use [12].

While Prout's discovery held promise, the large-scale production of murexide would not materialise until the mid-19th century. The key factor in this transformation was the discovery of a reliable method for extracting uric acid from guano, specifically the uric acid-rich guano deposits found in South America [15]. This readily available source of raw material, coupled with advancements in processing techniques, paved the way for the widespread adoption of murexide as a dyestuff.

The guano processing itself was a multi-step process. The initial step involved treating guano with a dilute acid to remove unwanted ammonium salts. The remaining residue was then treated with sodium hydroxide to dissolve the uric acid. Finally, the isolated uric acid was treated with specific acids and then excess ammonium carbonate to yield murexide [4].

The newfound ability to produce murexide in larger quantities led to its rapid adoption as a dye in the 1850s. Initially used for

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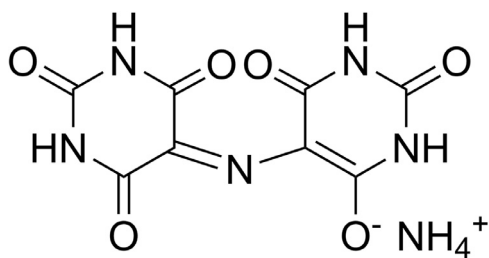


Fig. 1. Structure of murexide ammonium salt.

dyeing wool with tin as a mordant, its application expanded to silk (using a mercury mordant) and cotton (with lead mordant) [15].

Guano initially contained around 20 % uric acid, but this level dropped when the richest deposits have been exhausted. In Manchester in 1857–59, a major manufacturer, Robert Rumney, processed 12 tons of guano per week to obtain 1200 Kg of murexide. Guano was priced at £6000 a year and murexide was priced at around £100,000 [4].

However, murexide's time as a leading dye was destined to be short-lived. The late 1850's witnessed the discovery and rapid rise of synthetic aniline dyes, particularly Mauveine. These synthetic dyes offered superior properties in terms of cost, lightfastness and vibrancy, ushering in a new era in the world of dyeing. By the mid-1860s, murexide, with its limitations in lightfastness and the presence of more versatile alternatives, faded from commercial use.

1.2. Uses of murexide

The ammonium salt of murexide is used not only as a dye but also as a complexometric indicator in analytical chemistry since it is soluble in water. It is most often used to titrate calcium ions, but also for copper, nickel, cobalt, thorium and rare-earth metals. It functions as a tridentate ligand.

In contrast, as early as 1859, Salter documented the exceptional brilliance and purity of murexide as a pigment. While he complained about its susceptibility to light and air, limiting its widespread use, he also described a remarkable range of colours achievable with murexide, including red purpurates of lead, purple and rose-coloured of mercury, a purple-red of silver, a dark red-brown of strontium, a crystalline red of cobalt, a scarlet of platinum, a yellow of zinc and even a green of barium [14]. This diversity highlights the versatility of murexide as a pigment base.

Interestingly, historical records from the French manufacturer Lefranc & Cie mention "Jaune Peruvien" or Peruvian yellow, a pigment they claimed contained murexide obtained from snake excrement [5].

1.3. Challenges in characterisation

Despite its potential for vibrant colours, murexide presents challenges for characterisation, especially when used with mordants. While the pure ammonium salt readily dissolves and can be analysed by mass spectrometry [17], mordant-treated murexide pigments, particularly the yellow zinc salts, pose significant difficulties. Attempts to characterise these pigments using various analytical techniques haven't been successful due to their insolubility in common solvents (e.g. DMSO, diluted HCl and concentrated NH_3) [5]. In addition the pigment could not be characterised using Raman or FTIR spectroscopy. This lack of solubility and inability to generate vibrational spectroscopic data might explain the absence of documented historical use of such mordant-treated murexide pigments.

1.4. New tools for analysis

Recent advancements in pigment analysis offer promising solutions for studying murexide. Surface-Enhanced Raman Spectroscopy (SERS) has emerged as a powerful technique for analysing organic pigments and dyes. Traditionally, SERS involves extracting dye molecules from a sample for analysis. However, this process often loses information about how the dye interacts with the mordant [7].

To overcome this limitation, researchers are exploring in-situ SERS analysis, where the pigment sample itself is directly exposed to the nanostructured substrate used in SERS without extraction. Combining this method with portable XRF analysis, which provides non-invasive elemental analysis, offers a powerful approach. This combined approach allows scientists to characterise both the organic (murexide) and inorganic (mordant) components of the pigment molecule.

This research builds upon these advancements in pigment analysis. In this work, the identification of two samples from different Lefranc catalogues labelled as "Jaune Peruvien" are presented. By employing in-situ SERS analysis for the organic part and portable XRF for the inorganic part, in this way, a deeper understanding of these historical pigments can be gained.

2. Aim

To characterise two historical yellow samples labelled as "Jaune Peruvien" or Peruvian yellow using in-situ SERS and non-invasive elemental analysis for the mordant element.

3. Materials and methods

3.1. Samples

Murexide standard was obtained from BDH Chemicals Ltd (product 27,197). The standard was dissolved in deionised water. Subsequently Whatman paper (#3 qualitative) was soaked in the murexide standard solution and left to dry overnight. SERS nanoparticles were applied directly to the dried paper.

Two catalogues from Lefranc & Cie and Lefranc were used for analysis, both samples were labelled as "Jaune Peruvien". The catalogues were sourced from a private collection in the UK. The catalogues are not dated but are believed to have been made in the early 20th century since they contain cadmium based pigments. Pictures of the cover and the pigment washes are shown in Figs. 2 and 3.

3.2. Fourier transformed infrared spectroscopy (FTIR) diffuse reflectance (DRIFTS) and attenuated total reflectance (ATR)

FTIR analysis was done using an Agilent 4300 portable handheld instrument (Agilent Technologies). The instrument has deuterated tryglycine sulphate (DGTS) detector and a ZnSe beam splitter, the range used was 650–4000 and 4 cm^{-1} resolution Sampling was done using the DRIFTS accessory collecting the average of 80 background as well as sample spectra. A coarse gold reference cap was used for the background calibration for every sample. For ATR, an average of 32 background and sample spectra was collected. All FTIR spectra show absorbance units in the y-axis. Software used for collection was MicrolabPC. The DRIFTS accessory reduces the collection of the specular reflectance element to about 4 % depending on the refractive index of the material.

In order to allow direct comparison and for consistency, the x-axis in all spectra shows increasing wavenumbers from left to right.

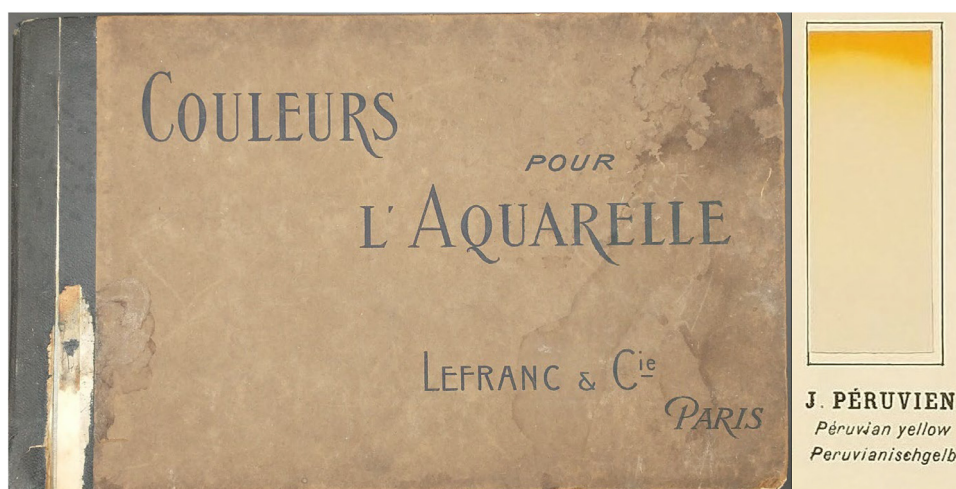


Fig. 2. Catalogue 1 Lefranc & Cie, sample labelled "Jaune Peruvien".



Fig. 3. Catalogue 2 Lefranc, sample labelled "Jaune Peruvien".

3.3. In-situ surface enhanced Raman spectroscopy (SERS)

SERS spectra were acquired using a modified protocol from Cesaratto et al. [3]. Briefly, silver nanoparticles were obtained by microwave reduction of Ag_2SO_4 in the presence of glucose and sodium citrate. The nanoparticles were concentrated 5 times (5X) by centrifugation and reconstitution to a smaller volume. These freshly prepared nanoparticles were deposited directly into the samples (1 μL), then left to dry to perform the analysis. It was not required to add an aggregating agent [3].

The Raman spectra was then collected using a Labram Aramis (Horiba Scientific) using a 532 nm laser (10 % power), a 50X objective, 600l/mm grating, 50 μm pinhole, 0.01–1 s integration time and average of 40 spectra accumulations, spectra was baseline corrected. Instrument was calibrated using a silicon wafer and automatic software correction (Labspec 6).

3.4. X-ray fluorescence (XRF)

XRF analysis was performed using a portable Niton XL2 XRF (Thermo Scientific) with camera. An integration time of 20 s was used for all samples. The instrument is fitted with a 50 kV X-ray

tube (100 μA , 2 W maximum) and a silicon drift detector (SDD) with an active area of 5 mm² fitted with a polymer window (MOX-TEK AP 3.3 film). The X-ray beam's focal-spot size is about 3 mm in diameter.

4. Results and discussion

4.1. FTIR, drifts and ATR

Fig. 4 presents the FTIR spectra obtained for both the murexide standard (using ATR) and the two "Jaune Peruvien" samples (using DRIFTS). While the spectra of the standard show characteristic bands for C–N amine stretches in the range of 1250–1000 cm^{-1} and C=N imine and oxime stretches between 1690 and 1640 cm^{-1} , definitively identifying murexide within the pigment samples based solely on the DRIFTS spectra proves difficult.

This observation aligns with previous findings [5], the FTIR spectra of mordant-treated murexide combined with a binding medium (likely gum Arabic in this case, as the pigments were used in watercolours) often lack the necessary detail for unambiguous pigment identification. The interaction between the pigment, mordant, and binder can mask certain characteristic peaks, making it challenging to isolate the unique spectral signature of murexide.

4.2. In-situ SERS

Fig. 5 displays the SERS spectra obtained for the murexide standard. Notably, the SERS spectra of ammonium purpurate has been previously reported [6,9]. In such research, several bands exhibit significant enhancement, particularly those at 337, 462 cm^{-1} , and the range of 640–680 cm^{-1} . Additionally, a distinct band at 720 cm^{-1} is observed. These bands at 720 cm^{-1} and 640–680 cm^{-1} have been attributed to skeletal deformations or out-of-plane bending vibrations involving N–H bonds.

Previous research has also explored SERS analysis of murexide-metal complexes, including those with Fe, Cu, and Zn ions [9]. Interestingly, it was found that only the murexide-Zn(II) complex demonstrated a strong adsorption affinity to the silver surface used in SERS substrates, allowing for successful recording of the SERS spectrum. Other metal complexes did not exhibit this behavior.

In the present study, similar results have been observed. The bands at 675 and 731 cm^{-1} were the most prominent in the SERS spectra, particularly for the samples from the pigment catalogues. Bands at 258 and 335 cm^{-1} , observed in the samples are associated with the " γ -out-of-plane bending" vibration in the imidazole ring

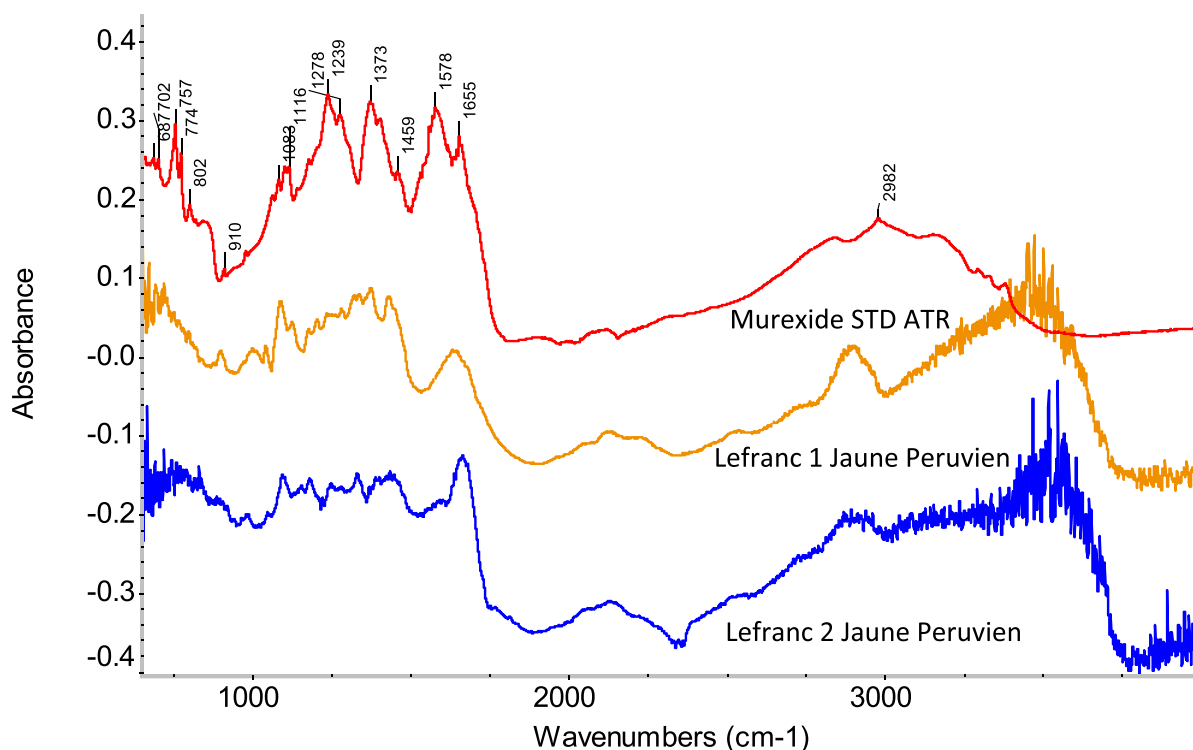


Fig. 4. FTIR spectra of murexide standard (ATR), Lefranc & Cie Catalogue 1 and Lefranc Catalogue 2 Jaune Peruvien samples (DRIFTS).

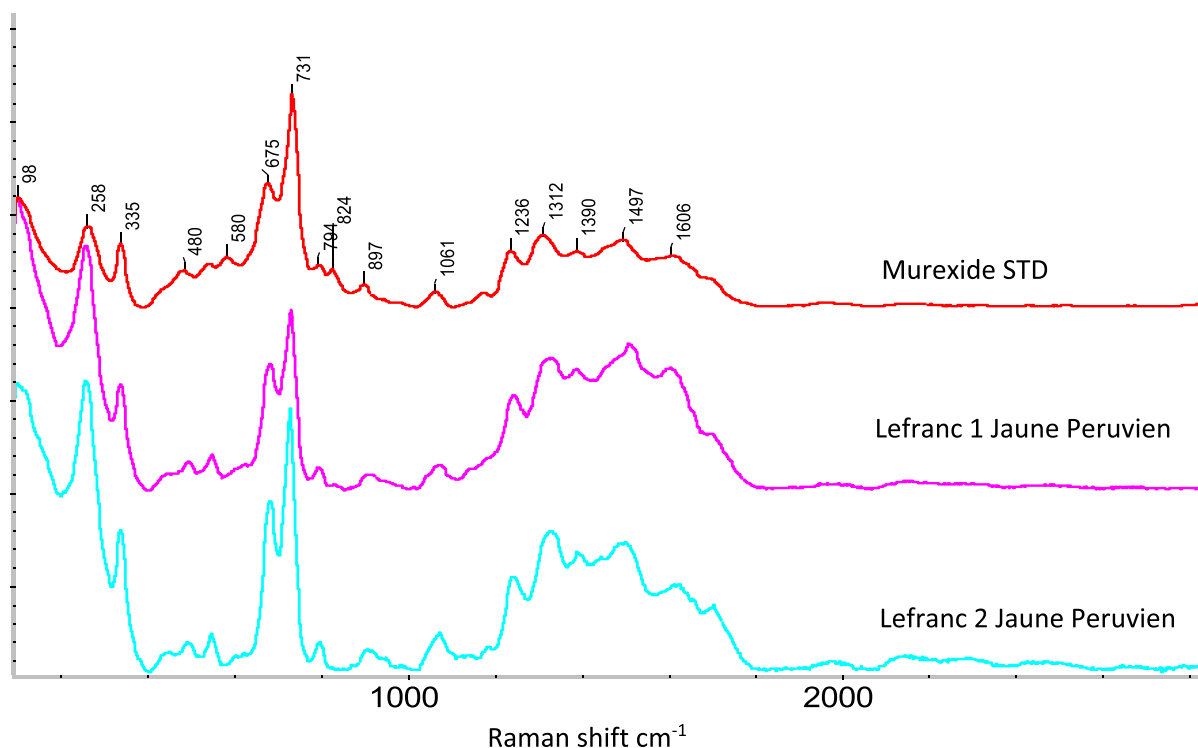


Fig. 5. SERS spectra of murexide standard applied as ammonium salt on wathmann paper, catalogue 1 Lefranc & Cie “Jaune Peruvien” and catalogue 2 Lefranc & Cie “Jaune Peruvien”. Y axis has arbitrary units, baseline subtraction was applied to all samples.

moving out of the plane of the ring. While other yellow organic pigments may exhibit bands at 675 and 731 cm^{-1} , the presence of bands at 258 and 335 cm^{-1} is highly indicative of murexide. These unique vibrational features can serve as a reliable marker for the

identification of this pigment in various samples. These observations alongside with the yellow colour of the pigment suggest a potential presence of murexide-Zn(II) complexes within the historical pigments.

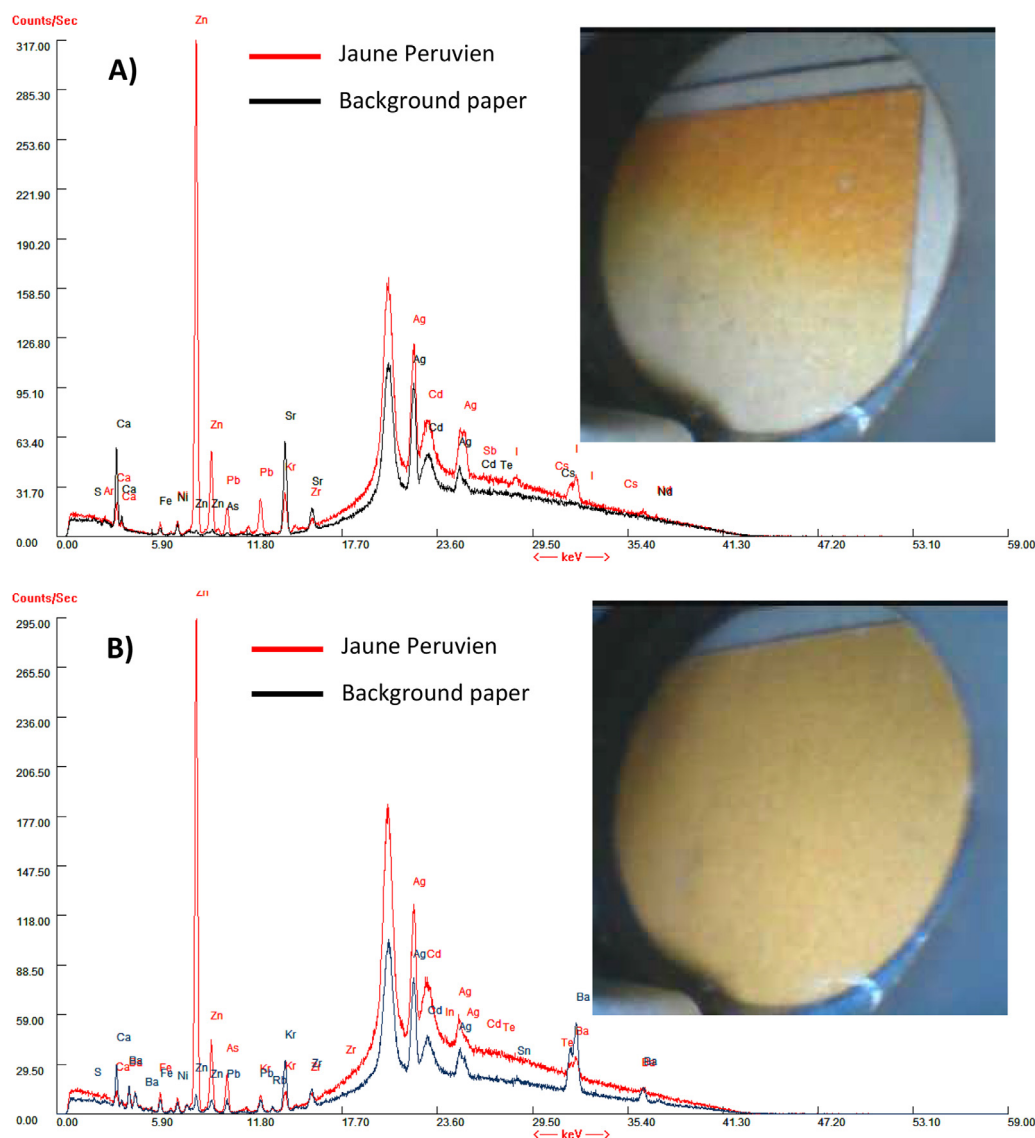


Fig. 6. X-ray fluorescence spectra of A) Lefranc & Cie catalogue 1, sample labelled as “Jaune Peruvien” (red and, background paper signal (black). B) Lefranc catalogue 2, sample labelled as “Jaune Peruvien” (red) and background paper signal (black).

4.3. X-ray fluorescence (XRF)

To identify the mordant used to render the “Jaune Peruvien” pigments insoluble in the watercolour samples, non-invasive X-ray fluorescence (XRF) analysis was used. The results are presented in Fig. 6A and B. These figures clearly show zinc (Zn) as the most prominent element in the samples, with characteristic energy lines detected at $K\alpha = 8.6$ keV and $K\beta = 9.5$ keV. The signal from the paper substrate was acquired in each catalogue showing negligible amounts of Zinc.

This finding of zinc aligns well with the potential presence of a murexide-Zn(II) complex suggested by the SERS analysis. Traditionally, identifying the organic components of pigments often requires harsh extraction methods using strong acids like hydrofluoric acid (HF), nitric acid (HNO₃), sulfuric acid (H₂SO₄), or hydrochloric acid (HCl) [2,13,16]. These techniques necessitate taking micro-invasive samples from the object being studied, potentially damaging the historical artifacts. These harsh extraction techniques may also compromise the organic components of the dye.

Another approach involves using gels for micro-invasive dye extraction [1]. On the other hand, advancements in SERS analysis

offer a more promising solution: in-situ analysis. Recent studies have demonstrated the successful application of in-situ SERS substrates for analysing various organic pigments directly on the object, including anthraquinones (alizarin, purpurin), indigoids (indigotin, indirubin), and protoberberine-type dyes [11].

In this study, a micro-invasive strategy was performed by applying only 1 microliter of the SERS substrate containing a small amount of silver particles to the pigment samples. This approach leverages the reported good affinity between Zn(II)-mordanted murexide and silver particles, facilitating the generation of reliable SERS spectra. Importantly, this work demonstrates the feasibility of analysing this type of pigment samples directly on their substrate, even when they contain a binder.

It's important to acknowledge that traditional techniques like spontaneous Raman, FTIR, or HPLC have often struggled to characterise many yellow organic pigments found in historical objects. Fortunately, the use of Macro-XRF analysis is becoming more widespread and accessible. While XRF offers a valuable tool, interpreting the data requires a deep understanding of the object under study. This is because XRF can penetrate beyond the surface and analyse deeper layers depending on the material's composition and thickness.

Furthermore, the presence of zinc and a yellow colour when using XRF should not automatically lead to the conclusion of certain inorganic pigments, including zinc phosphate or yellow ochres that sometimes contain trace amounts of zinc, especially in pigment mixtures. Here, it is demonstrated that one of such yellow pigments could be murexide. This is a crucial point to consider when analysing historical objects that may contain multiple pigments.

The identification of murexide and a zinc mordant in the “Jaune Peruvien” samples offers a glimpse into historical artistic practices. While historical records from Lefranc & Cie mention using snake excrement for pigment extraction [5], the findings presented in this research, provide the first scientific evidence to support this claim. Further research into historical pigment manufacturing techniques and documentation could shed light on the prevalence of such unconventional sources and identify if snake excrement was indeed used. Understanding the specific mordant used, in this case, zinc, also contributes to our knowledge of the technical choices made by artists and the challenges they faced in achieving desired colours and durability. Because 19th-century literature suggests efficient methods for extracting nearly pure murexide from snake excrement [14], definitively proving the source material in these samples remains a challenge. This is because specific biomarkers for identification may not exist due to the purity of the pigment itself.

The successful application of in-situ SERS and XRF analysis for “Jaune Peruvien” opens doors for further investigation into the characterisation of elusive historical pigments. These techniques offer a minimally invasive approach, allowing for pigment analysis directly on the object whilst being micro-invasive. Future studies could explore the applicability of this combined approach to a wider range of historical pigments, particularly those that have resisted traditional characterisation methods due to the presence of mordants or binders. Additionally, further research could refine the capabilities of SERS analysis to improve its specificity in identifying specific organic molecules within complex pigment mixtures. In addition, it is important to understand the pigment's lightfastness and potential degradation pathways. It is therefore suggested that future studies could explore this aspect in more detail.

The successful use of in-situ SERS analysis for pigment characterisation aligns with recent advancements in this field. Studies by Mingyi et al. [11] demonstrate the potential of SERS for analysing various organic pigments directly on the object [11]. This research builds upon such work by demonstrating the effectiveness of combining in-situ SERS with XRF analysis for a more comprehensive characterisation, including both the organic pigment (murexide) and the inorganic mordant (zinc). This combined approach offers a valuable tool for heritage scientists investigating historical pigments.

The micro-invasive nature of in-situ SERS analysis using a small amount of silver particles presents an advantage over traditional methods that require sample removal. However, it's important to acknowledge the ethical considerations of using any technique, even minimally invasive, on irreplaceable historical objects. Careful consideration should be given to the object's value and fragility, and the potential benefits of the analysis should outweigh any potential risks.

5. Conclusion

The identification of murexide and a zinc mordant in the “Jaune Peruvien” samples sheds light on a fascinating chapter in historical

pigment use. This discovery not only adds to our understanding of artistic practices but also highlights the potential of new analytical techniques like in-situ SERS in combination with XRF for unlocking the secrets of historical pigments.

However, further research is needed to expand the application of SERS and XRF. This in order to refine these techniques for broader applicability to various historical pigments and possibly offer a powerful tool for non-invasive characterisation.

This research could also help to investigate unidentified yellows. Focusing research efforts on heritage objects documented with uncharacterised yellow pigments holds some potential for uncovering new information about historical colour palettes and artistic choices.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [Gemini / Google] in order to improve clarity and readability. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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