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**Methods of dye analysis used at the Shenkar College  
Edelstein Center in Israel**

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# Methods of dye analysis used at the Shenkar College Edelstein Center in Israel

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## Introduction

The four most common techniques that are, or have been, used for the analysis of ancient textile dyes are infra-red (IR) spectrometry, thin-layer chromatography (TLC), ultra-violet/visible (UV/Vis) spectrophotometry, and high-performance liquid chromatography (HPLC). The application of the infra-red technique was successfully demonstrated by Abrahams and Edelstein in the early 1960s,<sup>1</sup> when dyes from textiles found in the Cave of Letters in the Judean Desert were analysed. These textiles probably belonged to the Jewish leader Bar-Kokhba and his followers, who rebelled against Roman rule in Judea in 132-135 CE. The TLC technique was applied to dye analyses by Schweppe,<sup>2</sup> Hofenk de Graaff,<sup>3</sup> and Walton Rogers and Taylor,<sup>4</sup> among others. The visible spectrophotometric technique has been successfully used by Saltzman<sup>5</sup> and by Taylor and Walton Rogers.<sup>6</sup> In 1985, Wouters<sup>7</sup> introduced the HPLC technique to the analysis of ancient textile dyes.

## Dye extraction techniques

The following methods of extracting the dye from the fibres were adapted from the various techniques described in the literature (see Notes). About 1-2 centimetres of dyed thread is used for each analysis.

### *Extracting red, yellow, or brown dyes*

A sample of the dyeing is treated with about equal parts of 3M hydrochloric acid and methanol and the resulting mixture is heated for a few minutes at about 100° C. Additional methanol is added to replace that which has evaporated during the heating stage. A mordant red or yellow dye or a direct yellow or brown dye should generally be extracted from the fibre with this treatment. The resulting acidified methanolic mixture is then evaporated by gentle heating to remove the volatile acid. The dye residue is then dissolved in methanol and filtered through a 13-mm 0.45-micron teflon or nylon syringe filter, which is housed in a polypropylene body, and the resulting methanolic filtrate is used in the subsequent analysis.

### *Extracting blue or purple indigoid dyes*

The indigoid dye might be the sole dye present on the fibre or it may have been combined with, for instance, a yellow dye to yield a green color, or a red dye to produce a purple shade. Thus, after the aforementioned treatment to remove a mordant dye, if present, is completed, extraction of the blue indigotin dye is performed via near-boiling N,N-dimethylformamide (DMF),<sup>8</sup> which is also the technique of choice for extracting a 'true purple' brominated indigotin dye. This solution is filtered as discussed above. Because the resulting blue indigoid solution is degraded by light radiation, the subsequent analysis of this solution is performed immediately after the DMF extraction.

## Standard dyes

The commercially available dyestuffs include: carminic acid (Fluka), alizarin (Merck), purpurin (Aldrich), and indigotin (BASF). A solution of each of the first three dyes was prepared by dissolving in room-temperature methanol (analytical), while indigotin was dissolved in room-temperature DMF (analytical). A methanolic solution of kermesic acid and flavokermesic acid was obtained by a warm methanolic extraction of these components from a dried berry-shaped *Kermes vermilio* (Planchon) Targ insect, which was obtained from Algeria, according to the supplier (Jenny Dean, Ashill Colour Studio, Shefford, Beds., England). Additionally, a solution of these insect acids was obtained from a woollen-dyeing produced from this species, graciously provided by Su Grierson (Perth, Scotland), using the red mordant dye extraction procedure described above. A blue DMF solution of 6,6'-dibromoindigotin (DBI)

was obtained from a 'true purple' cotton-dyeing produced with caracol, *Purpura patula pansa* Gould (Oaxaca, Mexico), which was generously provided by Max Saltzman (Los Angeles, USA). (The HPLC analysis method discussed below was applied to this dyeing and showed that the only colorant was DBI.) The related 6-monobromoindigotin (MBI) dye was generated by irradiating the DBI solution overnight at 288 nm in a closed UV/Vis spectrophotometer cell compartment.

### Fourier Transform Infra-Red (FTIR) Spectrometry

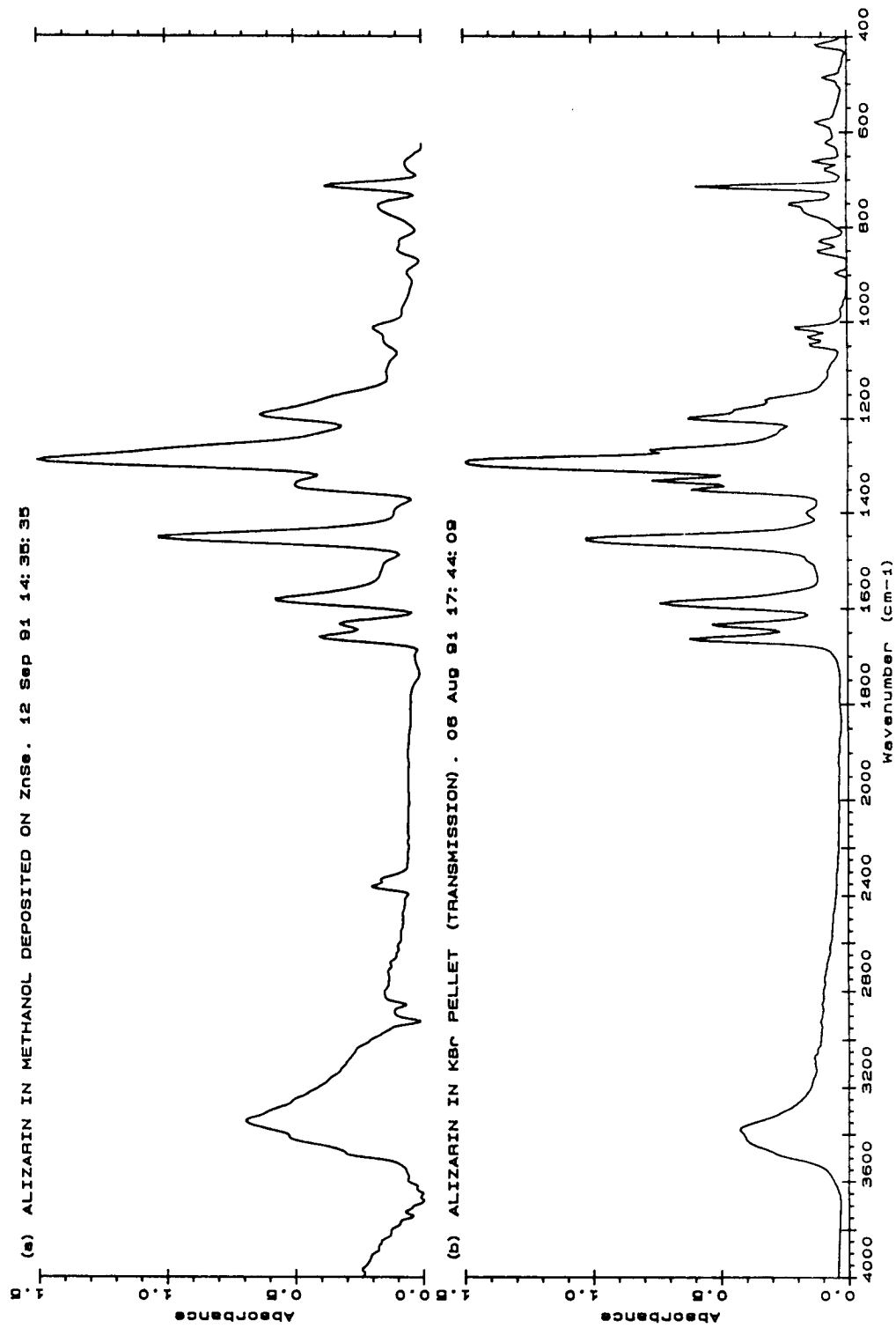
The IR technique of dye analysis, as performed by Abrahams and Edelstein,<sup>9</sup> preceded the advent of computerised instrumentation that was based on the Fourier transform (FT) algorithm. Thus, this early form of dye analysis consisted of careful chemical purifications of the desired dye substance by the selective precipitation technique. The resulting purified dye solid was then pressed into a potassium bromide (KBr) pellet for the subsequent IR examination. This technique, while representing a classically elegant separation method, involves a significant amount of sample preparation time. An additional drawback of this method is the relatively large dyeing sample needed for the dye analysis.

The major advantages of the 'FT' method, as integrated into the FTIR spectrometer, are that smaller samples may be used due to the increased sensitivity of the instrument and that an almost instantaneous spectrum can be produced for each sample. In addition, this computerised method allows for many kinds of reprocessing of the stored IR spectra, such as, smoothing, baseline adjustment and subtraction of two spectra. In order to (1) use as small a sample as possible, (2) minimise the sample preparation time, and, equally as important, (3) minimise the post-run cleaning time, the ATR (attenuated total reflectance) technique, which is also known as MIR (multiple internal reflectance), was employed. This technique involves the use of a zinc selenide (ZnSe) crystal from Spectra-Tech, which allows for numerous penetrations of the reflected infra-red beam into the sample, instead of the classically obtained IR spectra, which used the single-penetration transmission mode. Hence, with this 'multiple strike-force' technique, a smaller sample may be used. In addition, this crystal may be safely washed with common cleaning solvents, such as water, alcohol, or acetone. The ZnSe crystal is configured as either a trough for liquids or as a flat surface for solids, both of which can be easily cleaned.

The common solvents used for dye analysis, which may be an alcohol (for anthraquinones) or pyridine or DMF (for indigoids), strongly absorb in the IR region. For this reason, a computerised subtraction of the solvent spectrum from the dilute sample spectrum is needed. For dye analyses of important archaeological textiles, where only a very small dyed thread is often available to be sacrificed, relatively dilute dye solutions are generally obtained. The subsequent arithmetic subtraction technique performed on the liquid state spectra, while good in theory, does not always yield a clear difference spectrum that represents the dye alone. The interactive subtraction feature available with most FTIR instruments can artificially create peaks or, conversely, eliminate important small peaks. Thus, it can sometimes be very difficult to obtain unambiguous results with this technique, unless the dye solution is significantly concentrated, which is not always possible.

A related technique that was examined involved the FTIR analysis of the dye as a solid and not as a dye solution as was described above. For this purpose, the flat ZnSe crystal was used, which is easier to clean than the trough. In order to introduce the dye as a solid on to the crystal's surface, the dye was dissolved in, for example, methanol or acetone, and spotted along the crystal's surface by means of a capillary. This deposition technique yields the dye as a solid after the relatively quick evaporation of the solvent. However, this technique also produces residues that are impurities in the solvent and in the dye, which further add to the IR spectrum. This phenomenon leads to a blurring of the dye's IR spectrum, unless the dye, as indicated above, is present in a relatively large concentration. (The old adage, 'just about everything has an IR spectrum', is to blame here.) Subtraction of the spectra of the residue (formed from the evaporated solvent) from that of the evaporated dye solution can also lead to ambiguous results. However, figure 11 shows the close match between the FTIR spectra of solid alizarin as (a) deposited from a methanol solution, and as (b) prepared as a pressed KBr pellet. The relatively minimal sample preparation time associated with the attenuated multiple internal reflectance spectrum (a) compares quite favourably with the classical time-consuming KBr technique, which yields the corresponding transmission spectrum (b).

**Fig. 11: FTIR spectra of alizarin:**  
**(a) in the ATR (or MIR) mode by deposition from a methanol solution on a flat ZnSe crystal**  
**(b) in the transmission mode, as pressed into a KBr pellet.**  
**Both spectra obtained with the Nicolet 205 FT-IR spectrometer**  
**Number of scans = 32, resolution = 4  $\text{cm}^{-1}$**   
**For (a), gain = 4, processing using smooth = 17, slope, level and thickness = 11.429;**  
**for (b), gain = 1, processing using slope, level and thickness = 0.6540**



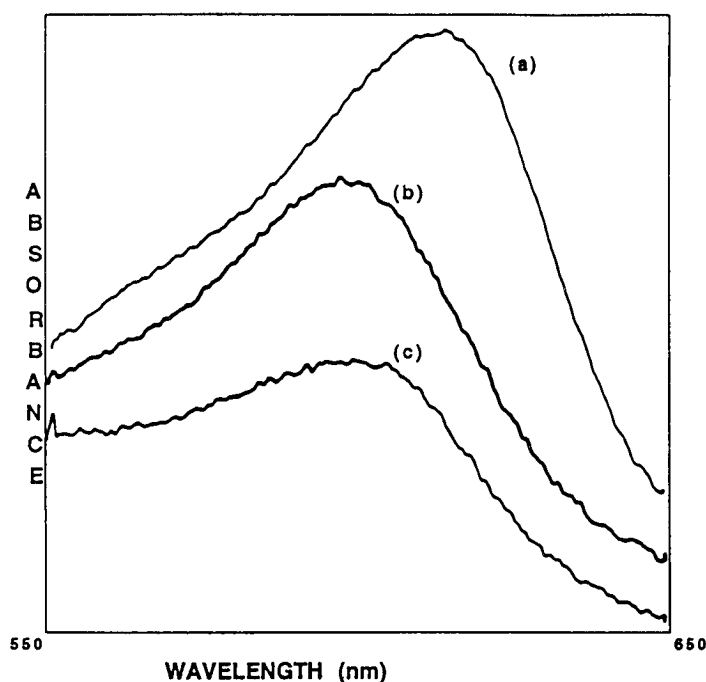
In summary, the results obtained from a dye analysis using the FTIR technique are to be interpreted cautiously. Another drawback of this spectrometric method is that, without prior separation, it is virtually useless in detecting various components of a dyestuff. The chromatographic techniques discussed below are advantageous in this regard.

### Visible Spectrophotometry

Because of its simplicity, this method has been used at the Edelstein Center primarily in the preliminary detection of a possible molluscan 'true purple' dyeing, which would contain the DBI dye.

An example of this method is shown in figure 12, which depicts the visible spectra of DMF solutions of (a) synthetic indigotin, (b) DBI as extracted from the true-purple cotton-dyeing obtained from Max Saltzman, and (c) the extraction of a dark stain on a small piece of limestone found in the conduit between two pits at Tel Dor, south of Haifa, Israel. The purple-dyeing installation at this Mediterranean coastal site dates from the 6th century BCE.<sup>10</sup> Hot DMF extraction of this stain yielded a characteristic blue indigoid solution. The corresponding wavelengths at the maximum absorbances ('lambda-max') are:

- (a) synthetic indigotin = 613 nm
- (b) natural DBI = 598 nm
- (c) Tel Dor sample = 600 nm.



*Fig.12: Visible spectra of DMF solutions of (a) synthetic indigotin, (b) natural DBI, and (c) a dark stain on a small piece of limestone from the conduit at the Tel Dor archaeological site*

The difference in the lambda-max between DBI and indigotin is fairly constant at about 15 nm. The Tel Dor sample, in comparison with the DBI spectrum, clearly indicates that the dark stain was an extract from a 'true purple'-producing sea snail. The slight increase of 2 nm in the characteristic wavelength for the archaeological sample over that of the pure DBI reference probably indicates that the Tel Dor 'purple' also contains a certain amount of higher-wavelength absorbing indigoid, such as MBI and/or indigotin.

The limestone present in the Tel Dor conduit was probably used to obtain an alkaline environment, which is necessary during the reduction of the dye solution to its soluble leuco form.

### Thin-Layer Chromatography (TLC)

The Schweppe method of TLC analysis<sup>11</sup> has been applied to the determination of the dye components of red dyeings. This consisted of a polyamide plate and a toluene/glacial acetic acid (9/1) eluent system. An example of this analysis can be seen in figure 13, which depicts the chromatograms of extracts from (a) a red-dyed thread from a Byzantine(?) textile probably belonging to the workers in the copper mines at Wadi Amram in Israel's Negev area, and from (b) a modern Israeli madder dyeing obtained from a Jordan Valley grown plant (*Rubia tinctorum* L.). (The modern dyeing was performed by Judith Safrai of Kibbutz Sde Eliahu, who graciously contributed it to this research.) It is clear that the ancient red dyeing was produced with madder, but besides the presence of the two major dyes in madder, alizarin and purpurin, two other minor components are also detected.

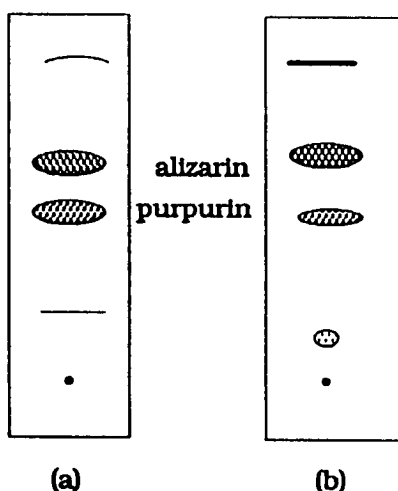


Fig. 13: TLC chromatograms of extracts from (a) a red dyeing from Wadi Amram, and (b) modern Israeli madder dyeing

### High-Performance Liquid Chromatography (HPLC)

The HPLC technique has been the 'workhorse' for dye analyses performed at the Edelstein Center. This choice is due to the outstanding sensitivity of this method in that it can detect dyes that may be present in nanogram quantities. The additional advantage of this chromatographic method, as compared with spectrometric techniques, is in the manner in which the information regarding dye analysis is produced. In a spectrometric method, there is an 'overlap' of information in a fixed wavelength range, whereas a chromatographic scheme presents a 'separation' of information. Hence, in a spectrometric analysis, minor components of a dyestuff may be masked by the dominant dye substances present. However, in a separatory analysis, such as HPLC, these minor components may become detectable. As is often the case, 'HPLC sees the invisible.'

Various elution schemes have been utilized for dye analyses. In 1985, Wouters<sup>12</sup> introduced a gradient elution scheme for the determination of plant and insect mordant dyes. This method produced a separation of these dyes after more than 35 minutes. The more current elution schemes that have been used for dye analyses are depicted in figure 14. These are based on variable methanol and water volumes and a fixed phosphoric acid volume (10% of a 5% solution). Accordingly, the gradient method used primarily for scale-insect dyes<sup>13</sup> required about 30 minutes of separation time. In 1991, an updated Chinese gradient method for plant and sea-snail indigoids was used.<sup>14</sup>

#### *Isocratic elution method*

In this laboratory, it has been found that a good separation of the alizarin and purpurin dye components of the madder dye can be obtained with an isocratic elution method consisting of 70% methanol and 30% (of a 5% solution) of phosphoric acid (fig.14). The purpurin

elutes in less than six minutes. The major components and conditions of the Varian VISTA 5500 LC chromatographic system consisted of a 15-cm x 4-mm Lichrosorb RP-18 column (7-micron particle-size), a 10-microlitre sample loop, a 1 ml/min flow rate and a Varian UV-200 detector. The isocratic method allows for zero instrument turn-around time and, thus, for faster analyses than the gradient method described below.

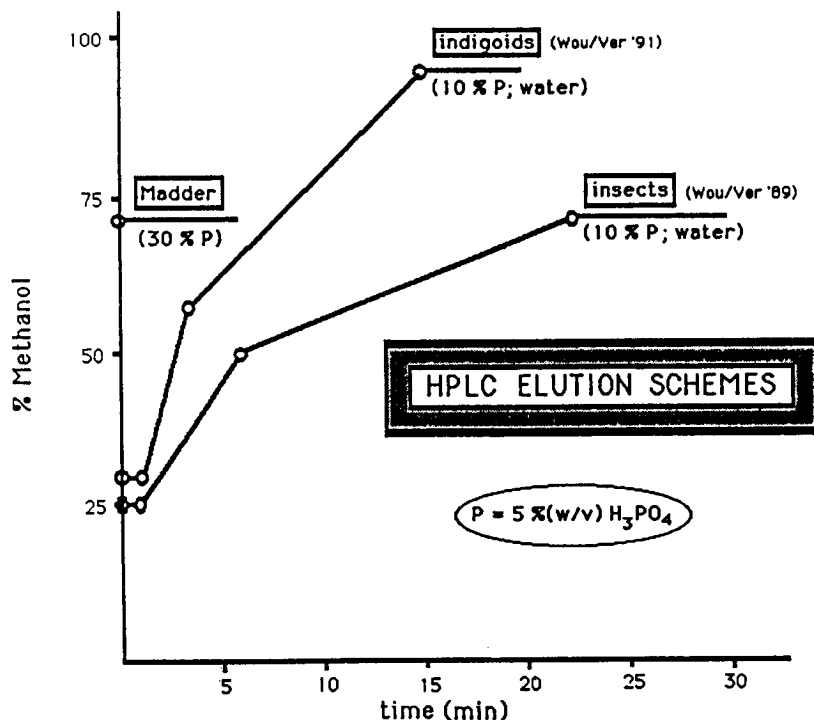


Fig.14: HPLC elution schemes for the investigation of red plant and insect dyes and blue and purple plant and molluscan dyes

The isocratic method has been applied to some of the madder dyeings excavated at Masada,<sup>16</sup> as, for example, shown in figure 15. These textiles probably belonged to the Jewish resistance who occupied this mountain hideout from 66 to 73 (or 74) CE. As described by Josephus, after being besieged by the Roman general Flavius Silva, almost one thousand men, women, and children decided to commit mass suicide rather than fall into the hands of the Romans.<sup>16</sup> This Judean desert fortress and palace, over-looking the Dead Sea, was built by King Herod of the Jews in case a retreat by him and his family was necessary. When he died in 4 BCE, a Roman garrison was stationed there until the resistance fighters over-powered them at the beginning of the rebellion against Roman rule in Judea.

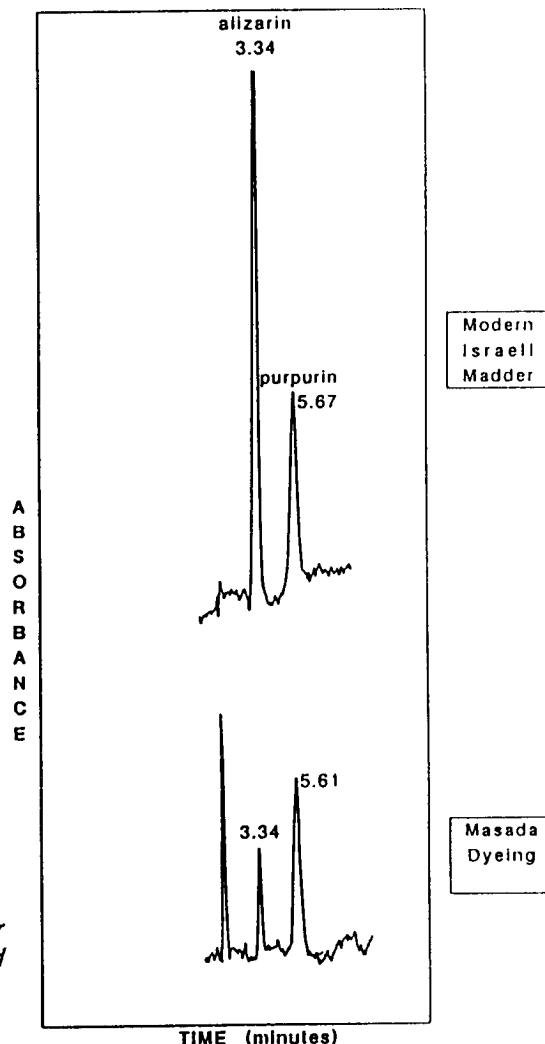


Fig.15: HPLC chromatograms of a modern Israeli madder dyeing and of a Masada dyeing; isocratic elution method at 430 nm

### Gradient elution method

A unified gradient elution method for the detection of both plant and scale-insect anthraquinonoid dyes as well as the blue and purple indigoidal dyes has been used in this laboratory. The chromatographic system is as described above. This scheme consists of the indigoidal elution method<sup>17</sup> (fig.14). Table 9 indicates the typical retention times obtainable from such a system and it is important to emphasize that the relative retention times (RRt) are reproducible to within 1%. According to the procedures described in this article, two selective extractions and, thus, two analyses would need to be performed in the case of bichromic dyeings that include indigotin.

Table 9: Retention times of some important ancient dyes  
(see the text for the chromatographic system and conditions)

Dye	Typical Rt (min)	Relative Rt
carminic acid	5.9	0.63
flavokermesic acid	8.2	0.88
kermesic acid	8.4	0.90
alizarin	9.40	1
indigotin	11.09	1.18
purpurin	11.56	1.23
monobromoindigotin	13.91	1.48
dibromoindigotin	15.89	1.69

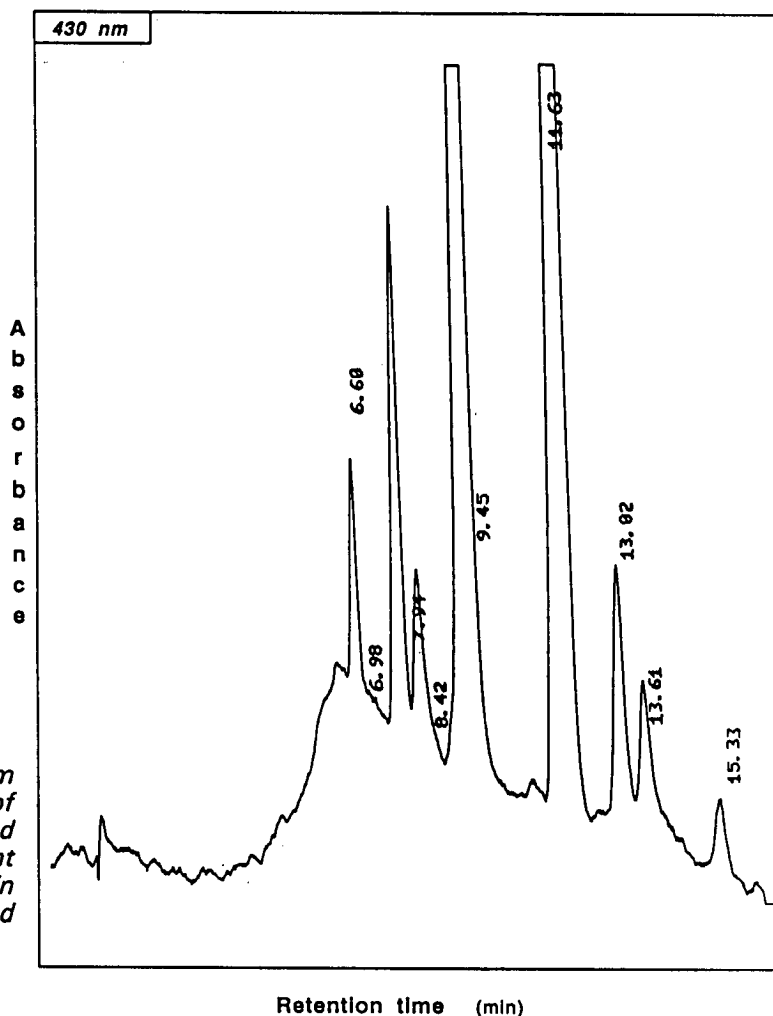


Fig.16: HPLC chromatogram of a methanolic extract of Israeli madder roots obtained after acid hydrolysis; gradient elution method yields alizarin eluting after 9.45 min and purpurin after 11.63 min

This elution scheme has also been applied to the dyes from the pulverised roots of modern madder (*Rubia tinctorum* L.) grown in Israel. The extraction technique consisted of the HCl/methanol method indicated in the red mordant-dye extraction section above. The chromatogram of the resulting HPLC analysis, figure 16, shows about ten colorants that have been separated from the madder root, foremost of which are the characteristic alizarin and purpurin peaks. All of these anthraquinonoid dyes are effectively separated in less than 16 minutes. It has been found that the optimum visible detector wavelength for the clear detection of these madder dye components is 430 nm.

### Conclusion

The dyes discussed in this article are the ones most typically found in Israel on ancient textiles and on fragments of dyeing vats.<sup>18</sup> Of all of the dye identification methods discussed, the HPLC 'indigoid gradient elution method' is the one most universally useful for the detection of a wide variety of dyes.

### Acknowledgements

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