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THE USE OF MASS SPECTROMETRY TO DIFFERENTIATE BLUE DYES FROM INDIGO AND WOAD

Precious I. Humphrey
Dr. Ruth Ann Armitage, Mentor

ABSTRACT

Indigo has a long and prestigious history, from the ancient past to our blue jeans today. Indigotin is a dark blue colorant molecule that is produced from many plants, including indigo (Indigofera), woad (Isatis), and knotweed (Polygonum) when the leaves are crushed, fermented, and then reacted with oxygen. All of the indigo-producing plants undergo the same chemistry, so they are difficult to differentiate from each other in archaeological textiles. There are many analytical approaches to the characterization of blue dyes in conservation science, though few are able to reliably distinguish between blues obtained from the various plant sources. Archaeologists and conservators are interested in sourcing the colors that ancient people used, as this provides insight into their selection of materials and the chemical technology necessary to produce dyes. We present here the initial stages of work to use direct analysis in real time (DART) mass spectrometry (as well as other ionization methods) to differentiate blue dyes made from indigo, woad, and knotweed on a variety of fiber substrates. Based on the results for the lab-prepared materials, we will apply the best method to archaeological fibers previously shown to contain indigotin.

INTRODUCTION

Indigo has been used throughout history and across all cultures. It is considered one of the oldest dyes used for textiles. The earliest form of indigo dyeing has been traced in Huaca Prieta, a ceremonial site in Peru. Blue pigment in a

6,000-year-old cotton fabric was successfully characterized as indigo from this excavation site.[1] There are three main indigo-producing plants: woad, indigo, and knotweed. Each of these plants is native to a different part of the world. Indigo (*Indigofera tinctoria*) is native to India, which is where it gets its name, and other tropical locations with long growing seasons. Woad, from the plant *Isatis tinctoria*, is found natively in colder climates, such as northern Europe. There are many different species in the genus *Polygonum,* commonly known as "knotweed," that can be used to produce blue dye. Knotweed-derived blue dyes have been used in South America and in Asia.

The blue color, mostly from the chemical compound indigotin (**Figure 1**, right) is not present as the whole molecule in the plants. Instead, the leaves contain a molecule called indican (**Figure 1**, left), which can be released by crushing the plants to break down the cells. When this molecule is reduced, either chemically or by fermenting the crushed plant material in an alkaline solution, it produces indoxyl, which is soluble. Any fibers or fabric can be dyed in this solution, but the resulting color is a pale green, not blue at this stage. The indoxyl gets oxidized in the air to make the blue indigotin. This chemical reaction is shown in **Figure 2**. Sometimes a structural isomer of indigotin, indirubin, is formed in the reaction; this compound (**Figure 3**) gives a reddish tinge to the resulting dye. Since all indigo-producing plants undergo this same chemical reaction, it is generally not considered possible to differentiate the source of these blue dyes in archaeological textiles.[2]

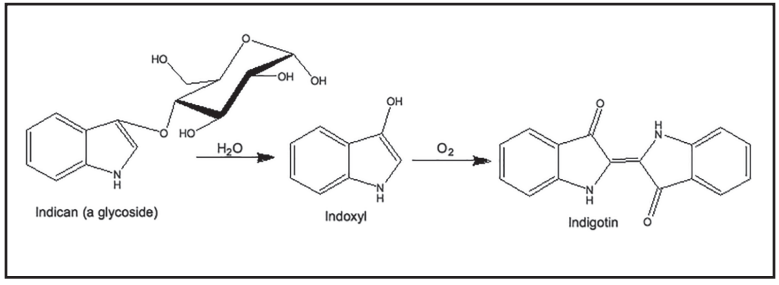

Figure 1. The structure of indigotin (right) and indican (left). The glucose molecule bonded to the oxygen makes indican a *glycoside.*

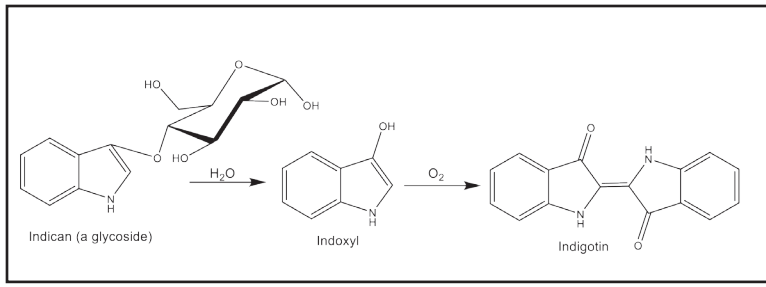

Figure 2. The chemical reactions that give indigo blue dye.

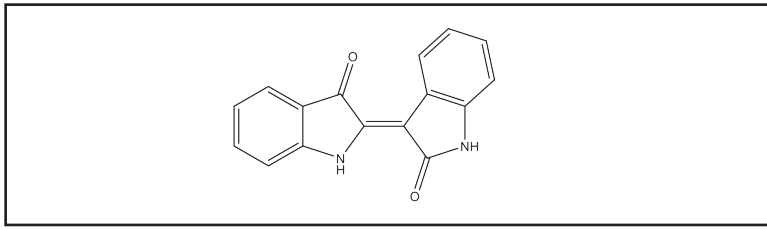

Figure 3. The structure of indirubin, a structural isomer of indigotin.

PREVIOUS STUDIES

It is impossible to source the exact plant used to produce blue dyes in ancient textiles without chemical analysis. Previous research has been able to identify the chemical structure and colorant compounds of these plants, although this does not really help archeologists understand the plant species from which the dyes originated. Knowing the exact plant origin of the blue dyes in archaeological textiles can help archaeologists understand why ancient people chose one plant over another, and the chemical process of dyeing with blue dyes. Also, since some plants are only native to certain areas, the origin of the indigo-derived dye can give archaeologists insight on any possible trade within regions.

Over the past several decades, a number of analytical chemistry methods have been used for the characterization of blue dyes in conservation science. Many of these methods involve chromatography, a method for separating mixtures. To identify the molecules, spectroscopic methods that measure how the molecules absorb light can be used. Spectroscopic methods may use a single wavelength of ultraviolet or visible light, or can use a diode array detector, which provides a spectrum (showing how the molecule

absorbs light over a range of wavelengths) for each compound. While a UV-visible spectrum can help to identify a molecule from among a number of possibilities, it is not good for identifying completely unknown compounds. For that, it is best to use mass spectrometry (MS), which gives a fingerprint of sorts that can be used to pick out structural information about the molecules present.

High-performance liquid chromatography (HPLC) has been used to analyze ancient textiles that consisted of blue dyes. Pulchaska et al.[3] utilized a liquid chromatography system with mass spectrometry detection (LC-MSD) to identify and separate indigoid dyes. Mass spectrometric detection is useful for the detection of colorless compounds that do not absorb well in the visible region. A small amount of indirubin was found in the woad samples in this study. However, the origin of indigotin dyes could not be determined. Blue fibers from a Japanese tapestry were also extracted and analyzed using the HPLC method. This method was able to identify indigotin as a compound in these samples.

HPLC with photodiode array and mass spectrometry detection was used by Mouri et al. to analyze extracts of plant material, dyed silk and wool from the Chehr Abad salt mine in Iran[2]. One of the samples consisted of a blue-dyed yarn. This sample showed the presence of indigotin and significant amounts of indirubin. This textile was described as "pseudoindirubin", which the authors had seen in a number of ancient textiles and in modern samples of woad. However, the "pseudoindirubin" was not an indirubin isomer, and its structure could not be determined. If this molecule can be isolated and identified, it may provide a way of differentiating between woad and other indigo sources.

Blue dyed fibers from San Pedro de Atacama were also analyzed by HPLC-DAD[3]. The San Pedro de Atacama region has been known in history for its network of trade, goods, and ideas. There are numerous records of textiles from different cultures that were traded throughout the region around San Pedro de Atacama. The blue textiles all contained a mixture of indigo and indirubin. A plant source of the indigo present was not characterized.

Degano et al.[4] studied a variety of pre-Columbian Peruvian blue-colored textiles using HPLC as their analytical method. A combination of indigotin and indirubin was present

in the samples. The study suggests that *Indigofera tinctoria* or *Indigofera suffruticose* may have been the plant source, due to local availability. However, their study was unable to identify a distinct plant source based on the chemical composition.

Wallert et al.[5] performed UV-vis absorption spectrometry (UVAS) on blue-dyed textiles from Chiribaya Alta and El Algodonal. The blue textiles showed the presence of indigo. The study could not distinguish whether or not the blue dye originated from *Indigofera tinctoria* or from the leaves of *Indigofera suffruticosa*.

In a report from 2015, gas chromatography-mass spectrometry (GC-MS) was used to detect and identify blue dyes in textiles from an ancient tapestry.[6] Reference wool and historical samples were used in the laboratory. Indigotin, the main color compound of both *Indigofera tinctoria* and *Isatis* was easily recognized through the GC-MS method. However, indirubin was not detected by this approach, nor were any of the glycoside compounds that might differentiate woad and indigo. This GC-MS method was considered an effective and convenient one for identifying indigo dyes on textiles. This method was able to detect indigo in blue fabric samples from the 17th and 18th centuries.

All mass spectrometry methods need an *ionization source*, a step that gives the molecules of interest – in our case, indigotin and the other dye colorants – a charge so that it can be measured. When used with HPLC, the most common method involves electrospray ionization, while in GC-MS, electron impact ionization is common. Since 2005, a number of methods for making ions in the open air (called *ambient ionization*) have been developed. One such method is DART, or direct analysis in real time ionization. This method has proven very useful in identifying a number of dyes, including indigo. Much of this work was done at Eastern Michigan University. Of particular note was the identification, without any sample preparation, of indigotin in a clump of a few fibers from a collection of reference materials dating to the 19th century.[7] A related method, atmospheric solids analysis probe (ASAP-MS), was used by Kramell et al.[8] to analyze blue colorants in a variety of ancient textiles. ASAP-MS was able to identify the isomeric compounds of indigo and indirubin compounds.

Neither of these ionization methods were able to characterize the plant source of the indigo compounds.

Sometimes combining mass spectrometry and spectroscopy provides additional information about dyes. Lee et al.[9] used time-of-flight secondary ion mass spectrometry (TOF-SIMS) and Fourier transform infrared spectroscopy (FTIR) to characterize blue dyes. These two analytical methods are considered non-destructive and efficient. In the study, three ancient blue textiles from an 18th-century Korean tomb were analyzed using TOF-SIMS. All three ancient textiles contained indigo. The indigo plant used to produce these Korean textiles could not be distinguished.

Overall, there is a common theme throughout all previous studies of ancient blue dyes in textiles. Numerous analytical methods have been developed to detect indigo-related colorants. However, a method to source the origin of the plant species has yet to be developed, since all indigo-producing plants undergo the same chemical reaction. Kaempferol and other flavonoids and their glycosides might help reveal more differences between the various blue dye source materials in the future. The ability to distinguish between them would help archaeologists better understand any possible trade, and the chemical process of indigo dyeing, throughout different regions and cultures.

As described earlier, HPLC can provide information about glycoside compounds present in dyed textiles when the correct sample preparation method is applied. But long extractions and even longer separations are necessary. Studying the glycoside molecules in the dyes without using HPLC is difficult. Neither DART nor ASAP are capable of ionizing these molecules directly, so other methods must be used to study glycosides by direct mass spectrometry. Two that have potential for dye analysis include paper spray (PS-MS) and matrix-assisted ionization (MAI).

PS-MS is an analytical method that can be used to identify glycoside compounds in textiles. Recognizing glycosides compounds in indigo and woad dyes may help contribute to differentiating the source of blue dyes in ancient textiles. PS-MS is used for various quantitative and qualitative applications. It allows direct chemical analysis from small molecules to large biomolecules. PS-MS has a very simple setup. A triangular paper

substrate is held by a metal clip connected to a DC high voltage power supply, placed in front of a mass spectrometer. Once the power supply is turned on, it generates charged droplets, causing the analytes from the sample to become charged ions and pass into the mass spectrometer for identification.[10]

Matrix-assisted ionization (MAI) also allows simple and fast mass spectrometric characterization of compounds. MAI converts compounds to gas phase ions when they are mixed with a matrix and then exposed to low pressure in the sample opening of a mass spectrometer. There is no need for high voltages or heat. MAI enables the analysis of low and high mass compounds with minimal sample preparation, and has proven to be effective for identifying synthetic dye compounds.[11]

This paper presents our work so far on using DART and paper spray ionization to study indigo, woad and knotweed dyes on a variety of different fiber substrates, including cotton, wool (both from sheep and alpaca), and silk.

MATERIALS AND METHODS

Materials

The natural dyes woad, indigo, and knotweed were gathered from various suppliers. Natural indigo (*Indigofera*) was obtained from Maiwa (Vancouver, Canada). The knotweed was purchased from Kremer Pigments (New York, USA), and the woad came from Dragon Dyeworks (Portland, OR, USA). Natural fiber yarns of wool (sheep), cotton, and silk, and cotton fabric (TestFabrics, West Pittston, PA) and alpaca yarn (from a local fiber fair) were used in this study.

Vat dyes of indigo, woad, and knotweed were prepared using standard recipes. The dye bath was prepared by adding the natural dye powders to a beaker of warm water. Sodium hydroxide (to make the solution basic) and sodium dithionite (a reducing agent) were slowly stirred into the solution, at which point the indigotin was reduced to give a solution containing indoxyl. The solution was kept warm for thirty minutes and frequently stirred until everything was dissolved. The dye bath was considered ready when it appeared as a clear greenish-yellow color.

The wool, alpaca, silk and cotton reference yarns were first wetted and then immersed in the dye bath for ten minutes. The cotton fabric was prepared for tie-dye, with portions of the fabric gathered together and "tied" with rubber bands before introducing the fabric into the dye vat. The samples were taken out of the vat and rinsed with aerated tap water to help with oxidation. The samples were left to dry at room temperature. The resulting samples are shown in **Figures 4-6**. Some of the difference in the colors obtained is due to how long the fibers were allowed to sit in the dye vat. The tie-dyed cotton was made because it is known that Andean people prepared complex colors by this method using overdyeing.

The resulting fiber bundles were sampled for mass spectrometric analysis. Clumps of fibers from each sample were placed in microcentrifuge vials for extraction, which was necessary for paper spray mass spectrometry. A 1:1 ratio of dimethylformamide and 0.1% Na_2EDTA in water was used to extract the colorants from the fibers; this solution preserves glycosides, which are cleaved when acidic extractions are used. The samples were sonicated at 60°C for an hour.

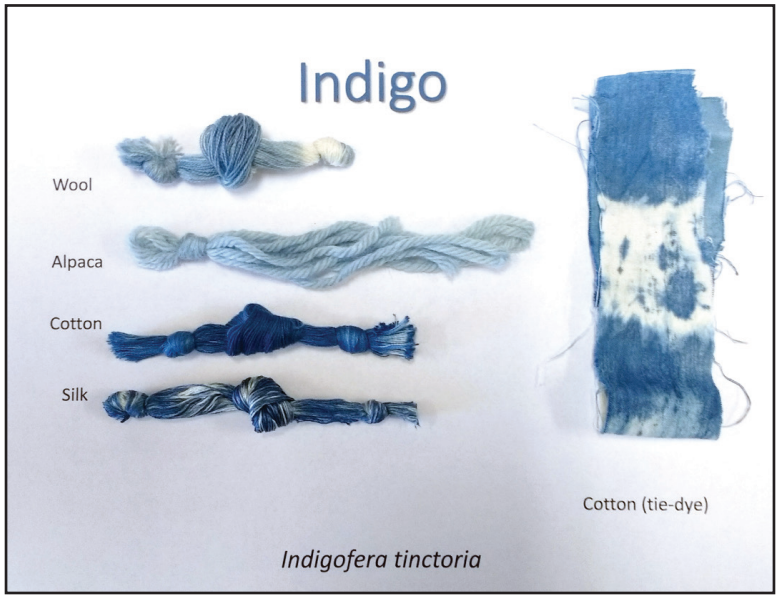

Figure 4. Images of the fibers dyed with indigo (Maiwa).

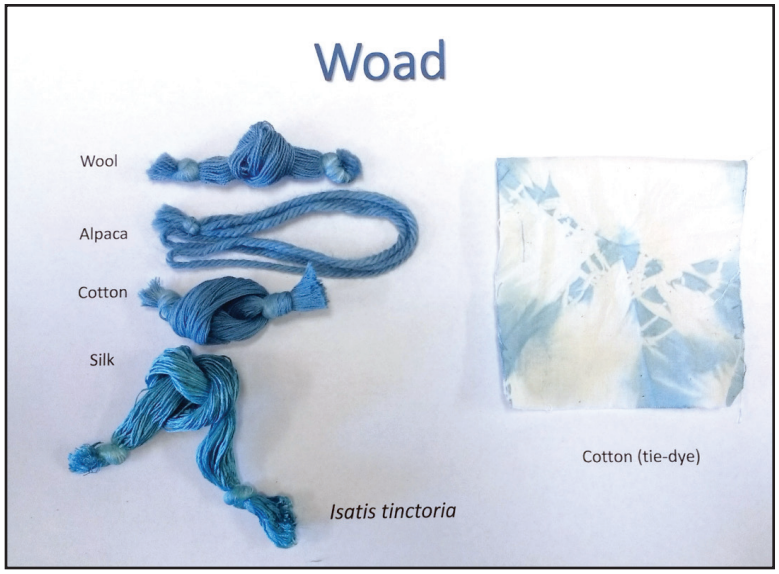

Figure 5. Images of the fibers dyed with woad (Dragon Dyeworks).

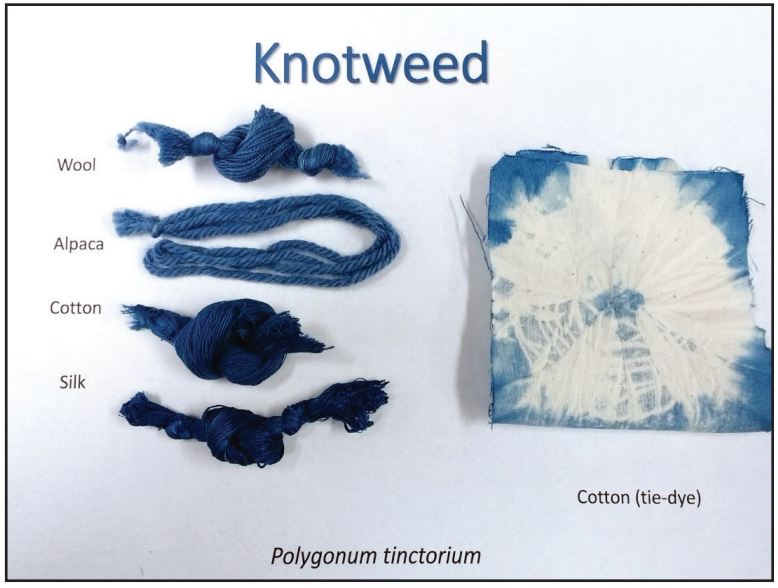

Figure 6. Images of the fibers dyed with *Polygonum tinctorium* (Kremer).

Methods: DART-MS

Approximately 1 mm of yarn was removed from each sample. The fibers were held in tweezers and introduced into the gap of the DART ionization source. Samples were examined in positive ion mode at 350 ºC.

Methods: PS-MS

Paper spray is a type of electrospray ionization for mass spectrometry. The extract of each dye in the 1:1 DMF:EDTA solution was pipetted onto the paper electrode of the PS ion source. The electrode voltage was 3500V and the samples were examined in positive ion mode.

RESULTS

Indigo

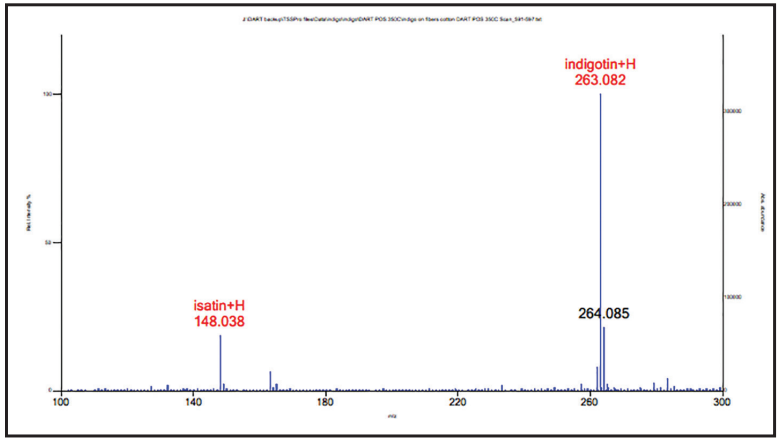

Figure 7. DART mass spectrum of indigo-dyed cotton fibers showing primary colorant indigotin and isatin, a side product of the indigo dye reaction.

DART-MS

The direct analysis in real time mass spectra (DART-MS) all showed a major peak for indigotin (as the MH^+ ion) at *m/z* 263.08 for indigo-dyed wool, alpaca, cotton, and tie-dye samples of indigo, as shown in **Figure 7**. A smaller peak at *m/z* 134.06, corresponding to the MH^+ ion of indoxyl, was present in the samples of alpaca, silk, cotton, and tie-dyed fibers as well.

PS-MS

For reasons that remain unclear, PS-MS on extracts from indigo-dyed fibers showed none of the expected indigoid compounds. Further studies are necessary to determine why this occurred.

Woad

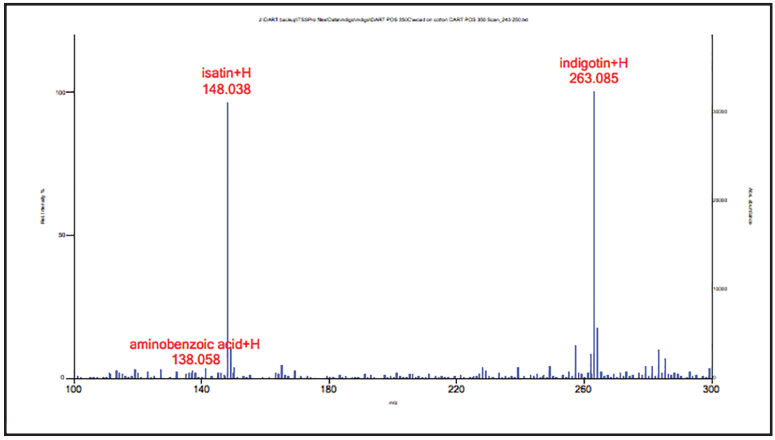

Figure 8. DART mass spectrum of cotton yarn dyed with woad. Aminobenzoic acid is a degradation product often observed in indigo-based dyes.

DART-MS

The DART mass spectra for woad were primarily the same as those observed for indigo. The base peak of the spectra was in all cases at *m/z* 263.08, consistent with the MH^+ ion of indigotin. The silk sample showed a small peak at *m/z* 134.06, identified as the MH+ ion of indoxyl. All of the samples also showed a significant peak at *m/z* 148.03 which indicated the presence of isatin (as the MH^+). An example DART spectrum for a woad sample is shown in **Figure 8.**

PS-MS

PS-MS showed a small peak in the extract from the alpaca wool for the glycoside compound kaempferitrin, indicated by a peak at *m/z* 569.1. In one of the replicates obtained on the extract from cotton dyed with woad, a peak at *m/z* 366.10, indicative of the compound isatan A, thought to be characteristic of woad, was observed.

Knotweed

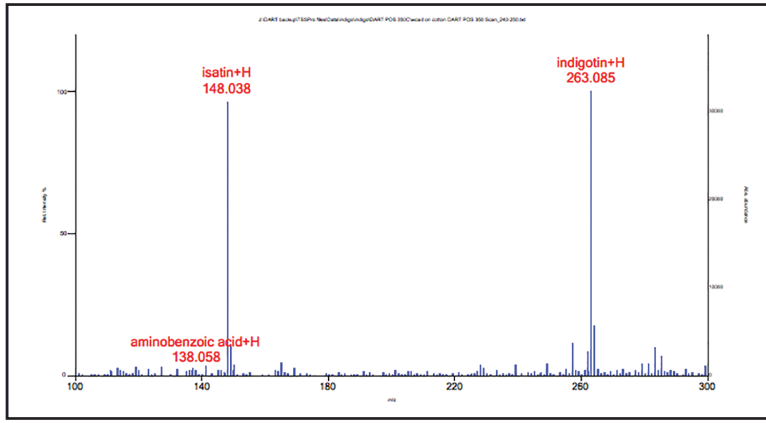

Figure 9. DART mass spectrum of cotton dyed with knotweed. Isatoic anhydride is another degradation product of indigo dyes.

DART-MS

As expected, the DART mass spectra for fibers dyed with knotweed were basically indistinguishable from those obtained for the other indigo sources, as shown in **Figure 9**. Indigotin (MH+) dominated the spectra of all the samples, and isatin was also observed consistently. Indoxyl was only observed in the tie-dyed and cotton samples.

PS-MS

The tie-dyed cotton extract showed a small peak consistent with the mass of the glycoside afzelin at *m/z* 432.09. Another glycoside, astragalin, is indicated by a peak at *m/z* 448.09, in the extract of the cotton sample prepared with knotweed.

SUMMARY OF RESULTS

The DART-MS and PS-MS methods show that different molecules can be identified in the various samples using the two methods. This shows that the methods are complementary, and that they are best used together to fully characterize dyed fibers. **Table 1** shows the complete results for the suite of compounds we were looking for in the indigo dyes.

Compound name	Formula	Indigo (*Indigofera*)					Woad (*Isatis*)					Knotweed (*Polygonum*)				
		W	C	S	A	TD	W	C	S	A	TD	W	C	S	A	TD
indigotin (indirubin)	$C_{16}H_{10}N_2O_2$	P D	D	PD	PD	D	D	D	D	PD	D	D	D	PD	PD	D
indoxyl	C_8H_7NO		D	D	D	D			D	P			PD	D		D
indican	$C_{14}H_{18}NO_6$	P	D	P		P									P	
isatin	$C_8H_5NO_2$	PD	PD	PD	PD	PD	PD	D	D	PD	D	PD	PD	PD	PD	D
isatoic anhydride	$C_8H_5NO_3$	PD	PD	D		PD	PD	PD	D	PD	D	PD	PD			D
aminobenzoic acid	$C_7H_7NO_2$	PD	D	D	D	D	PD	D	D	PD	D	D	D			D
kaempferol	$C_{15}H_{10}O_6$		P		P	P				P						
afzelin	$C_{21}H_{20}O_{10}$				P	P		P								P
astragalin (trifolin)	$C_{21}H_{20}O_{11}$												P			
kaempferitrin	$C_{27}H_{30}O_{12}$				P	P				P						
robinin	$C_{33}H_{40}O_{19}$															
isatan A	$C_{17}H_{17}O_9$							P		P			P			
isatan B	$C_{14}H_{15}O_6$	P		P						P		P	P			

Table 1. Summary of all mass spectrometry results for this project. Some compounds have isomers (same mass, different structure) that cannot be differentiated using these methods; those compounds are shown in parentheses. (W = wool; C = cotton; S = silk; A = alpaca; TD = tie-dyed cotton; P = paper spray; D = DART)

DISCUSSION

Our study shows that DART-MS and PS-MS can be used as efficient analytical methods to detect indigo-producing colorants in lab-prepared textiles. In the indigo dyed samples, a major peak in DART-MS was indigotin or indirubin, an isomer of indigotin. A minor peak for indoxyl, which is half of an indigotin molecule, was also recognized. For the indigo samples, the PS-MS results were not very reproducible. As expected, PS-MS showed flavonols and glycosides such as kaempferol, kaempferitrin, and afzelin in the woad samples. Isatan A, a woad precursor, were sometimes observed by PS-MS. The DART-MS results for woad and knotweed were similar to those obtained for the indigo samples. PS-MS observed flavonol glycosides, such as afzelin and astragalin, in the knotweed samples.

Although DART-MS and PS-MS were useful for identifying the colorants compounds, the ability to source the origin of the plant species was not feasible based on the list of compounds shown in **Table 1**. Further studies on other non-color related compounds, such as kaempferol and other flavonoids and their glycosides, may possibly help reveal more differences between indigo and woad in the future.

Future Work on Secondary Colors

To date, the majority of work on identification of dyes in archaeological materials has focused on the primary colors red, blue and yellow. Secondary colors – green and purple – can be made by overdyeing fibers first dyed red or yellow with indigo. South American people, especially the Wari, made complex and multicolored fabric objects. Knowing how they made the different colors can help archaeologists and museum conservators better understand the Wari's technology and even trade routes.

To evaluate the utility of our methods for identifying multiple colorants in secondary colors, we performed DART-MS and PS-MS on lab-prepared materials, including indigo over-dyed samples on red obtained from cochineal insects and madder root, as well as on a yellow sample prepared with quercitron bark powder. Our preliminary results show that multiple colorants can be identified at the same time without any sample preparation. The DART-MS method was not capable of identifying cochineal

insect dye in samples because the main colorant from those insects, carminic acid, is not volatile enough for analysis by DART-MS. Extraction and paper spray analysis will be needed to confirm the presence of that dye in historic and archaeological materials.

Acknowledgements

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REFERENCES

Splitstoser, J., Dillehay, T., Wouters, J., Claro, A. (2016). Early pre-Hispanic use of Indigo blue in Peru. Sci Adv. doi:10.1126/sciadv.1501623

Kramell, A., Porbeck, F., Kluge, R., Wiesner, A., and Csuk, R. (2015). A fast and reliable detection of indigo in historic and prehistoric textile samples. J. Mass Spectrom., 50: 1039–1043. doi:10.1002/jms.3625

Mouri, C., Aali, A., Zhang, X., & Laursen, R. (2014). Analysis of dyes in textiles from the Chehrabad salt mine in Iran. Heritage Science, 2(1), 20-28. doi:10.1186/s40494-014-0020-3

Niemeyer,H., Agüero, C., (2015). Dyes used in pre-Hispanic textiles from the Middle and Late Intermediate periods of San Pedro de Atacama (northern Chile): new insights into patterns of exchange and mobility. Journal of Archaeological Science. doi:10.1016/j.jas.2015.02.003.

Degano, I., Colombini, M., (2009). Multi-analytical techniques for the study of pre-Columbian mummies and related funerary materials. Journal of Archaeological Science, 36(8), 1783-1790. doi:10.1016/j.jas.2009.04.015

Wallert, A., Boytner, R., (1996). Dyes from the Tumilaca and Chiribaya cultures, south coast of Peru. Journal of Archaeological Science, 23(6), 853-861. doi:10.1006/jasc.1996.0079

Selvius DeRoo, C.; Armitage, R. A., (2011). Direct identification of dyes in textiles by direct analysis in real time-Time of Flight Mass Spectrometry. Anal. Chem. 83 (18), 6924.

Degani, L., Riedo, C., & Chiantore, O. (2015). Identification of natural indigo in historical textiles by GC–MS. Analytical and Bioanalytical Chemistry, 407(6), 1695-1704. doi:10.1007/s00216-014-8423-2

Kramell, A., Porbeck, F., Kluge, R., Wiesner, A., and Csuk, R. (2015). A fast and reliable detection of indigo in historic and prehistoric textile samples. J. Mass Spectrom, 50: 1039–1043. doi: 10.1002/jms.3625

Lee J., Kim M.-H., Lee K.-B., van Elslande E., Walter P., and Lee Y. (2014). Analysis of natural dyes in archeological textiles using TOF-SIMS and other analytical techniques, Surf. Interface Anal. 312–316. doi:10.1002/sia.5594

Devereaux, Z. J., Reynolds, C. A., Foley, C. D., Fischer, J. L., Deleeuw, J. L., Wager-Miller, J., .Trimpin, S. (2016). Matrix-assisted ionization (MAI) on a portable mass spectrometer: Analysis directly from biological and synthetic materials. Analytical Chemistry, Just Accepted Article. doi:10.1021/acs.analchem.6b00304

Wang, H., Jiangjiang, L., Cooks, R., Ouyang, Z. (2010). Paper spray for direct analysis of complex mixtures using mass spectrometry. 122(5). doi:10.1002/ange.200906314