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Investigating the viability of electrochemiluminescence for archaeological material Analysis: A pilot study

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ABSTRACT

Archaeometry is a challenging field that requires scientific methodologies to conduct archaeological analysis. Electroanalytical methods have been used in archaeometry for over a century, but recent advances in electrochemistry have yet to be fully integrated into the field. The emergence of new electroanalytical techniques, such as electrochemiluminescence (ECL), offer the potential for increased sensitivity and non-destructive analysis of solid samples. Here, we present the first pilot study to investigate the feasibility of voltammetry of immobilised particles (VIMP) ECL for the analysis of archaeological fibrous quids. We successfully demonstrate the ability of VIMP ECL to be employed as qualitative analysis method, which when paired with statistical analysis and cultural context, allows for the identification of *Datura wrightii* within archaeological quid samples. Our results demonstrate that VIMP ECL can be used to analyse solid samples with minimum destruction. These findings highlight the potential of ECL as a successful tool in archaeometry, particularly for the analysis of solid-state samples. This study provides a strong proof-of-concept for the potential of VIMP ECL to enhance the capabilities of archaeometry and opens up new avenues of research in the field.

1. Introduction

The area of archaeometry centres around the application of scientific technique toward the analysis of archaeological artefacts or sites. Utilising physiochemical techniques, such as X-ray fluorescence and radiocarbon dating, researchers aim to uncover the information about the societal roles these objects played and shed light on the technology, culture and economic patterns of ancient societies. [1–3] Archaeometry therefore, represents a highly interdisciplinary area, where scientific findings must be combined with historical knowledge; otherwise material composition findings may not be useful on their own. [1,2].

Analysis of archaeological artefacts brings a number of unique but critical challenges, analysis must be either non– or minimally destructive whilst facing challenges of multicomponent systems contained within complex and potentially interfering matrices. [3] Electrochemical methods first crossed into archaeometry as far back as the 18th century where they were used for the preservation and restoration of metal artefacts, the technique made famous by Rathgen [4] in 1898 was then widely adopted replacing prior technical and chemical methods throughout the 20th century. [3,5,6] Expansion of the analytical aspects of electrochemistry has seen a shift in employment of electrochemical strategies within the archaeometry field with electroanalytical aspects dominating in this area in the last 50 years. [3,7,8] This change was fundamentally driven by the development of the solid-state or abrasive voltammetry technique of voltammetry of immobilised particles (VIMP). Coined by Scholz *et al.*[9–11], VIMP allowed for the obtainment of analytically useful information from sparingly solid samples using masses of a low as a few nanograms of material. The solid material is abrasively attached to the electrode surface before it is placed in contact with the electrolyte. Despite the solid matrix the same electroanalytical interrogations can be performed, that is the redox processes can still be observed thus allowing for qualitative identification of the species and quantification of the composition. [10] The ability to perform solid state voltammetry made VIMP an ideal tool toward archaeological analysis, where only trace amounts of material from the artefact are required for identification and quantification purposes. [3,12] To this end VIMP became widely used within the archaeological and conservation community, where it has since been employed for the identification of pigments in paintings and fibres, corrosion components or for the dating of metal or alloy artefacts. [3,12–15].

The success of VIMP within the archaeological field has revolved around the employment of voltametric or impedimetric sensing with

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both methodologies demonstrating great success in the field. Electrochemiluminescence (ECL) is an alternative electroanalytical method which may also demonstrate success within this area. ECL combines the advantages offered by electrochemical techniques with those from photoluminescence, but does so without the need for an external light source thereby offering reduced instrument complexity and footprint promoting portability and lowering cost. [16–28] Working under the same redox principles as traditional voltametric studies, ECL could offer the same analytical information as VIMP but may provide an increased sensitivity or the ability to indirectly detect species which have minimal electrochemical behaviour at the electrode surface. While voltametric and electrolysis techniques have both been widely adopted into archaeometry, the use of ECL has to the best of our knowledge not been applied in this area, in-spite of the benefits it offers. We have previously demonstrated the success of abrasive ECL for the analysis of plant material[29], which acted as a spring board for the body of work discussed herein. where the same ideology was applied to the analysis of archaeological samples. The archaeological samples in question comprise of fibrous quids excavated from the Pinwheel Cave (CA-KER-5836) located in Chumash borderlands of interior south-central California.[30,31] The quids were extracted as part of a larger archaeological project “Unravelling the Gordian knot: Integrating Advanced Portable Technologies into the Analysis of Rock-Art Superimposition” where the pinwheel cave’s rock art motif was under investigation. [31] Quids are typically made up of yucca, agave, tule or tobacco and are believed to have been chewed upon. [30] Their fibrous nature and composition of plant materials makes them ideally suited toward VIMP ECL. We have previously reported on the in depth analysis of the quids via a host of analytical methodologies to determine their elemental and chemical composition. [30] The results of which provided for the first time a physical link between the hypothesised theory of the connection between rock art and hallucinogenic substances, a hugely important finding to the rock art research community. [30] Given the well characterised composition of these samples, they were an ideal candidate for evaluating the feasibility of VIMP ECL as a tool for archaeometry, and for exploring the technique’s advantages and limitations in this context. Therefore, we report here the findings of our pilot study, which represents the first application of ECL toward archaeometry using VIMP ECL.

2. Reagents & apparatus

2.1. Materials & chemicals

Tris (2,2′-bipyridyl) – dichlororuthenium(ii) hexahydrate ($[Ru(bpy)_3]^{2+}$), atropine sulfate, (−)-scopolamine hydrobromide trihydrate, lithium perchlorate (LiClO4), and 117 Nafion (~5% mixture of lower aliphatic alcohols and water) were purchased from Sigma-Aldrich. Absolute EtOH was purchased from VWR Chemicals. All chemicals were used as received and all solutions were prepared in Milli-Q water (18 MΩ cm^{-1}). *Datura* plant species were grown in house from seed and harvested as required. *Solanum lycopersicum* (tomato plant), *petunia hybrida* (petunia plant) and *Nymphaea ampla* (waterlily) were commercially purchased.

2.2. Instrumentation

All electrochemical and photoluminescence measurements were made via the coupling of the PalmSens 4 potentiostat to a H10723-20 photomultiplier tube (PMT). All ECL measurement were performed within a light-tight Faraday cage, with a specially designed sensor holder which positions the PMT window directly above the working electrode surface. GSI Technologies carbon paste screen printed electrodes (SPE) with a 4 mm carbon working, carbon counter and Ag paste pseudo-silver reference were used throughout with a maximum sample volume of 100 μL.

2.3. Fabrication of the ECL sensor

The ECL sensors were fabrication via our previously published methodology. [29,32–36] In brief this involves the drop casting of a mixture of 0.5 mM $[Ru(bpy)_3]^{2+}$ encapsulated within a 0.2 %w/v nafion conductive film. 6 μL of the mixture was drop cast upon the working electrode and dried under heat to ensure swift evaporation of any residual solvent. When not in use sensors were stored under darkness to prevent photodegradation.

2.4. Conducting VIMP ECL

VIMP ECL was conducted for the analysis of all plant material and fibrous quids. For plant material, a small portion of the leaf’s were used and mechanically rubbed upon the modified working electrode surface. This physically deposited a small quantity of the material onto the working electrode for analysis before a small portion of the leaf was placed on top for analysis. The same methodology was used for quid analysis where a few stand of the fibrous material was removed from the bulk quid prior to physical deposition of the material to the electrode surface. After physically depositing the material to the working electrode surface, 60 μL of the 0.1 M $LiClO_4$ electrolyte was drop cast on top to complete the electrochemical cell prior to initiating measurement.

3. Results & discussion

3.1. ECL detection of hallucinogenic components

By utilising samples of known composition, we can assess the fundamental applicability of ECL for the analysis of archaeological samples and, as such, comment upon whether the technique could be appropriate for use within this field. To do so, analysis was conducted on archaeological quids, known to contain *Datura wrightii.* [30] In order for ECL, or any qualitative analytical technique, to be useful within this domain, guidance must be sought from the archaeological context to understand which compounds are of analytical significance and direct the method development. In this pilot study, the theory that the quids contained *Datura wrightii* arises from the plant’s archaeological significance to the Chumash tribe. LC-MS and SEM analysis previously conducted on the same quid material has confirmed that the species was present within the quids and made them the ideal sample type on which to conduct this pilot study. [30] The benefit of ECL and electrochemical techniques in this context is their strength for the detection of organic compounds. Many organic compounds, particularly those associated with psychoactivity, often contain electroactive functionalities, primarily amine groups. As such, many drug substances have been electrochemically detected via these functional groups, including amphetamine-type substances[37–40], synthetic cannabinoids [41–43], opioids[44,45], cathinone’s [46,47], and alkaloids.[29,33,48–50] Many natural hallucinogenic species, including *Psilocybe cubensis, Banisteriopsis caapi* and *Lophophora williamsii*, likely associated with archaeological samples also contain these amine functionalities, making them prime candidates for ECL and electrochemical detection. *Datura wrightii* hallucinogenic properties arise from the active hallucinogenic tropane alkaloid compounds, atropine, and scopolamine. Like cocaine, their psychoactivity arises from their tropane alkaloid functionality, which is also responsible for their electroactivity. Therefore, for ECL to assess the likely presence of *Datura wrightii* within the quids, the key lies in the identification of its two tropane alkaloid species. We have previously demonstrated the ability of atropine and scopolamine to behave as suitable ECL co-reactants.

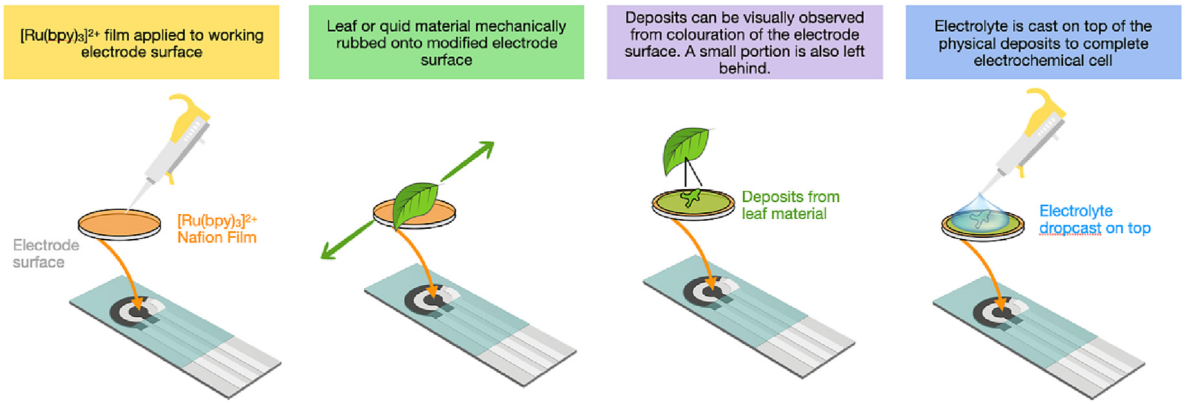
[29,33,34,36] However, unlike definitive analytical techniques such as mass spectrometry or NMR, electrochemical techniques require a reference standard material for qualitative analysis. Therefore, Identification relies upon the use of accurate reference materials. In the context discussed within this manuscript, this would rely upon the archaeological knowledge of the artefact, so that an estimation of the components likely to be present could be made, ensuring the correct reference material could be sourced.

3.2. Abrasive ECL

With the knowledge that ECL can be successfully used for the screening of the hallucinogenic compounds, atropine and scopolamine, the next stage toward identification of the plant material within the quids involves the analysis of *Datura wrightii* itself. The VIMP ECL methodology used here and depicted within Scheme 1 follows the initial methodology proposed by Scholz *et al.* [9] for the conductance of VIMP. The solid material is mechanically deposited on to the electrode surface. The deposited material is then brought into contact with an electrolyte solution to provide the required conductivity to facilitate measurement. Scholz *et al.* [9] developed a pressing method for solid powders, which saw the electrode surface continually pressed onto a small amount of powder, this resulted in a portion being physically adsorbed onto the electrode surface. However, due to the structural makeup of plant material, performing this step is more challenging. Therefore, we modified the application process by rubbing the leaf material across the electrode surface before placing a small portion on top for analysis. The deposition of leaf material to the electrode surface was visually observed via the green-coloured deposits left behind on the electrode surface. After the leaf material was deposited onto the electrode surface, the electrolyte solution was carefully cast on top of the material to initiate the measurement process. Fig. 1 (a) shows the ECL response achieved via this abrasive voltammetry method for analysis *Datura wrightii.* Interrogation of the resultant ECL signal reveals that *Datura wrightii* does produce a notable anodic ECL signal with a maximum ECL intensity observed at mean potential of 1.06 V vs pseudo-Ag. Comparison of the obtained ECL signals for the plant material was made with that of the pure reference standards of the two tropane alkaloids known to be responsible for the observed ECL intensity [29], atropine, and scopolamine, see Fig. 1 (c). The pure reference standards were collected under ideal electrochemical conditions, that is the two compounds where measured when present within their solution phase. We have extensively studied the behaviour of these two tropane alkaloids via ECL within the solution phase with the same ruthenium based ECL sensor utilised here for VIMP and thus are confident in the analytical performance of the sensor under typical operating conditions. [29,32–34,36] By comparing the electrochemical behaviour of the tropane alkaloids in solution to their behaviour within the plant material, we observed a shift in the potential of the maximum ECL peak intensity and the anodic and cathodic peak potentials of the ruthenium redox couple. The maximum ECL potentials of atropine and scopolamine under ideal conditions were 0.84 and 0.87 V, whereas within the plant material the maximum potential was observed at 1.06 V. The potential shift was also evident in the corresponding CV's (Fig. 1 (b)), with a similar shift in the i_{pa} and i_{pc} values for the ruthenium redox couple observed, suggesting the overall electrochemical behaviour of the system is hindered by the solid-phase sample. We believe the deposition of the solid material is in effect partially blocking areas of the electrode surface, thereby reducing the electroactive area of the working electrode. Reduction of the electroactive area therefore accounts for the potential shift observed during VIMP analysis and the poorer electron transfer kinetics and mass transport to the electrode surface observed. This poorer electrochemical behaviour is confirmed by examining the peak to peak separation (ΔEp) and the full-width at half maximum (FWHM) of the Ru(II)/Ru (III) redox couple. Under solution-phase conditions, the CVs show the expected behaviour with values between 124 and 127 mV and FWHM values between 218 and 220 mV, consistent with our previous studies. [29,32–34,36] However, during VIMP analysis, larger values were found with a ΔEp of 175 mV and a FWHM of 268 mV, indicating a greater deviation from ideaility and poorer electrochemical behaviour. These findings confirm that the potential shift observed within the ECL measurements is due to the poorer electrochemical behaviour of the VIMP system, likely associated with the deposition of the solid material partially blocking the electrode surface.

One major concern about the abrasive ECL technique used is its irreproducibility intrinsic to the VIMP technique, which results from the difficulty of ensuring the same amount of physically deposited material due to the mechanical application of the sample to the electrode surface. As previously established in our prior publications the ruthenium-based ECL sensor utilised here demonstrates good analytical performance. [29,32–34,36] However, the inherent variability in the dispersion of tropane alkaloid amount across the plant material [51] combined with the VIMP methodology resulted in a high degree of variability within the seven replicate measurements of plant material, as shown in Fig. 2. The observed variations, with a relative standard deviation (RSD) of 26%, can be attributed to this combination of the VIMP methodology and the inherent variability in the plant material. Therefore, this high degree of variation indicates that the VIMP ECL technique, in conjunction with the findings of Scholz *et al.* [10], would be suitable solely for qualitative analysis.

A further concern with adopting the ECL VIMP technique for qualitative analysis is the lack of specificity, a common issue with all ECL systems. The ruthenium luminophore complex utilised within



Scheme 1. VIMP ECL technique, where leaf or quid material is mechanically applied via rubbing the solid sample across the surface. This leaves physical deposits of the sample behind. The electrolyte is then cast on top to complete the electrochemical cell.

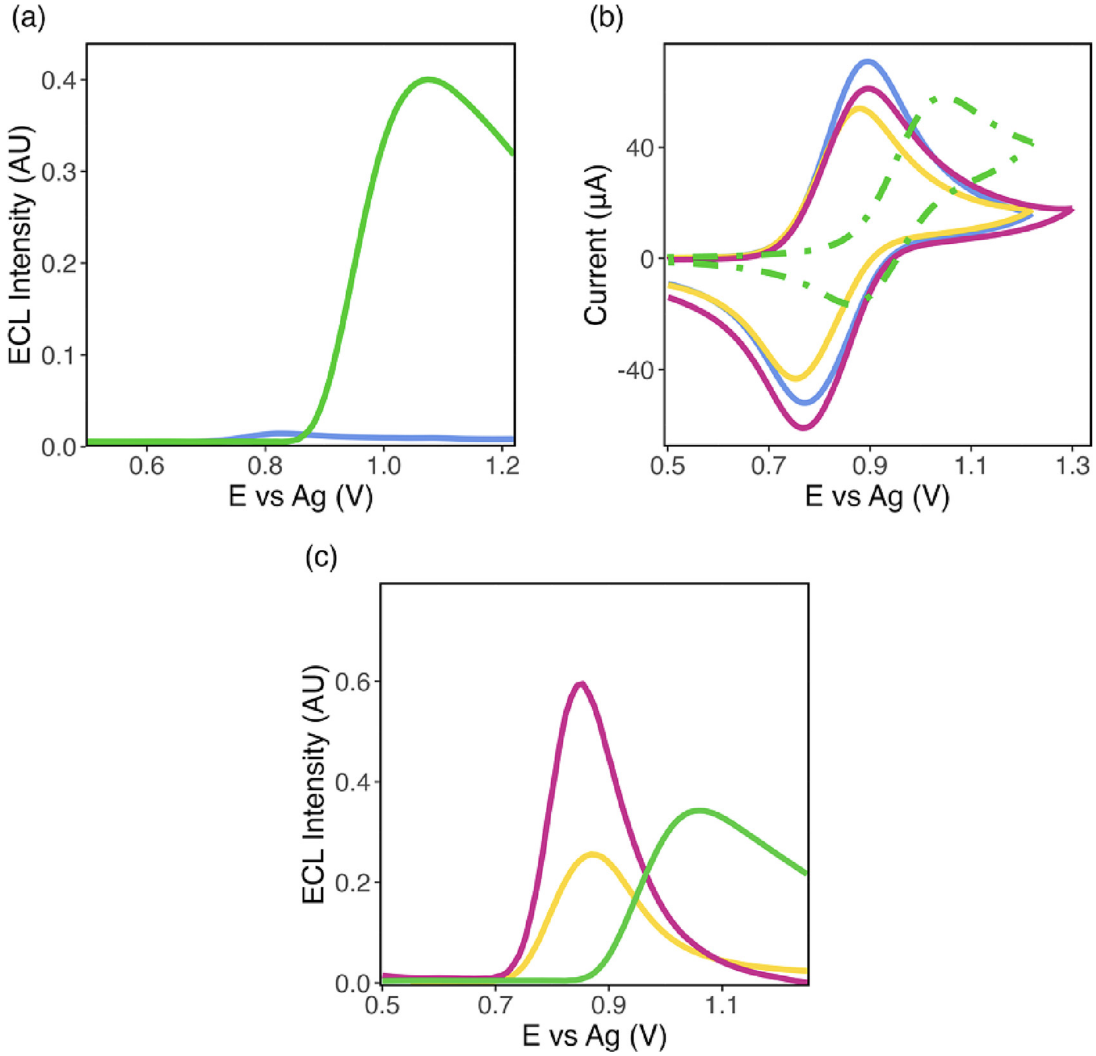


Fig. 1. (a) ECL responses obtained from analysis of *Datura wrightii* (green) and blank electrolyte (blue) via abrasive ECL methodology. (b) CV plots corresponding to blank electrolyte (blue), 10 μM scopolamine (pink), 10 μM atropine (yellow) in solution-phase and *Datura wrightii* (green) in solid-phase. (c) Corresponding ECL responses of 10 μM scopolamine (pink), 10 μM atropine (yellow) and *Datura wrightii* (green). All measurements were collected upon the $[Ru(bpy)_3]^{2+}$ film modified working electrode with 0.1 M $LiClO_4$ as the electrolyte across a potential range of $0.5 \leq E \leq 1.3$ V vs pseudo-Ag at a scan rate of 100 mV s^{-1} and a PMT bias of 0.6 V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this study reacts with a wide variety of different species, raising questions about whether the observed signal from *Datura wrightii* arises from the associated tropane alkaloids. Analysing a similar sample of the same taxonomy makeup without the tropane alkaloids is required to determine the origin of the signal. Since *Datura wrightii* belongs to the *solanaceous* taxa, analysis other members of this taxa are the best approach to understand whether the plant material itself or the tropane alkaloids are responsible for the observed ECL signal. Our previous study showed minimal ECL signals at lower potentials from *Solanum lycopersicum* (tomato) and *Petunia hybrida* (petunia). [29] Fig. 3 (a) shows the resultant ECL signals from *Solanum lycopersicum* and *Petunia hybrida* upon the ruthenium modified electrode, compared with *Datura wrightii*. Confirming the ECL signal observed from *Datura wrightii* is primarily composed of the tropane alkaloids with minor contributions from other taxa components. However, considering the proposed use of ECL VIMP within this pilot study for archaeometric analysis it is crucial to consider the impacts which other taxa. Therefore, we choose to include *Nymphaea ampla* (waterlily), from the *nymphaeaceae* family, in our analysis. The waterlily shares similarities with *Datura* and is associated with shamanic use among indigenous tribes within the Amazon basin. [52–54] Its hallucinogenic properties also arises from the alkaloids present within, nupharine and nymphaeine. [55–57] The waterlily was observed to produce an ECL signal around the same potential as *Datura wrightii*, see Fig. 3 (b) and Figure S1 which shows all overlays of each replicate for each plant taxa analysed. It is not entirely surprising given that the electroactive components arise from the same structural functionality of the alkaloid groups highlighted in Fig. 3 (c). This suggests that distinguishing between two taxa of hallucinogenic nature which contain similar chemical components responsible for their psychoactivity is unlikely with the ECL VIMP technique alone. However, distinguishing between other taxa within the same family that do not contain the psychoactive components is possible. Statistical analysis in the form of a one-way ANOVA and a post-hoc Tukey honest significant difference (HSD) test was conducted to confirm this conclusion. The resultant outputs from the Tukey HSD test can be found within Table S1. Fig. 3 (d) shows the resultant ANOVA box plot of the different species. Interrogation of the statistical outputs alongside Fig. 3 (d) confirm that there is no significant statistical difference between *Datura wrightii* and *Nymphaea ampla* (waterlily), with a p-value of 0.561. However, both *Datura wrightii* and *Nymphaea ampla* were found to be statically different from *Solanum*

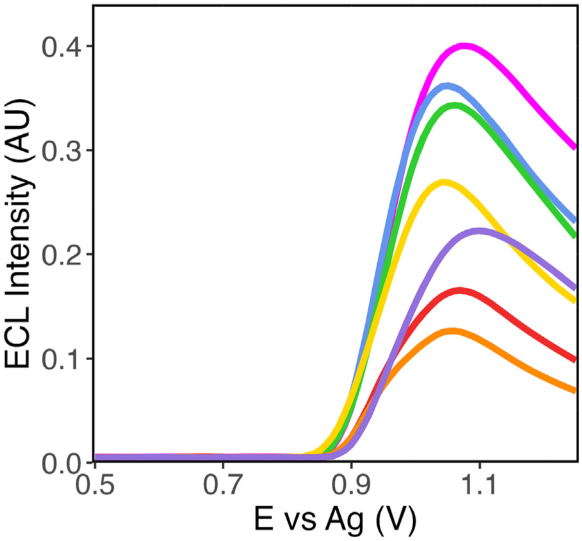


Fig. 2. ECL responses across seven replicates of *Datura wrightii* plant material via VIMP upon a $[Ru(bpy)_3]^{2+}$ film modified working electrode with 0.1 M $LiClO_4$ as the electrolyte across a potential range of $0.5 \le E \le 1.3$ V vs pseudo-Ag at a scan rate of 100 mV s^{-1}at a PMT bias of 0.6 V.

lycopersicum (tomato) and *Petunia hybrida* (petunia) at 95% confidence intervals.

3.3. Application of ECL toward archaeological quids

The key to the ability of the ECL VIMP technique to distinguish between hallucinogenic and non-hallucinogenic plant species with statistical confidence lies in the psychoactive chemical functionality. Given that ECL has had considerable success in the detection of hallucinogenic and illicit substances, primarily due to their amine functionality, it therefore stands to reason that this characteristic benefit could be exploited for archaeometric analysis. The fibrous quid Ker-5836 subsample 2 (pictured within Fig. 4) was used as a pilot study for the assessment of the ECL VIMP technique toward archaeometric analysis. We know from our prior LC-MS studies that this quid does contain the hallucinogenic tropane alkaloids, atropine, and scopolamine, responsible for the ECL emission of the *Datura wrightii.* [30] Ten small strands of the fibrous material were removed from the parent quid for VIMP analysis. Fig. 4 (b) shows the resultant ECL outputs from all 10 replicate measurements. As expected, given our findings with the plant material (see Fig. 2), examination of the data reveals a large degree of variation in signal intensity, with an RSD value of 43.8% across all 10 replicates. As with the plant material this variation is the combination of the nature of the VIMP technique, with different amounts of the material physically deposited each time as well as the inherent nature of the sample. Here, the inherent variability in the sample will have a greater bearing given the nature of the sample when considering not only the samples age but also the expected maceration of the quid during spiritual rituals. Given this, a high variability in the amount of hallucinogenic species present within different sub-sections of the quid will see a huge degree of spread across replicates. However, as identity is not dictated by signal intensity this variation will not negatively impact the technique, but as previously discussed render it solely toward qualitative analysis. More important in assessing the feasibility of the technique is the variation in peak potential of the maximum ECL intensity, given this factor is used to distinguish whether a hallucinogenic species would be present. The variation of the maximum peak intensity gave an RSD value of 2.2%, with the spread of the maximum potential values visually represented within Fig. 4 (c). This RSD value is in line with that observed for the *Datura wrightii* replicates (RSD = 2.45%), both of which lie within the typical analytical standard of below 5%.

Visual comparison of the resultant ECL signals generated by the quids and *Datura wrightii* demonstrated good agreement, particularly in terms of their maximum ECL potential (see Fig. 5 (a)), with mean values across all replicates of 1.05 V and 1.06 V, respectively, vs pseudo-Ag. A comparison of all replicates for all taxa and the quids is shown within Figure S2. While visual comparison was promising, qualitative analysis required statistical evaluation to establish the necessary confidence. To gain this, we again performed a one-way ANOVA analysis with a post-hoc Tukey HSD, the full outputs of which can be found within Table S2, and visually shown in Fig. 5(b). The Tukey HSD revealed no statistical difference between the quids and *Datura wrightii* with a p-value of 0.922, which was expected since they contain the same chemical functionality. However, the same was also found between the quids and the *Nymphaea ampla* (waterlily) with a p-value of 0.483. Given the prior inability to distinguish between the *Datura wrightii* and *Nymphaea ampla* (waterlily), this result was not unexpected. Nevertheless, the quids could be confidently distinguished from *Solanum lycopersicum* (tomato) and *Petunia hybrida* (petunia). As a result, we can assign the resultant ECL signals to the presence of hallucinogenic species containing characteristic alkaloid functionality. As our prior LC-MS analysis showed the observed ECL signals in the quids arise from the same chemical functionality as that seen for *Datura wrightii*, we can confidently state that the ECL observed in the quids is due to tropane alkaloid functionality from confirmed presence of atropine and scopolamine.[30] However, without prior knowledge of the quids' composition, interpretation of the resultant signals would only conclude that hallucinogenic species were present, but could be either *Datura wrightii* or *Nymphaea ampla* (waterlily). In this case, the archaeological context of the sample is crucial, as knowledge that *Nymphaea ampla* is not native to Southern California or associated with Chumash culture would allow us to confidently assign the resultant signals to *Datura wrightii*. Therefore, this highlights that qualitative determination in archaeometric analysis cannot be made confidently with just scientific techniques but requires cultural expertise to establish the appropriate context.

4. Conclusions

Overall, this pilot study successfully demonstrated the feasibility of employing VIMP ECL within the archaeometric field. Electroanalytical techniques like VIMP and electrolysis have a long history of in archaeology and conservation science, and it's reasonable to expect that advancements in electroanalytical science could be equally successfully in this area. We have successfully analysed solid-phase samples from both plant species and archaeological quids (Ker-5386 sub-sample 2) using a ruthenium modified working electrode. While analysing solid samples is achievable via VIMP ECL, a reduction in the electroactivity of the sensor is witnessed compared to its performance under ideal electrochemical cell conditions. Therefore, when analyzing solid-state samples, consideration must be given to the chosen reference standards used for qualitative identification purposes. In practice, reference standards must also be analysed within their solid-phase to avoid false negative or positive identification. The VIMP ECL technique employed allowed us to perform analysis upon a minimum amount of the archaeological sample, requiring removal of only a few strands. This represents a huge benefit of the technique toward archaeological sample analysis, where minimising sample destruction is crucial. As with all ECL techniques, the inherent lack of specificity as a result of its operation relying upon functional group presence must be considered. We demonstrated how this could influence the proposed methodology within the archaeological context through the analysis of similar taxa which could lead to false identification of *Datura wrightii* within the quid samples. By coupling the VIMP ECL

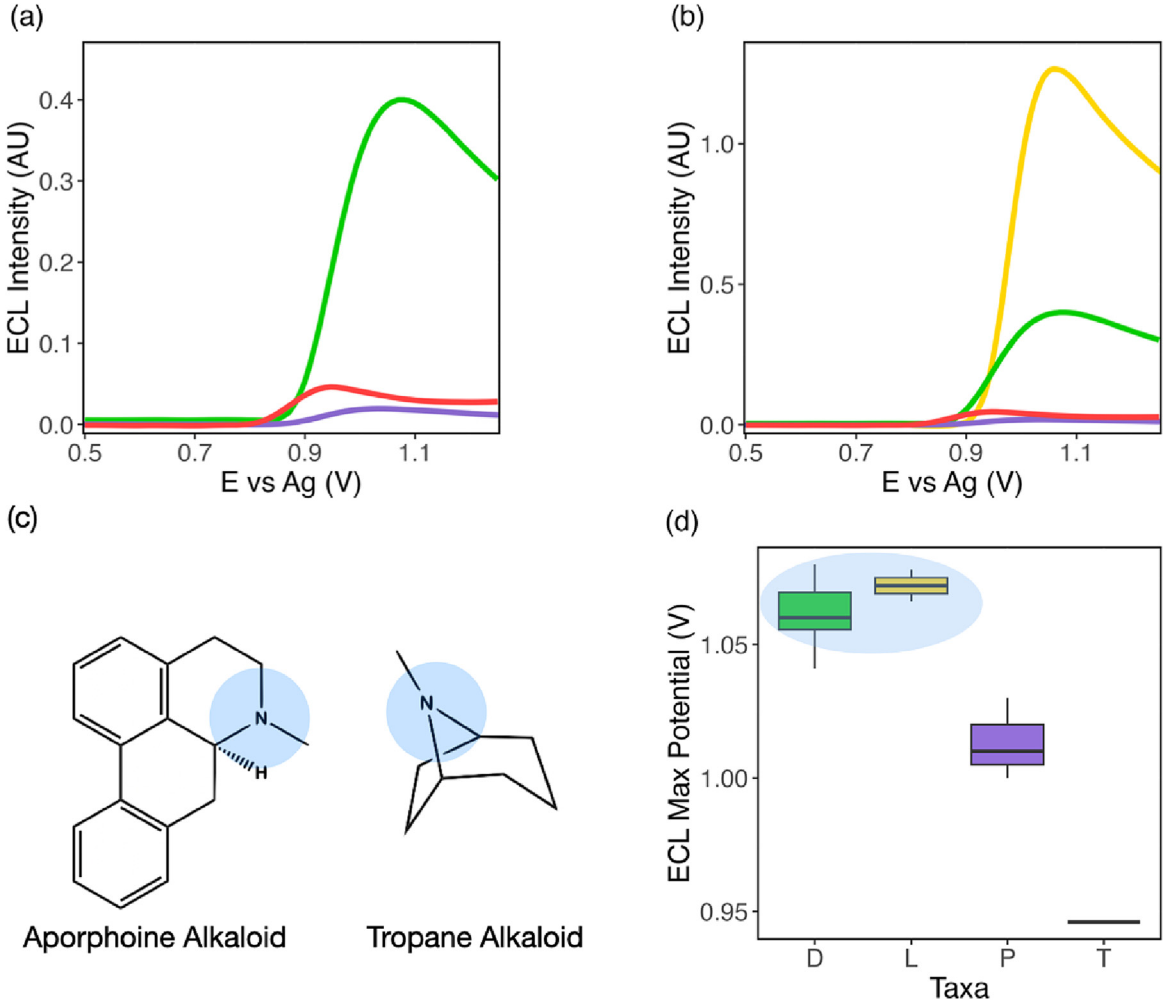


Fig. 3. (a) ECL responses of Datura wrightii (green), *Solanum lycopersicum* (tomato) (red) and *Petunia hybrida* (petunia) (purple). (b) ECL responses of Datura wrightii (green), *Solanum lycopersicum* (tomato) (red), *Petunia hybrida* (petunia) (purple) and *Nymphaea ampla* (waterlily) (yellow). (c) Chemical functionality responsible for the electroactive behaviour of *Nymphaea ampla, aporphoine alkaloid and Datura wrightii*, tropane alkaloid. (d) Box plot of ANOVA Tukey HSD results showing the inability to distinguish between Datura and waterlily (D and L) but the statistical significance of both from Petunia and Tomato (P and T). All measurements were collected via the ECL VIMP technique with the $[Ru(bpy)_3]^{2+}$ film modified working electrode with 0.1 M $LiClO_4$ as the electrolyte across a potential range of $0.5 \leq E \leq 1.22$ V vs pseudo-Ag at a scan rate of 100 mV s^{-1}at a PMT bias of 0.6 V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

technique with statistical analysis, we were to distinguish between species which contained psychoactive components, *Datura wrightii* and *Nymphaea ampla* (waterlily), and those without, *Solanum lycopersicum* (tomato) (red) and *Petunia hybrida* (petunia), demonstrating that in spite of the complexity of the plant matrix we can target successfully target the active hallucinogenic components. Utilising the same combination of statical methods with VIMP ECL, we determined that the quids were not statistically different from the *Datura wrightii* and *Nymphaea ampla* (waterlily), likely indicating their presence within the archaeological sample. These results highlighted that while VIMP ECL when coupled with statistical methods could lead to positive qualitative identification based upon functional group presence, confirmatory assignment of a specific species is not currently achievable. In this case, confirmation that the quids contained *Datura wrightii* was possible through consideration of the archaeological context of the sample, since *Nymphaea ampla* (waterlily) is not native to the region or associated with Chumash tribal cultural. Therefore, if VIMP ECL is used in combination with cultural context and statistical methods positive identifications could be made. Nevertheless, the technique serves as valuable triage tool to determine whether further confirmatory analysis is necessary.

CRediT authorship contribution statement

Kelly Brown: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Rowan S. Blake:** Investigation, Visualization. **David W. Robinson:** Conceptualization, Resources, Supervision, Funding acquisition. **Lynn Dennany:** Conceptualization, Supervision, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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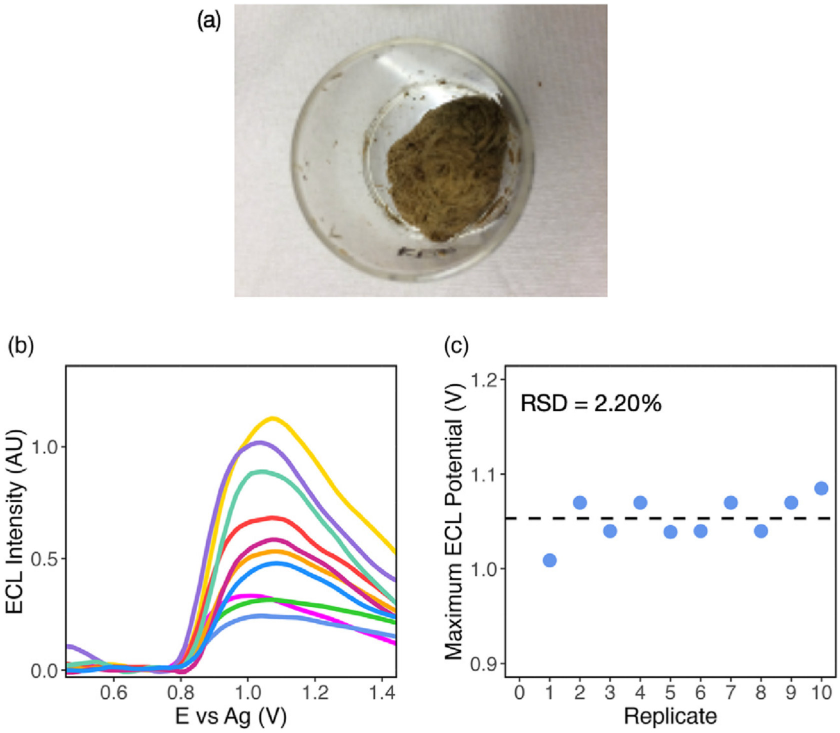

Fig. 4. (a) Photograph of fibrous quid Ker-5386 sub-sample 2. (b) ECL responses across 10 replicates of quid Ker-5836 subsample 2 via VIMP ECL with the $[Ru(bpy)_3]^{2+}$ film modified working electrode with 0.1 M $LiClO_4$ as the electrolyte across a potential range of $0.5 \leq E \leq 1.5$ V vs pseudo-Ag at a scan rate of 100 mV s^{-1} and a PMT bias of 0.6 V. (c) Spread of maximum potential values across all 10 replicates, where dashed line represents the mean value.

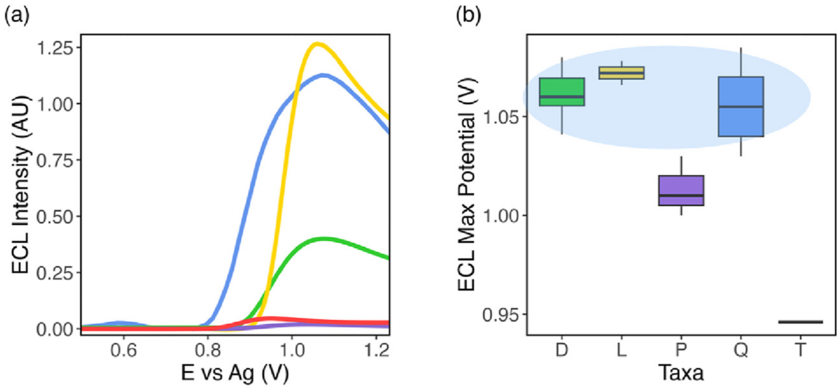

Fig. 5. (a) Comparison of ECL responses from quid Ker-5836 subsample 2 (blue), *Datura wrightii* (green), *Solanum lycopersicum* (tomato) (red), *Petunia hybrida* (petunia) (purple) and *Nymphaea ampla* (waterlily) (yellow). All measurements were collected via the ECL VIMP technique with the $[Ru(bpy)_3]^{2+}$ film modified working electrode with 0.1 M $LiClO_4$ as the electrolyte across a potential range of $0.5 \leq E \leq 1.22$ V vs pseudo-Ag at a scan rate of 100 mV s^{-1}at a PMT bias of 0.6 V. (b) Box plot of ANOVA Tukey HSD results showing the inability to distinguish between Datura and waterlily (D and L) but the statistical significance of both from Petunia and Tomato (P and T). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rary Native Americans of the region. We acknowledge the deep importance of the ancestors who made the remarkable archaeological record and encourage the respect and care for sites of Native heritage. We also thank Landon Peppel, Melissa Dabulamanzi, Daisy Carrillo, and all the staff at the Wind Wolves Preserve for their support. Thanks to Jennifer Perry, Matthew Baker, Clare Bedford, Eleni Kotoula, Devlin Gandy, Jon Picciuolo, Jack Sprague, Fraser Sturt, Julie Bernard, the Pinwheel excavation team and the RAD Group. We also thank the Arts and Humanities Research Council for the funding to conduct this project "Unravelling the Gordian Knot: Integrating Advanced Portable Technologies into the Analysis of Rock-Art Superimposition" (Project Reference AH/M008894/1). All data underpinning this publication are openly available from the University of Strathclyde KnowledgeBase at https://doi.org/10.15129/05dbaf7f-4f75-475c-aa8b-0c698dc8f498.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelechem.2023.117521.

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