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Coupling of Dye Analysis and Compound Specific Radiocarbon (^{14}C) Analysis (CSRA) in Heritage Sciences

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Abstract: Natural organic dyes and pigments have been used for millennia to bring colour into our daily lives. Being sourced from a variety of natural sources, they form an extremely varied and large class of compounds, all of which retain the atmospheric $^{14}CO_2$ of their year of growth. As such these compounds represent ideal candidates for radiocarbon (^{14}C) dating, allowing the identification of or providing information towards the period in which the coloured artefact was created. However, up to now no such analysis has ever been conducted solely on organic colourants within an object. The complex nature of the samples and the sample size limitations with respect to precious and rare art artefacts requires innovative inter- and multidisciplinary approaches. Here we discuss preliminary results in the development of a compound-specific radiocarbon analysis (CSRA) methodology for the analysis of anthraquinone derived red dyes extracted from dyed wool yarns. The aim of this research project is to introduce new routes to date cultural heritage objects, in particular to overcome their intrinsic complexity through the development of CSRA strategies.

Keywords: Chromatography · Heritage sciences · Natural organic dyes · Radiocarbon (^{14}C)

Laura Hendriks is a Branco Weiss fellow at the School of Engineering and Architecture Fribourg (HEIA-FR), University of Applied Sciences and Arts Western Switzerland, since Fall 2020. As a graduate student she worked at the interface of chemistry, physics and heritage sciences focusing on the development of micro-invasive strategies for radiocarbon (^{14}C) dating of paintings. In January 2020, she graduated with honors from ETH Zurich, receiving both the ETH Silver medal and Dimitris N. Chorafas prize. With the support of a Branco Weiss – Society in Science Fellowship, she is investigating novel routes to ^{14}C date artworks, in particular how compound specific radiocarbon analysis (CSRA) may benefit heritage sciences.

Cyril Portmann is an associate UAS Professor in Analytical Chemistry at HEIA-FR since 2020. Dr. Portmann's teaching and research activities are based on his scientific background and professional experiences in analytical chemistry, natural product chemistry and drug development. He acquired his PhD with Prof. Karl Gademann at the EPFL University and his postdoc with Prof. Jon Clardy at Harvard Medical School. Prior to joining HEIA-FR, he worked in biotech start-up companies developing small molecules and biological drugs from discovery to Phase 3 clinical development. Currently, Dr. Portmann is consulting for a biotech company in Switzerland.

Negar Haghipour graduated in 2013 at the Department of Earth Sciences at ETH Zurich. Since 2013 she is laboratory coordinator at the Geological Institute, where she runs the cosmogenic nuclides lab and is responsible for gas ^{14}C measurements on the MICADAS at Laboratory of Ion Beam Physics. She is interested in Geochronology and CSRA method development.

Thomas M. Blattmann obtained his PhD in the Department of Earth Sciences in January 2019 and was awarded the Silver Medal from ETH Zurich. He then started postdoctoral research at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) as Young Research Fellow for two years where he focused on compound-specific radiocarbon analysis of amino acids. Currently, he is working as scientific collaborator at ETH Zurich managing the laboratories of Biogeoscience Group of the Geological Institute and is interested in a wide range of Earth Science and analytical topics surrounding organic geochemistry and mineralogy.

1. Introduction

Among the variety of analytical tools available to study artworks, radiocarbon (^{14}C) dating holds great promise in providing missing information about an object's historical context.[1] Nowadays, the substantial decrease in sample size requirements for such analysis has supported the development of new ^{14}C dating strategies in the field of cultural heritage. While working on elucidating the origin of a Nazca tunic, Smith *et al*. put forward a novel combined, sequential use of dye analysis and ^{14}C dating.[2] With this strategy it was possible, from a single yarn, to gain both composition and age information. The sampling requirements were reduced by a factor of two and the risk of additional damage to the textile was also proportionally limited. This example demonstrated the complementarity of the two methods in terms of information output and compatibility. It furthermore paved the way towards the present research in which not only the textile but also the extracted dyes may be ^{14}C dated. This approach is both innovative and original in the context of heritage science

studies, as the chemical identification of the dye may support geographic provenance but limited chronological information is gained as natural dyes have been used from 5000 BCE until the present day.[3,4] Natural organic dyes, extracted from a multitude of natural sources, represent an extremely large class of possible compounds, including anthraquinones, flavonoids, indigoids or tannins.[5] All of these compounds retain a snapshot of the atmospheric $^{14}CO_2$ during their year of growth and so are potential ^{14}C measurement candidates. This information may be retrieved by capitalizing on compound-specific analysis as opposed to bulk sample analysis as already presented in a perspective paper.[6] Therein, the associated ^{14}C constraints within the different steps of the methodology were highlighted covering the chemical extraction, chromatographic separation, and final ^{14}C dating (see schematic workflow in Fig. 1). Here we present follow up results, focusing on anthraquinone-derived red dyed wool substrates.

2. Materials and Methods

2.1 *Red Dyes and Chemicals*

Madder roots (*Rubia tinctoria L.* and *Rubia Cordifolia L.*) and cochineal (*Dactylopius coccus L.*) were purchased from MAIWA and used as modern standards ($F^{14}C \geq 1$). The corresponding synthetic counterpart of the main coloring agents were used as depleted standards ($F^{14}C \geq 0$), namely alizarin ($C_{14}H_8O_4$, MW = 240.21 g/mol, Acros organics, purity 97%), purpurin ($C_{14}H_8O_5$, MW = 256.21 g/mol, Cayman, purity 95%) and carminic acid ($C_{22}H_{20}O_{13}$, MW = 492.39 g/mol, Sigma Aldrich, purity 90%). Analytical grade solvents were employed for all HPLC related analyses, namely methanol (Optima, LC-MS grade, Fischer scientific), acetonitrile (Rotisolve HPLC gradient grade, Carl Roth GmbH, Karlsruhe, Germany) and phosphoric acid (85 wt. % in H_2O, Merck, Darmstadt, Germany) as mobile phase modifier. For the dyed yarn hydrolysis, HCl 37% (Rotipuran, Carl Roth) was used while oxalic acid dihydrate was purchased from Sigma Aldrich.

2.2 *Sample Preparation*

Dyed wool threads (2–3 mg) were placed in 500 µL hydrolysis solution and heated in a reactitherm heating module (ThermoFischer, Massachusetts, USA). The traditional acid hydrolysis conditions[7,8] involving 37% hydrochloric acid (HCl)/methanol/water (2:1:1 v/v/v) for 10 minutes at 100 °C were varied with respect to acid strength (10%, 5%, 2%), time (10–60min) and temperature (60–100°C). The hydrochloric method was further compared against a milder protocol involving 4 mM oxalic acid in MeOH:water (1:1) (OAMW)[2] and again time and temperature were varied. The hydrolysis step was quenched by cooling in an ice bath and the samples were dried down under a N_2 stream at 50 °C. The samples were then reconstituted in a known volume of methanol and filtered through a 0.22 µm PTFE syringe filter (13 mm ϕ, BGB Analytik AG, Switzerland) prior HPLC analysis.

2.3 *Chromatographic Separation*

The separation was conducted in parallel at ETH and HEIA using two different systems. At ETH the separation was conducted using a Zorbax Eclipse C18 column (4.6 x 100 mm, 3.5 µm) fitted with a Supelco Column saver (0.5 µm filter) on a 1260 serie HPLC system (Agilent, Santa Clara, USA). The compounds were eluted by a binary systems of solvents, A = milliQ water/0.1% phosphoric acid and B = methanol. At HEIA, the analyses were separated on a Adamas C18-Extreme column (4.6×100 mm, 3.5 µm) on a Ultimate 3000 HPLC system (ThermoFischer, Massachussets, USA) and eluted with a gradient of milliQ water/0.1% H_3PO_4 and acetonitrile. The analyses on both system were monitored at 280 and 430 nm. Repeated injections were performed and allowed to collect ~5–30 µg C of the targeted analytes.

2.4 *Radiocarbon Analysis*

All samples following preparative HPLC collection were transferred to pre-cleaned tin cups (V = 0.025 ml, Elementar Analysensystem GmbH, Germany) and measured at the Laboratory for Ion Beam Physics at ETH Zurich on the ^{14}C dedicated accelerator mass spectrometer (AMS), also known as Mini-Carbon Dating

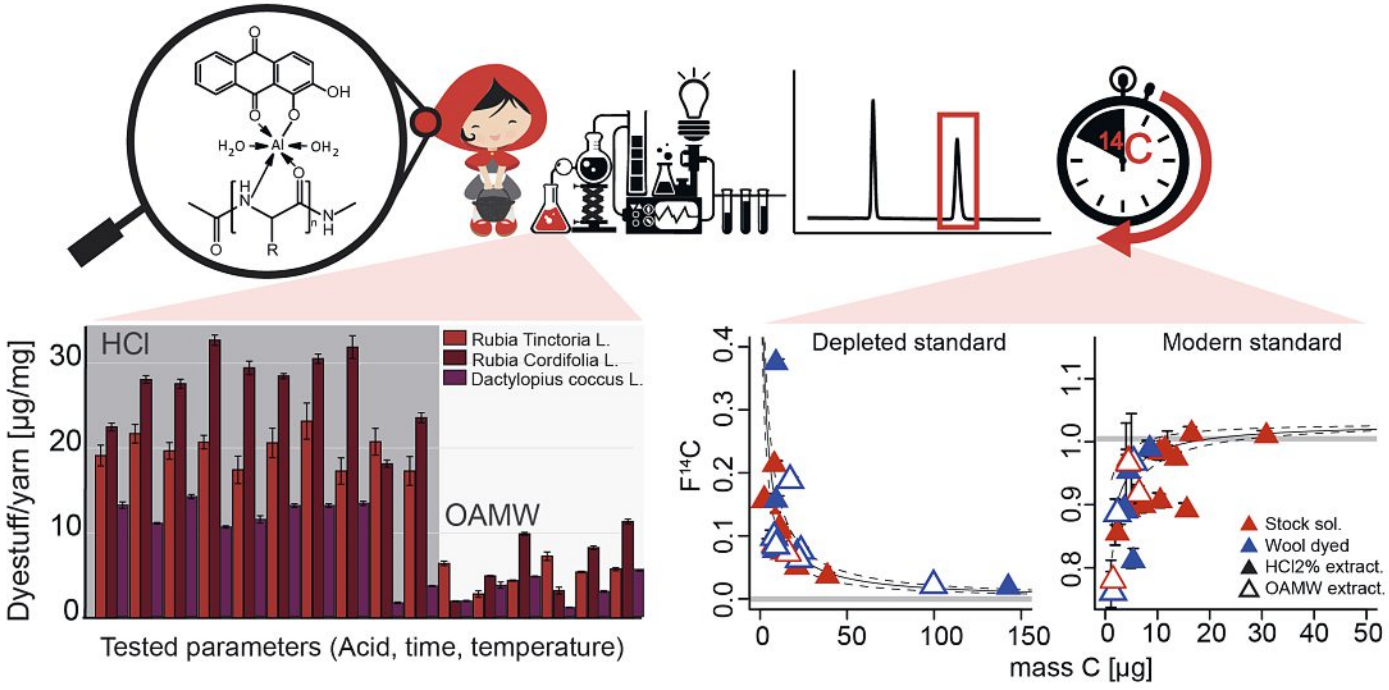

Fig. 1. Schematic diagram of the workflow combining dye analysis with CSRA. Left: Average dyestuff recovery and standard deviation (n = 3) of hydrolysed extracts of dyed wool with two madder species and one scale insect source. Right: Measured set of standards for constant contamination modelling, including stock solution (red filled triangles) and dyestuff isolated from dyed wool yarns following acid hydrolysis (blue triangles) with HCl (filled) and OAMW (empty). The best fit is represented by the solid curve and the dashed lines the corresponding 1σ uncertainty.

System (MICADAS).[9] The samples were combusted in an elemental analyzer (EA) before direct transfer to the AMS source.[10]

3. Results and Discussion

In order to successfully couple dye analysis and CSRA analysis targeting natural organic dye molecules, the amount of dye extracted must be maximized while also minimizing exogenous carbon contamination (C_{Ex}). Limiting and effectively monitoring C_{Ex} during the whole sample preparation process represents the biggest challenge as the latter may have different sources, from the chemical extraction ($C_{Chemistry}$), the sample matrix (C_{Matrix}), the chromatographic separation (C_{Chrom}), combustion and ^{14}C analysis ($C_{EA\text{-}AMS}$). A quantification including both the mass (µg C) and respective isotopic ratio ($F^{14}C$) of the added contamination per ^{14}C analysis is possible *via* a model of constant contamination. Within the pilot study C_{Ex} parameters were found to be 3.1 ± 0.8 µg C with a $F^{14}C_{Ex}$ of 0.45 ± 0.07,[6] which are comparable with other blank quantification done for other compounds, such as for amino acids with 2.2 ± 1.3 µg C and a $F^{14}C_{Ex}$ of 0.25 ± 0.09,[11] lignin with 2 ± 0.5 µg C and a $F^{14}C_{Ex}$ of 0.48 ± 0.10 or even down to 0.9 ± 0.2 µg C with $F^{14}C_{Ex}$ of 0.81 ± 0.15,[12,13] benzene polycarboxylic acids (BPCAs) with 1.6 ± 0.7 µg C and $F^{14}C_{Ex}$ 0.90 ± 0.50.[14] Nonetheless, when attempting to reproduce results from the pilot study, 6.5 ± 1.5 µg C contamination were observed, regardless of the laboratory where the samples were prepared, either at ETH or HEIA. A thorough examination of the different sets of reference materials, which were independently subjected to the different steps of the process allowed to narrow the contamination source to $C_{EA\text{-}AMS}$. The common denominator was found to be linked to a specific batch of tin caps, which showed anormalous high carbon content, with 2.5 ± 0.5 µg C as opposed to the typical 0.5–1.0 µg C.[15]

C_{Chrom} is comparable between the two laboratories, system and columns, with 3.5–4 µg C and $F^{14}C_{Ex}$ of 0.50 ± 0.1. When considering the sample preparation step, C_{Chem} and C_{Matrix} are here interlinked. Indeed, in the case of anthraquinone-based red dyes, in order for the dyestuff to bind to the textile it is necessary to use a metal mordant to enhance the dye's binding affinity towards the fiber yarn. Commonly, an acid hydrolysis in a methanolic solution allows to break the dye–metal bond and free the colorant.[16] While promising for downstream ^{14}C analysis, HCl-based protocols may result in different undesired forms of degradation of the chromophores[7,17,18] as well as depolymerization of protein-based fibers, leading to an increased C_{Matrix} contribution. Hence, milder alternatives were investigated with two aims; prevent irreversible sample destruction while still ensuring maximal recovery of the organic dye. As displayed in Fig. 1 and already observed by different groups, HCl-based protocols lead to higher extraction yields of dye per mg textile.[19,20] Between 2 to 4 fold increase is observed in comparison to OAMW protocols. Among the different parameters tested, the acid strength showed to have the most influence on the dye extraction efficiency. Less aggressive amounts of 5% or 2% HCl resulted in similar yields with the advantage of causing less fiber hydrolysis. Increased temperature or longer reaction times were shown to have little impact other than causing an increased baseline in the respective chromatogram, indicative of fiber degradation. Regardless of the chosen acid, when plotted along the model of constant contamination in Fig. 1 (right), all hydrosylates fell in line with stock solution samples, which had only been subjected to the chromatographic step, indicating that $C_{Chem} << C_{Chrom}$. This also accords with earlier observations reported by Smith *et al*., where despite involving the use of organic solvents and acids in the extraction step, no cross contamination was observed in the ^{14}C ages.[2] Hence, the major contribution to C_{Ex} is C_{Chrom}.

4. Conclusions

The development of CSRA strategies in cultural heritage research follows the growing interest in multi-analytical approaches coupling isotopic studies.[21] From the primary identification of natural organic dyes and pigments, their subsequent dating by ^{14}C analysis has the potential to provide important clues as to the chronological context of the object. CSRA appears as a promising tool to confront many of the different questions arising in the study of cultural heritage objects, regarding the origin, art historical, technical, social, cultural background of the object. The authors will report on their progress in future contributions.

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[1] I. Hajdas, P. Ascough, M. H. Garnett, S. J. Fallon, C. L. Pearson, G. Quarta, K. L. Spalding, H. Yamaguchi, M. Yoneda, *Nat. Rev. Methods Primers* **202**1, *1*, 62, https://doi.org/10.1038/s43586-021-00058-7.
[2] G. D. Smith, V. J. Chen, A. Holden, N. Haghipour, L. Hendriks, *Heritage Sci*. **2022**, *10*, 179, https://doi.org/10.1186/s40494-022-00811-5.
[3] S. Kay-Williams, 'The Story of Colour in Textiles', A&C Black, **2013**.
[4] J. H. Hofenk de Graaff, W. G. Roelofs, M. R. van Bommel, 'The colourful past: origins, chemistry and identification of natural dyestuffs', **2004**.
[5] E. S. Ferreira, A. N. Hulme, H. McNab, A. Quye, *Chem. Soc. Rev*. **2004**, *33*, 329, https://doi.org/10.1039/B305697J.
[6] L. Hendriks, C. Portmann, *Helv. Chim. Acta* **2022**, *106*, e202200134, https://doi.org/10.1002/hlca.202200134.
[7] J. Wouters, *Stud. Conserv*. **1985**, *30*, 119, https://doi.org/10.1179/sic.1985.30.3.119.
[8] J. Wouters, A. Verhecken, *Stud. Conserv*. **1989**, *34*, 189, https://doi.org/10.1179/sic.1989.34.4.189.
[9] H. A. Synal, M. Stocker, M. Suter, *Nucl. Instrum. Meth. B* **2007**, *259*, 7, https://doi.org/10.1016/j.nimb.2007.01.138.
[10] M. Ruff, S. Fahrni, H. W. Gaggeler, I. Hajdas, M. Suter, H. A. Synal, S. Szidat, L. Wacker, *Radiocarbon* **2010**, *52*, 1645, https://doi.org/10.1017/S003382220005637X.
[11] T. M. Blattmann, D. B. Montluçon, N. Haghipour, N. F. Ishikawa, T. I. Eglinton, *Front. Mar. Sci*. **2020**, *7*, https://doi.org/10.3389/fmars.2020.00174.
[12] X. Feng, B. C. Benitez-Nelson, D. B. Montluçon, F. G. Prahl, A. P. McNichol, L. Xu, D. J. Repeta, T. I. Eglinton, *Geochim. Cosmochim. Acta* **2013**, *105*, 14, https://doi.org/10.1016/j.gca.2012.11.034.
[13] S. Sun, V. D. Meyer, A. M. Dolman, M. Winterfeld, J. Hefter, W. Dummann, C. McIntyre, D. B. Montluçon, N. Haghipour, L. Wacker, T. Gentz, T. S. van der Voort, T. I. Eglinton, G. Mollenhauer, *Radiocarbon* **2020**, *62*, 207, https://doi.org/10.1017/RDC.2019.108.
[14] M. Gierga, M. P. W. Schneider, D. B. Wiedemeier, S. Q. Lang, R. H. Smittenberg, I. Hajdas, S. M. Bernasconi, M. W. I. Schmidt, *Org. Geochem*. **2014**, *70*, 1, https://doi.org/10.1016/j.orggeochem.2014.02.008.
[15] C. Welte, L. Hendriks, L. Wacker, N. Haghipour, T. I. Eglinton, D. Günther, H.-A. Synal, *Nucl. Instr. Meth. Phys. Res. B: Beam Inter. Mater. Atoms* **2018**, *437*, 66, https://doi.org/10.1016/j.nimb.2018.09.046.
[16] M. Shahid, J. Wertz, I. Degano, M. Aceto, M. I. Khan, A. Quye, *Anal. Chim. Acta* **2019**, *1083*, 58, https://doi.org/10.1016/j.aca.2019.07.009.
[17] J. Sanyova, J. Reisse, *J. Cult. Heritage* **2006**, *7*, 229, https://doi.org/10.1016/j.culher.2006.06.003.
[18] L. L. Ford, Phd University of Leeds, **2017**.
[19] L. Valianou, I. Karapanagiotis, Y. Chryssoulakis, *Anal. Bioanal. Chem*. **2009**, *395*, 2175, https://doi.org/10.1007/s00216-009-3137-6.
[20] A. Manhita, T. Ferreira, A. Candeias, C. Barrocas Dias, *Anal. Bioanal. Chem*. **2011**, *400*, 1501, https://doi.org/10.1007/s00216-011-4858-x.
[21] A. G. Nord, K. Billström, *Heritage Sci*. **2018**, *6*, 25, https://doi.org/10.1186/s40494-018-0192-3.