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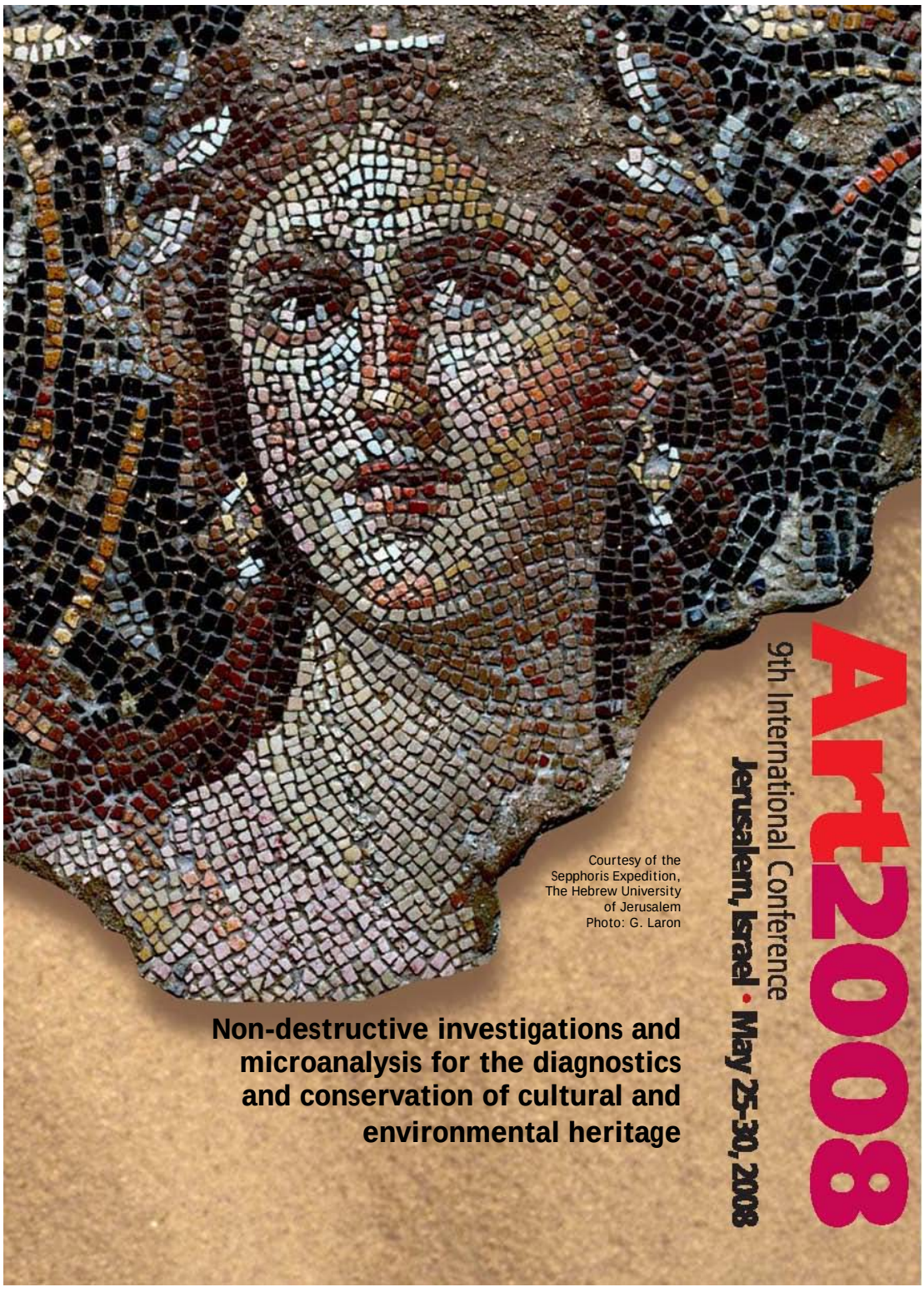

NON-DESTRUCTIVE VS. MICROCHEMICAL ANALYSES: THE CASE OF DYES AND PIGMENTS

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ABSTRACT

Non-destructive tests (NDT) of historical artifacts are the ideal scientific tools for the analyses of culturally important heritage objects. However, for the investigations of certain materials, especially natural organic dyes and pigments, it is important for curators, conservators, and scientists to be aware that such analyses are not always practical or prudent. At best, NDT can definitively detect the presence of the major component of a dyestuff or pigment, but will have inherent problems in positively identifying other colorants present in the natural pigment or dyestuff. The identification of these secondary components is necessary for the determination of the botanical, entomological, or malacological provenance of the dyestuff. As such, these techniques will only provide a global picture of the coloring material on an object and will have difficulty in identifying the specific species that produced the colorant. At worst, in some dye analyses, NDT may produce artificial or even erroneous results.

Microchemical tests (MCT), especially high-performance liquid chromatography (HPLC) coupled with photodiode array (PDA) and/or mass spectrometric (MS) detection, have pushed the limits of detection of organic colorants to picogram levels. Thus, only miniscule sampling of the material being examined is necessary, which affords the experimentalist an advanced instrumental tool that is nearly non-destructive and does not affect the structural or aesthetic integrity of the object. Such MCT can provide a wealth of multi-component information vital to the determination of the biological provenance of the dyestuff, which may also shed light on its geographical point of origin.

Several case studies of archaeological colorants, primarily from ancient Israel, are discussed in terms of the above arguments, and they include the dye sources mentioned above, including the famous rare royal purple pigment.

ORGANIC DYES AND PIGMENTS: WHAT'S THE DIFFERENCE?

This work will not discuss inorganic pigments, which are composed of various metal–non-metal ionic compounds that have been used in the **painting** of the surface of a substrate, such as a wall, sarcophagus, pottery, canvas, or even a textile (notably burial shrouds).

In this work, only organic colorants (dyes or pigments) used for the true **dyeing** of textiles are addressed [Koren 1993]. Various definitions have been given in the literature for these two types of colorants. The difference between them relates to the relative solubilities of these colorants in water, whether neutral, acidic, or basic (alkaline). A dye, without being complexed to another chemical species, is a textile colorant that is generally soluble in aqueous solution. A pigment is relatively water-insoluble and can thus also be used as a paint colorant. In any textile dyeing process the colorant must first be dissolved in water, and in this way the individual dissolved molecules can penetrate into the interiors of the fibers and attach themselves to them via physico-chemical bonds. A substance that is categorized as a dye readily dissolves in water in its original chemical form, by definition. For a water-insoluble pigment to be used as a textile "dye", it must first be transformed to a chemically soluble form. That is the case with the famous dark blue indigotin pigment that is produced via a complex reduction and oxidation process from the precursor molecules present in the

leaves of the indigo plant *(Indigofera tinctoria)* or from the woad plant *(Isatis tinctorum)*. That pigment is dissolved by chemically changing it to a reduced form in an alkaline solution. This slightly altered molecule is yellow-green in color and as it is "whiter" than the original navy-blue color of indigotin, it is referred to as leuco-indigotin. Once the original dye, or the chemically altered pigment, has been dissolved to form a dye bath, the textile is immersed in the heated solution to begin the dyeing process. However, the final product – the textile dyeing – has been attained whether a "dye" or a "pigment" was initially used.

The dyestuff sources of these organic colorants are flora and fauna, and the latter case includes certain red-producing scale insects yielding shades of scarlet (orangey-red) to crimson (bluish-red) and sea mollusks that produce purple and violet pigments. The red-producing plant roots of the madder species of plants, such as the famous "dyer's madder" *(Rubia tinctorum)* inhabiting both Europe and the Middle East, as well the bodies of dried female scale insects, need a fixative – a salt, such as alum or one containing iron, that acts as a mordant – in order to strengthen the attachment of the dye to the textile fibers. These are then denoted as mordant dyes. Molluskan pigments, on the other hand are not used with a mordant, but rather are treated similarly to plant-produced indigotin, that is first chemically reduced to dissolve them in a container or vat (this process is known as "vatting"). After the immersed textile is removed from the dye bath, the dissolved leuco-indigotin molecules undergo air-oxidation inside and on the fibers to re-form the indigotin pigment, but now on the textile. Hence both plant indigotin as well as molluskan pigments are known as vat dyes.

NON-DESTRUCTIVE METHODS USED IN DYE ANALYSES

Some of the non-destructive methods that have been used in the past for the study of dyes are described below.

Visible Reflectance Spectrophotometry

As dyes are by definition substances that absorb visible radiation of certain wavelengths and thus reflect light at other wavelengths, visible reflectance spectrophotometry was a logical candidate to attempt for the characterization and thus identification of the dye on a textile. In this method, the dyed textile is irradiated with visible light (in the approximate wavelength range of 400 – 700 nm) by means of a spectrophotometer and the intensity and wavelengths of the light that is not absorbed by the dye-textile matrix, but rather reflected, is measured. This produces a reflectance spectrum. This is a fairly simple and relatively harmless method, as long as the time of irradiation is relatively short.

However, this method is extremely risky for use in the identification of a specific dye in an archaeological textile. This is due to the fact that, for example, many red dyes, by the very nature that they are colored red, will produce in many cases such similar reflectance spectra that it is often very ambiguous to determine which specific dye produced that spectra. This problem is often compounded by the presence of other colorants that are typically present in most natural dyestuffs as well by the other impurities in the ancient textile that has been excavated from the soil. These components will contribute to the makeup of the overall reflectance spectrum making a clear identification very uncertain, and can lead to obviously erroneous results.

An additional drawback of this method is that it is nearly useless for the identification of minor colorants that are often also present in the dyeing. These components, though in minor composition, provide for the chemical fingerprinting of the dyestuff. Thus, one can tell the difference between, for example, various botanical species of the same overall dyestuff. A

case in point is the famous red-producing roots of the madder family of dyes. Many varieties of this exist, ranging for example from the Indian variety, known as *Rubia munjista*, to the European and Middle Eastern dyer's madder, *Rubia tinctorum*, as well as wild madder, *Rubia peregrina* of the Levant. While they all contain hydroxy-anthraquinones, and all will produce reddish hues, however the type and quantities of the components are all different. Thus, even if this optical method can determine the correct dyestuff from which the dye was produced, it cannot provide the fine detail that is necessary to quantify the various multiple components that constitute the dye or pigment.

An additional problem with this method is that often the warp and weft yarns are colored differently, with the warp often undyed. Hence, in order to use this method, the warp and weft yarns need to be separated from each other, and damaging the structural integrity of the fabric, which would call into the question the very name of this "non-destructive" technique.

The visible reflectance spectrophotometry method therefore cannot be used in identifying dyes. Though it may be non-destructive to the artifact, it is also in most cases, unfortunately, "non-informative". The reflectance spectrometric method was originally developed to provide, among other things, an objective mathematical characterization of the color or hue of a textile or any other substrate (plastics, paper, leather, etc.). This method thus eliminated the subjective determination of color that the old color schemes provided, such as the Munsell system [Billmeyer and Saltzman 1981]. Such a spectrometer has often been referred to as the "color computer" and it is an excellent technique for the characterization of the color observed according to a standardized coordinate system, called CIE L*a*b*. However, it should not be used for the determination of which chemical species produced that color.

3D Fluorescence Spectrophotometry

Another non-destructive method that has been studied to determine whether it can be used in the analyses of those natural dyes that show fluorescence is three-dimensional fluorescence spectroscopy [van Bommel et al. 2007]. Fluorescence is a luminescence whereby the electrons in certain molecules absorb radiation of a relatively high energy (short wavelength) – the excitation wavelength – and their subsequent emission of visible light (low energy, high wavelength) – emission wavelength. This method provides a 3D contour mapping depicting the fluorescence intensity as a function of the emission and excitation wavelengths, and can be characteristic of a fluorescing dye.

This 3D spectrofluorimetric method has been shown to be of extremely limited use in identifying not only the minor dye components, but also of even the major component in a dyeing. This is due to the great similarities between the resultant contour plots obtained from different dyes. Additional problems with this method are that though the probe tip's diameter is small (4 mm), it is not small enough to focus entirely on individual weft and warp yarns. Further, the reliability of this method for the identification of natural colorants has been brought into question due to the influence on the reflectance maxima by the fluorescence of the substrate itself, by the process whereby the colorant was prepared, the sensitivity of some fluorophores to pH, and by many other process variables [Clarke 2002].

It was concluded that this method, at best, can be used as a screening technique to aid in the micro-sampling of an object for micro-chemical analysis. Thus, if in the examination of different parts of a textile sample, the fluorometric plots are nearly identical, then one need only sample from one of those areas. However, the application of this sophisticated

instrument for that purpose can be "overkill" as a much more inexpensive optical microscope can – and does – provide the same screening objectives.

Infrared and Raman Spectrometry

Infrared and Raman spectrometry have been – and still are – very popular techniques for the study of organic substances. Optical fiber probes are available for these instruments so that objects can be analyzed *in situ* without detaching them from their physical source. However, these methods are optimal when working with a relatively pure organic substance. This is most definitely not the case when analyzing archaeological textiles. These ancient textiles contain various organic substances, including the dyestuff, which itself can consist of a number of different colorants, the impurities found in the textile (especially as it is often found buried in the soil), and worst of all, the textile itself (usually wool). An attempt to identify the dye (an organic substance) on a textile (another organic material), with the weight composition of the dye at about 1% of the weight of the textile, is precarious. Though sophisticated mathematical processing can be performed on the resultant spectra these algorithms can introduce artificial peak signals. The ability of this method to hone in on a specific organic substance, which is a very minor component in a field of a major organic material, is reminiscent of the proverbial "finding a needle in a haystack", though in the latter case, the needle is metallic and the haystack is organic.

DETACHMENT OF A SAMPLE FOR "NON-DESTRUCTIVE" ANALYSES

Some methods were applied for the analysis of ancient textiles – dyed and undyed – and reported as being "non-destructive", but the sample analyzed was physically removed from the main textile, which affects the structural integrity of the fabric. An example of this involves detaching the fibers and then pulverizing them together with KBr in order to produce the so-called KBr pellets for the infrared analysis of the textile and dye. Though the fibrous material will always remain as such within the pellet, needless to say, it can never be reattached to the fabric itself.

CHROMATOGRAPHIC METHODS

The most efficient technique for providing the maximum information regarding the identities of the dyes present on a textile is chromatography. The two main chromatographic methods that are still being used today for dye analyses are TLC and HPLC, as discussed below [Koren 1994]. TLC affords an aesthetic visual approach to the determination of the presence of the dyes, while the HPLC method produces more mathematically objective identification with much better sensitivity, but sacrificing some of the colorful niceties of TLC.

Thin-Layer Chromatography (TLC)

In the early days of chromatographic analyses of dyes, TLC (thin-layer chromatography) was used [Schweppe 1989]. This is a relatively inexpensive technique and can produce the necessary separation of the major components constituting the dyestuff.

This method involves the extraction of the dye from the textile by means of an appropriate solvent system that depends on the nature of the dye – mordant or vat. A drop of the extracted dye solution is spotted at one end of the surface of a plate (metal, glass, or plastic) that has been coated with a thin-layer of dye-retaining material. Once the spot has dried the plate is then placed in a closed vessel containing a shallow reservoir consisting of a solvent system for which the dye has some affinity. When the solvent ascends the plate by capillary action, it pulls with it various components that were in the original extracted drop. These constituents have different affinities to the stationary phase (the thinly layered coating on the

plate) and to the mobile phase (the solvent system) and these dual competitions yield the separations. The resultant set of separated component spots is known as a chromatogram. The distance travelled by each component from the starting spot, relative to the solvent front, can be characteristic of the dye and this can be used for the identification of the dyestuff.

This method can be used with generally good results when calibrated against known dye substances. However, there are several drawbacks to this method that limit its usefulness: (1) In order to visualize the separated component spots it is necessary to use a sampling size that is significantly larger than the other chromatographic method (HPLC) discussed below. (2) The sample size thus cannot in general detect all the important minor components of the dyestuff. (3) Though various instrumental systems have been developed with the appropriate software that can be used to produce computerized optical information regarding the separated component spots, this method is rather limited, again due to its sample size. (4) Sometimes the experimentalist encounters "unexpected" technical problems even when all procedures for producing a good TLC chromatogram were seemingly followed, but for some "unexplained" reasons the separations are not efficient. This problem may stem from a poor stationary phase surface or mishandling of the plates, which enforces the adage that "it often takes a good deal of TLC (tender-loving care) to produce a good TLC".

High-Performance Liquid Chromatography (HPLC)
This method is to-date the most efficient one to be used for the analyses of dyes.

All chromatographic methods are similar, as the technique consists of stationary and mobile phases. In the HPLC method, the stationary phase is a highly packed matrix composed typically of a non-polar material, such as the alkane (saturated hydrocarbon) having 18 carbon atoms, also known as C-18, housed in a stainless steel column. Hence, the mobile phase solvents are polar reagents typically composed of water, methanol, and a weak acid. This setup is known as "reverse-phase" HPLC for historical reasons, as the first methods developed involved an opposite set of polarities – a polar stationary phase and non-polar solvents, and this was then referred to as "normal phase". This method was also known as high-pressure liquid chromatography due to the high pressures required (up to about 300 atm) in order to push the sample and eluents through the column. The high surface area of the particles in the column provides for high resolution of the separated components, and hence the name "high performance". After the components elute (or wash) out of the column they are then subjected to ultraviolet and visible radiation in order to detect their presence through their absorption of radiation at certain wavelengths. The length of time that a component is retained in the column before eluting out of it is known as the retention time, denoted as R.T. or t_R. The resultant computer-generated graph (referred to as a chromatogram, as in TLC) that is produced with this method is a plot of the absorption intensity as a function of time, and shows a peak for each separated component at its respective retention time.

The advantages of the HPLC method over the other chromatographic method, TLC, are as follows: (1) Sample size is much less due to the greater sensitivity of the spectrometric detector as opposed to the naked-eye detection of separated TLC spots. (2) The reproducible chromatographic property produced – the retention time – can be indicative of the dye present. (3) Spectrometric data produced by the photodiode array (PDA) detector is also provided in the shape of a UV/Visible spectrum showing the wavelengths of maximum absorptions in the two regions. (4) The composition of each individual dye component in the dyeing can be quantified based on the relative area of its peak signal in the chromatogram.

(5) A digital file is produced and can be processed by various algorithms and stored in the computer.

In short, in the HPLC methodology, the chromatographic information (the retention time) concerning a dye is combined with its spectrometric property (UV/Vis spectrum). These dual properties help to uniquely characterize and thus identify the presence of that particular dye.

MICRO-SAMPLING NEEDED

The quantities needed for HPLC analyses are truly "nearly negligible". Good information regarding the identity of the dyestuff and its major and important minor components can be realistically obtained from a fragment of dyed yarn (or thread) composed of several single fibers that is only about a millimeter in length, with a weight on the order of a few micrograms (millionths of a gram). As the weight of a dye on the fiber is typically on the order of 1% of the weight of the textile, this translates into the ability to satisfactorily detect even nanogram (a billionth of a gram) levels of dye substance. In extreme situations, this nano-scale can be pushed even further into the picogram (a trillionth of a gram) region.

In many cases, the micro-sampling of a few millimeters of yarn can be performed at the periphery of the textile fragment where the weft and warp yarns are physically crumbling, and thus maintain the physical integrity of the textile material. In fact, in some cases, it may be only necessary to collect the fibrous powder naturally produced from the physical breakdown of the original textile fibers. Hence, the minimal micro-sampling needed is such a negligibly invasive technique that it can certainly be considered to be nearly non-destructive. The tremendous wealth of information obtained from the HPLC analyses of the dye extracted from such a minute quantity of textile material more than outweigh the nearly negligible sampling of the textile. The HPLC method "sees the invisible".

EXAMPLES OF ANALYSES OF ANCIENT DYES

Following are examples of the information that the HPLC method can yield.

The sample whose analytical results are shown in Figure 1, is a nearly 2,000-year old textile fragment excavated at the Masada hill-top in the Judean Desert outside of Jerusalem, Israel. The clear signals detected are those of alizarin and purpurin, which clearly indicate that the roots of a madder plant were used to produce that reddish color. As the two leading dye components detected show that purpurin is in major abundance over alizarin, the dyestuff that was used for producing this red-purple color was probably wild madder, which is a purpurin-rich source.

In another extraction of the same textile sample, the resultant HPLC chromatogram (Figure 1) shows the peak signals of the dark-blue pigment indigotin and its reddish isomer, indirubin. In the Middle east of the Roman Period the most likely native plant to produce the dominant blue coloration is woad.

The presence of dyes produced from two dyestuff sources is a classic example of double-dyeing or over-dyeing the same textile. Thus, the dyer deliberately used a blue-dye source (woad) and a red-dye source (wild madder), which when combined on one textile would yield purple or violet hues [Koren 1994, 2006].

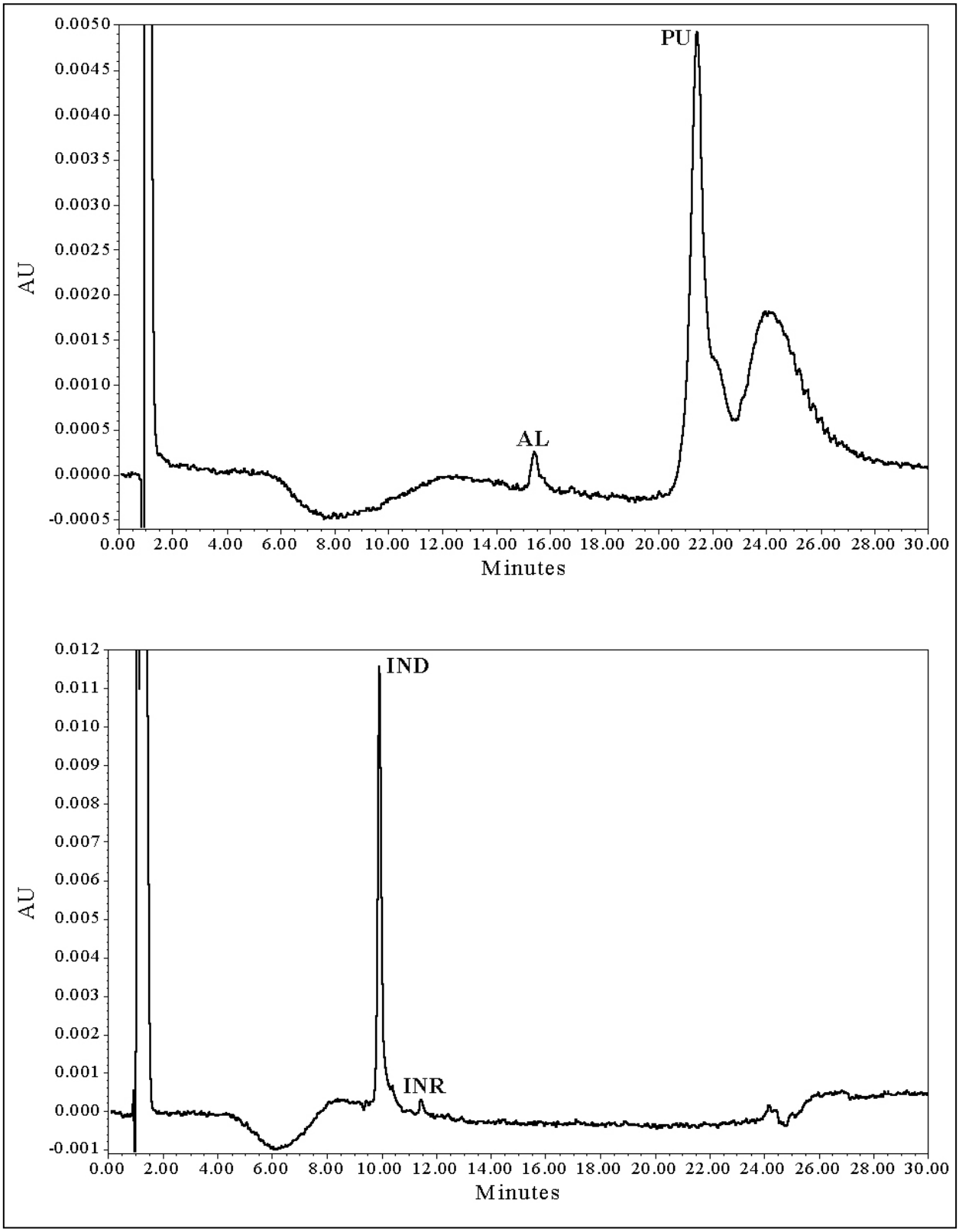

Figure 1. HPLC chromatograms of two extracts from the same purple-dyed yarn:
(top) an acidic methanol extract of the red-purple dye showing the presence of the minor alizarin ("AL") component and the major ("PU") purpurin dye, measured at 430 nm;
(bottom) a dimethylsulfoxide (DMSO) extract of the blue dye showing the presence of the major indigotin ("IND") component and the minor indirubin ("INR") dye, measured at 540 nm.
The UV/Vis spectra of these dyes are given in the next figure.

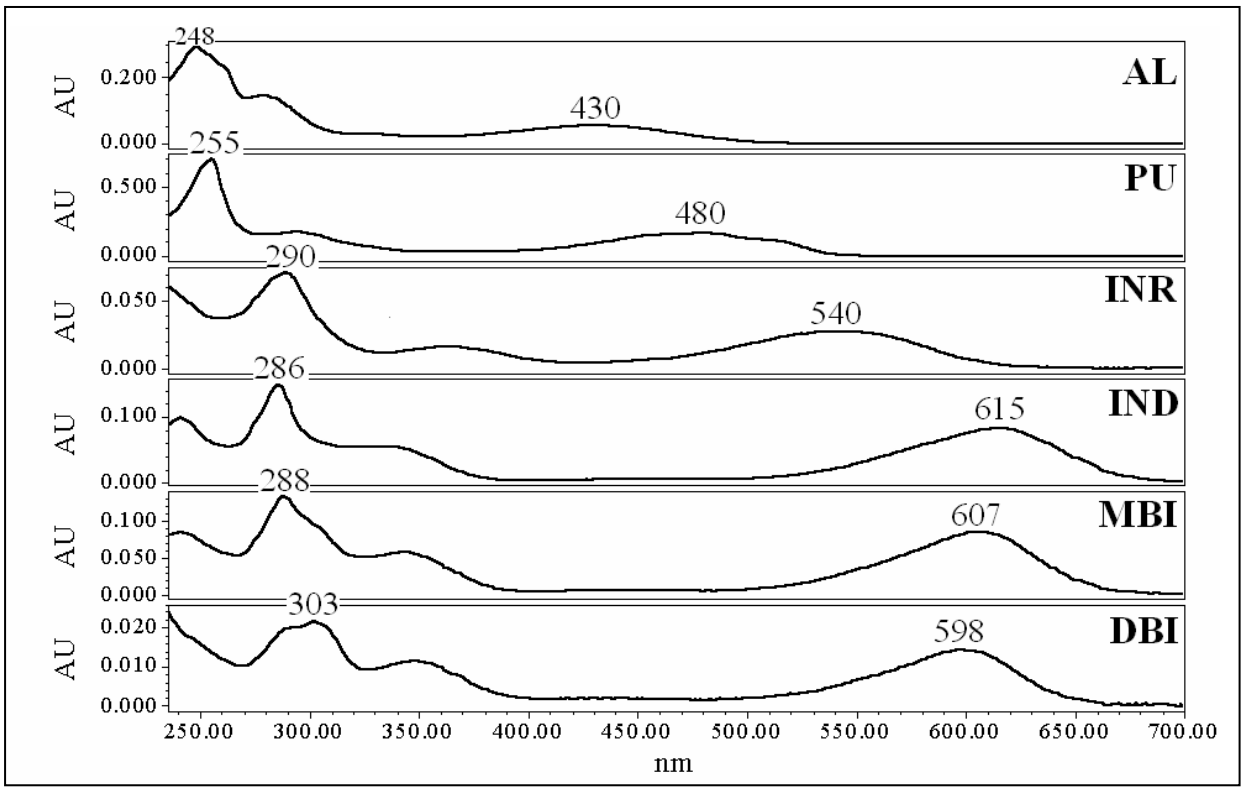

Figure 2. UV/Vis spectra of five dyes appearing in the chromatograms of Figs. 1 and 3, showing the wavelengths of maximum absorption in the visible and ultraviolet regions: AL = alizarin, PU = purpurin, INR = indirubin, IND = indigotin, MBI = 6-monobromoindigo, DBI = 6,6'-dibromoindigo

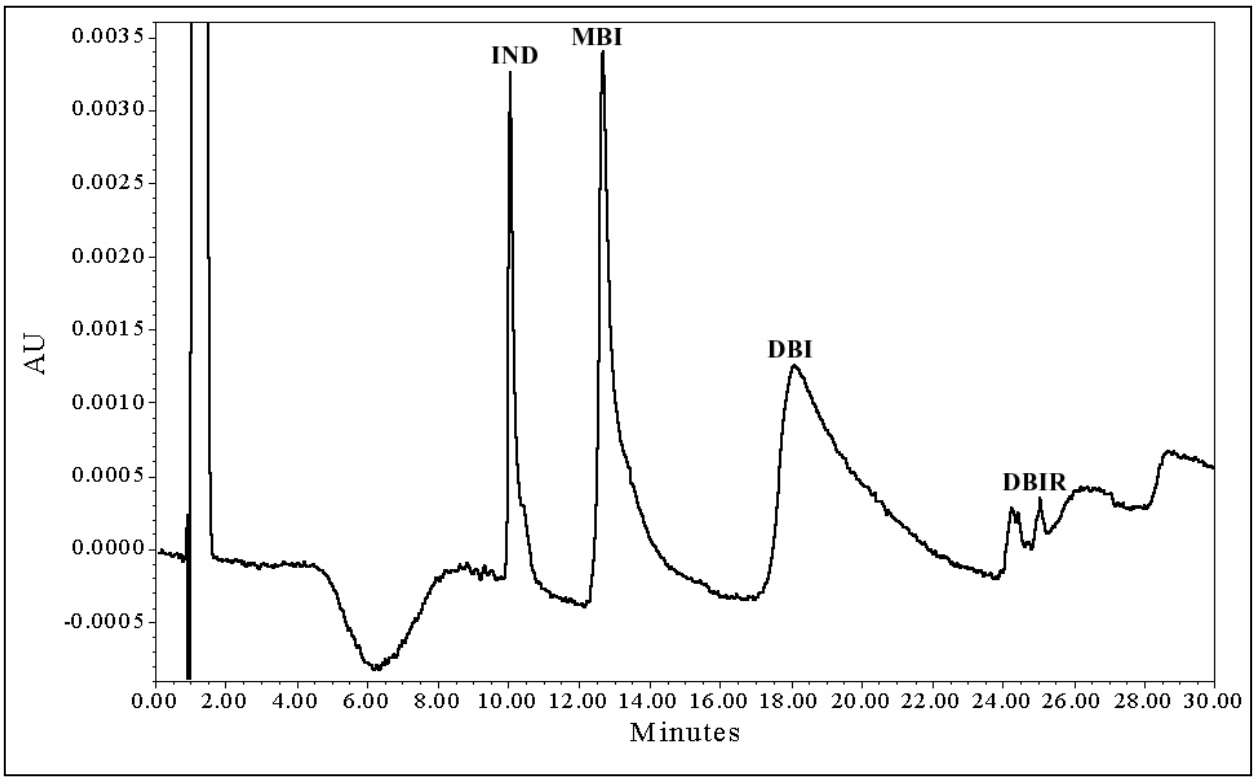

Figure 3. HPLC chromatogram at 600 nm of a DMSO extract of a real molluskan Royal Purple dye on a Herodian fabric from Masada showing the presence of four components: IND = indigotin, MBI = 6-monobromoindigo, DBI = 6,6'-dibromoindigo, and DBIR = 6,6'-dibromoindirubin.

In another rare archaeological sample excavated from the Herodian Period of Masada (late 1st cent. BCE), the chemical fingerprints of the molluskan purple pigment were detected [Koren 1997]. These dye markers are DBI, MBI, and indigo, as well as a fourth component, DBIR, and are identified in the chromatogram of Figure 3. The only source of such brominated colorants is a sea snail from the Muricidae family, and with the relative compositions indicated in the chromatogram, it can be surmised that the source of that pigment is a Murex trunculus sea snail that is rich in the DBI component [Koren 2008]. This textile dyeing has been referred to as Tyrian Purple or Royal Purple, which is certainly a color befitting King Herod – the builder of many palaces and structures in ancient Israel at the time before the historical turn of the millennium, 2,000 years ago.

CONCLUSIONS

All spectrometric techniques suffer from the same disadvantage: They provide an "overlap" of information. Thus, whether the object analyzed consists of one colorant or multiple colorants, only one graph or spectrum is produced, which is a superposition – a combination – of all of the components that constitute that dye. A chromatographic method, such as HPLC, is a microchemical technique that produces a "separation" of information and can detect various dye components on the nanogram scale, and in some cases even in the picogram region. The HPLC method coupled with a PDA detector and/or a mass spectrometric (MS) detector has thus become the workhorse for dye analyses. It extracts a wealth of information regarding the dyestuffs used for the production of color and no "non-destructive" technique can match or even come close to the resolving power of this method.

Museum curators and conservators should therefore not fear the micro-sampling procedure – when left in good and gentle hands – involved in the surgical extraction of a miniscule amount of yarn required for the subsequent microchemical HPLC analyses.

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