

Paper Number: 5B4

The Application of Liquid Chromatography-Mass Spectrometry and Accelerated Light Ageing for the Analytical Identification of Yellow Flavonoid Dyes in Historical Tapestries

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The chemical characterisation and identification of natural dyes using Photo Diode Array High Performance Liquid Chromatography (PDA HPLC) and Liquid Chromatography-Mass Spectrometry (LC MS) techniques are explored. The techniques have allowed the dye sources to be identified from many yellow and green historical fibres, sampled from a collection of well-provenanced European tapestries as part of the ‘Monitoring of Damage on Historic Tapestries’ (MODHT) project. These results are beginning to provide information regarding dyeing practices in different weaving centres at different times. The results from current accelerated ageing experiments to compare weld and sawwort are also reported.

Keywords: flavonoids; HPLC; mass spectrometry; accelerated ageing; MODHT; weld; sawwort; dyer’s greenweed

High performance liquid chromatography with photodiode array detection (PDA HPLC) is now an established routine method for the analytical identification of natural dyes in historical textiles (Wouters 1989; Koren 2001). Interpretation of the results to identify the biological origin of the dye, for example, the plant species, relies on matching data for major and minor organic compounds in the sample with data from known chemical references and authentic botanical sources (Ferreira 2004). The application of Liquid Chromatography-Mass Spectrometric (LC-MS) techniques to compliment PDA HPLC extends existing boundaries for the chemical characterisation and identification of natural dyes (Ferreira 2003b; Szostek 2003) and the combined techniques have been successfully applied to the characterisation of yellow flavonoid dyes and their altered molecular composition caused by light ageing effects (Ferreira 2003a). This analytical approach is now being applied in the EC-funded project, 'Monitoring of Damage to Historic Tapestries' (MODHT) to identify the dye sources in the tapestries under study and to relate the damage to the dyes with the overall damage to the objects.¹

The aim of the MODHT project is to create a scientific 'damage assessment' method for historical tapestries using physical and chemical markers at the macro- and micro-scale (Quye in press). A range of analytical techniques is being developed and applied to measure the chemical and physical properties of 17 well-provenanced tapestries from northern and southern European collections, about which the storage, display and conservation histories are known. Model tapestries replicated using authentic materials and methods, then subjected to accelerated ageing under controlled conditions, are also being analysed to study relationships between the materials used to make tapestries, and

the chemical changes to the dyes, wool, silk and metal threads and physical changes such as tensile strength caused by ageing. This novel approach to damage assessment will provide an objective evaluation method for conservators and curators when making decisions about the display, handling and conservation of tapestries, and indeed other historical textiles. The overall outcomes of this study will be published in due course, but information on the project and its progress can be found at the project website, <http://www.hrp.org.uk/webcode/content.asp?ID=706>. This paper is concerned solely with the analytical study of flavonoid dyes for MODHT.

The basic technique of dye analysis using HPLC, although destructive, requires only a small amount of fibre (approximately 1 mg or 5 mm in length). The fibre is broken down with acidified solvents, releasing the dyestuff into solution (Wouters 1989). Although this process has been optimised to leave many natural dye compounds largely unaltered molecularly, it must be appreciated that chemical changes have the potential to occur, and may make the analysis of certain dyestuffs using this method problematic (Ferreira 2001).

Most natural dyestuffs contain several major and minor organic chemical compounds which attach to the fibre during dyeing, either directly or via a mordant, to impart the colour. For a large number of natural yellow dyes, the colouring components are flavonols and flavones which belong to the class of molecules known as the flavonoids (some of the most important flavonoids present in natural European dye sources are shown in Figure 1). Although flavonoid molecules can be found in many plants, certain

components are more common than others, due to both a high naturally occurring frequency and a molecular arrangement conferring an excellent ability to adhere to the fibre (or mordant) from the dyebath. In many cases, different natural dye sources contain different flavonoids with differing relative concentrations. This can be the first clue in the identification of the botanical source used to dye an historical fibre.

{Insert 5B4-Fig1 here}

The PDA HPLC method allows dye components present in the solution to be separated, and in many cases, identified, by comparing their retention times (HPLC) and response to light over a range of wavelengths (PDA). The elution programme used in the present study is a modification of previously reported methodology (Ferreira 2003b) in an effort to maximise the flavonoid information (Table 1). Interpreting the results to identify the source of the dye relies not only on a good reference library of known chemical materials to match against both the major and minor organic compounds found in the sample, but also extensive knowledge regarding the chemical components (and sometimes their relative ratios) found on fibres dyed using authentic botanical sources. Figure 2 shows the chromatograms obtained from the acid hydrolysed extracts of reference dyeings of weld (*Reseda luteola* L.), sawwort (*Serratula tinctoria* L.) and dyer's greenweed (*Genista tinctoria* Gaud.). The flavonoids luteolin and apigenin are present in all three dyes, but they can be distinguished by other components: luteolin methyl ether in weld; quercetin and kaempferol in sawwort; and genistein in dyer's greenweed. **In favourable cases, the**

detection of characteristic minor components allows the presence of different dyestuffs to be inferred.

{ Insert 5B4-Table1 here } and { Insert 5B4-Fig2 here }

The use of a mass spectrometer equipped with an “ion trap” analyser has further confirmed the presence of these species by enabling a molecular mass to be assigned to each of the chromatographed peaks. The ion trap enables yet more information to be gained by breaking the molecular species into smaller fragments by multistep fragmentation, typically in two stages, called MS-MS (or MS^2) or three stages (MS^3). This fragmentation involves loss of characteristic groups in a unique way, thus giving a structural ‘fingerprint’ for each component (Figure 3). This technique is a powerful tool for enabling the full or partial chemical structure of previously unknown components to be elucidated. For example, the location of the methoxy group in the luteolin methyl ether was shown to be on the ‘B’ ring of the flavonoid (Figure 1) (Ferreira 2003a). The structure of unknown degradation products caused by light ageing can be found in a similar manner (Ferreira 2002).

{ Insert 5B4-Fig3 here }

The application of PDA HPLC and LC MS has enabled the identification of the dye sources in many of the yellow or green yarns sampled from the back of the tapestries selected for MODHT ~~samples from the MODHT tapestries~~ as being weld or dyer’s

greenweed. From the results for the 14 historic tapestries analysed so far (a total of 145 samples), some tentative trends for yellow dye sources are being explored (Table 2). For the nine tapestries originating from workshops in Brussels or Bruges in the first half of the 16th Century, the use of both weld and dyer's greenweed has been found. However, in the tapestries woven after 1600 from Bruges and Antwerp, and the tapestry woven in Arras before 1500, only weld was used with no evidence for dyer's greenweed. If this pattern continues with the analysis of further samples, previously unknown information regarding dyeing practices in different weaving centres at different times will emerge.

{ Insert 5B4-Table2 here }

Dye source identification is not, however, always so easy. Yellow flavonoid dyes can prove particularly challenging to identify because they are prone to fading caused by chemical damage when exposed to light. Experiments on authentic samples to examine how the dyestuff changes chemically upon ageing and what influences this process are an essential part of MODHT.

Recent research has shown that flavonols in flavonoid dyes on alum-mordanted wool degrade much faster than flavones when exposed to visible light (Ferreira 2002). Caution should therefore be exercised when using PDA HPLC results for the identification of historical dye sources. A study within the MODHT project employs accelerated conditions (experiments conducted at the National Galleries, London) (spectral characteristics of the light box given in Saunders 2001) to light-age alum-mordanted

wool dyed with weld and with sawwort. After exposure to approximately 10,000 Lux of simulated daylight through glass for 4500 hours (equivalent, very approximately, to 60 years exposure at 250 Lux for 8 hours each day), the two distinguishing flavonols (quercetin and kaempferol) degraded, leaving the PDA HPLC dye profile of sawwort looking very similar to weld (Figure 4). It is therefore possible that sawwort, a dye plant historically documented as an important alternative to weld (Cardon 1994), has been overlooked analytically for some time. Although the photo-degradation pathway is understood and the products of the reaction known to be detectable by PDA HPLC (Ferreira 2002), the initially small amounts of quercetin and kaempferol present in the MODHT samples make the detection of characteristic photo-degradation products difficult. Currently, LC-MS analysis of light-aged tapestry models and historical samples are underway to find other potentially distinguishing features, for example, whether the methyl ether derivatives of luteolin and quercetin are likely to survive at a detectable level after 500 years and so distinguish between these two dye sources. In the context of MODHT, this is important because although extreme photogradation as modelled in the accelerated ageing studies is unlikely to have occurred in the historical samples because they were taken from the reverse of the tapestries, several show the flavonoid components of weld with other minor coloured peaks. These minor peak are, as yet, uncharacterised, but may relate to sawwort or other flavonoid dyes.

{ Insert 5B4-Fig4 here }

Accelerated ageing experiments are also in progress for a wide variety of dyes, including young fustic (*Cotinus coggygia* Scop.), identified in a number of dyed silk cores from tapestry metal threads. The development of an analytical method for the identification of neoflavonoid compounds in the fugitive red dyes from brazilwoods (*Caesalpinia* species) and the black dye from logwood (*Haematoxylum campechianum* L.) is also underway. With a better understanding of the chemical changes to dyes caused by light ageing and the ability to detect analytically the degradation products or processes, comes the potential to use them as indicators of damage.

Acknowledgements

- Alan Taylor (*The University of Edinburgh*)
- Jo Kirby-Atkinson and David Saunders (*National Gallery Scientific Department, London*)
- Ester S.B. Ferreira (*AMOLF, FOM Institute for Atomic and Molecular Physics, Amsterdam*)
- Funding gratefully acknowledged from the European Commission FP5

Endnotes

1. MODHT is supported by the European Commission under the Fifth Framework Programme and contributing to the implementation of Key Action 4: 'The City of Tomorrow and Cultural Heritage, subsection 4.2.1 'Improved damage assessment of cultural heritage,' within the Energy, Environment and Sustainable Development.

Contract No. EV4K-CT-2001-00048. The project website can be found at

<http://www.hrp.org.uk/webcode/content.asp?ID=706>

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Short Biographies:

Dr Alison Hulme studied at the University of Cambridge, England, graduating B.A. in 1989 and Ph.D. in 1993. After post-doctoral work at Colorado State University she returned to a Research Fellowship at the University of Cambridge in 1994. In 1995 she moved to The University of Edinburgh where she is a **Senior** Lecturer in Chemistry. Her research interests include the asymmetric synthesis of bioactive natural products, the development of new chiral catalysts, and the chemistry of natural and non-natural dyestuffs.

Dr Hamish McNab studied at the University of St. Andrews, Scotland, graduating B.Sc. in 1971 and Ph.D. in 1974. After post-doctoral work at The Australian National University he returned to Scotland in 1976 as senior Demonstrator at The University of Edinburgh where he is now Reader in Chemistry. His research has involved the synthetic applications of flash vacuum pyrolysis (FVP) and the chemistry of conjugated systems such as dyestuffs. He was awarded the RSC Bader prize in 2003.

David Peggie graduated MChem from The University of Edinburgh in 2002. He is currently working towards a PhD degree, jointly supervised by Drs Hulme, McNab and Quye. His research centres around the 'Monitoring of Damage on Historic Tapestries' project, developing analytical methods for the identification of natural dyestuffs and exploring the effects of their degradation.

Dr Anita Quye CChem MRSC gained a chemistry degree from the University of Strathclyde in 1986 and a PhD in Forensic Toxicology from the University of Glasgow in 1989. From 1989 until present, she works as an analytical scientist in the Department of Conservation & Analytical Research of the National Museums of Scotland, applying established and novel analytical techniques to the identification of organic materials in historical and archaeological artefacts. Her specialist research interests include the study of degradative ageing processes on the identification, and preservation of natural textiles dyes and man-made plastics in museum collections.

Tables with legends:

Table 1: The Analytical Conditions for the identification of flavonoids using High Performance Liquid Chromatography (HPLC)

Column	Eluents	Eluent Programme	Flow Rate (mLmin ⁻¹)
5µm Sphericlone ODS2	A: 20% (v/v) MeOH _(aq) B: MeOH C: 5% (w/v) ortho-phosphoric acid _(aq)	0-3 mins 67A:23B:10C (isocratic), 3-29 mins 0A:90B:10C (linear), 29-30 mins 67A:23B:10C (linear) then 5 minutes equilibration at starting conditions before next injection	1.2

Table 2: Emerging trends in the historical use of yellow dyestuffs

Date / Workshop	<1500	1501-1550	1551-1600	1601-1650	1650-1700
Arras (1)	●	--	--	--	--
Brussels (8)	--	● ▲	--	--	--
Bruges (1) (3)	--	● ▲	--	●	--
Antwerp (1)	--	--	--	--	●

● Weld ▲ Dyers greenweed
(Number of tapestries)

List of Figure Captions:

Figure 1: Flavonoids present in some historically important European dye plants

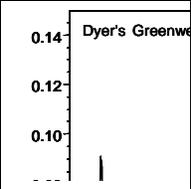


Figure 2: Chromatograms obtained from the acid hydrolysed extracts of alum-mordanted wool reference dyeings of weld (*Reseda luteola* L.), sawwort (*Serratula tinctoria* L.) and dyer's greenweed (*Genista tinctoria* Gaud.).

Figure 3: Ion trap fragmentation 'fingerprints' for luteolin, apigenin, luteolin methyl ether, genistein, quercetin and kaempferol

Figure 4: Comparative PDA HPLC dye profiles for weld and sawwort after exposure to accelerated light-ageing conditions

Total Word Count: 2,234