Extraction of the natural indigo carmine pigment from the Isatis plant, characterization and dyeing of wool

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Abstract. Natural indigo is hardly used anymore, replaced by synthetic indigo. Considered to be more powerful and more intense. The aim of this study is to optimize the method of extracting indigo carmine from the Isatis plant located in the Timhdit region of Morocco, during the fermentation period of the extracted liquid (pH = 10) and by adding 10% sugar it was possible to increase the yield by 0.5% indigo carmine blue powder to 0.9%. The main effect of applying centrifugation at 7000 rpm instead of conventional filtration is the reduction of the time from 30 min to 5 min as well as the reduction of the loss of traces of indigo in the filter papers by easily recovering the blue powder in the form of sediment in the centrifuge tubes. The extracted pigment is characterized by the infrared which confirmed the structure of the indigo carmine obtained, by visible UV spectroscopy, the maximum absorption of which is approximately 598 nm. This blue pigment was applied to the wool fibres by optimizing the dyeing procedure (T = 50 °C and pH=10) with a 1/100 bath ratio and a 35 min soaking time. Dyed wool sample has reached a rigid fixation confirmed by the test for fastness washing 105-C06 and to friction 105-X12.

1. Introduction
Indigo pigment was the first extracted dye used in natural dyeing. The chemical synthesis of indigo by Adolph Von Baeyer in 1883 extended the use of indigo as the preferred blue in dyeing [1]. Being a non-polluting dye extracted from a natural source that gives a deep and solid coloration, it is interesting to think about its reintegration. However, the best source of blue dyes resistant to discolouration is indigo plants, dispersed in many different botanical families present in very diverse natural environments, the main ones belonging to Indigofera, Isatisintctorial and Polygonum (Table 1) [1].

According to our work, indigo is extracted as pigment from the leaves of Isatis located in the Timhdit region of the Middle Atlas Mountains of Morocco. The extraction of indigo is done by macerating the leaves in hot water at 50°C for 2 hours, then oxidation, fermentation and finally filtration [2-4]. According to previous studies, during maceration, it is advisable to stay in a temperature range between 40 and 50 °C. This condition corresponds to the maximum plateau of activity of the enzyme responsible for the kinetics of the reaction that transforms the indican present in the leaves into indoxyl [3] and maceration at 48 °C over a period ranging from 1 h 30 to 2 hours would extract enough indigo while limiting the diffusion of impurities and as a last step following the technique of oxidation with compressed air, indigo is formed from two molecules of indoxyl during the fermentation period (Figure 1). By this method, we were able to extract 0.5% indigo pigment. Based on these experiments already in use, this next work aims to optimize the fermentation step by adding sugar to the fermented baths to improve the yield of the indigo extract. Subsequently, the
centrifugation technique facilitated the recovery of the indigo without any loss in place of the filtration used in previous experiments. Indigo is a vat dye, known for its water insoluble properties. However, under the action of a reducing agent, sodium dithionite (\(\text{Na}_2\text{S}_2\text{O}_4\)) and in an alkaline medium, indigo gives its water-soluble leuco derivative and once out of the bath, by oxidation in air or with an oxidizing agent, the initial water-insoluble dye is regenerated in the fiber [3, 4], the chemistry of the indigo dye involves the redox reaction [8]. In order to control the different factors for dyeing wool fibres with natural indigo, these factors need to be studied. In this work, we are interested in these main factors: the temperature of the dye bath, the pH of the solution, the dyeing time and the effect of the salt on the fixation of this pigment.

### Table 1. Sources of major natural blue colorants from plant species

| Botanical name       | Common name | Origin                            |
|----------------------|-------------|-----------------------------------|
| Indigofera           | Indigo, Nila| Tropical countries in Asia        |
| Isatis tinctoria L   | Woad        | Europe                            |
| Polygonum tinctorium | Polygonum, or Japanese indigo | Northern/Central Italy, Germany, Japan, China, Korea |

2. Material and methods

2.1 Extraction method

The leaves of Isatis were collected from cultivated plants in early May. The leaves (1 kg) were washed and cut into small pieces of 1 cm\(^2\). The dye was then extracted using 4 liters of hot water (50 °C) for 90 minutes, the sheets were removed and the sample was quickly cooled to 30 °C, then 40 ml of 25% ammonia was added to reach a pH of 9, the extraction liquor was then aerated for 1 h using a compressor to allow the complete oxidation of the indigo precursors, up to the appearance of the color blue. The liquid rested for 24 h, different concentrations of sugar (5%, 10%, 15% and 20%) were added to the water of the fermented baths (see figure 3) then centrifuged at a speed of 7000 rpm for 5 min, and finally dried in the oven for 12 h at a temperature of 105 °C. Once the pigment has been collected, it is stored in tubes in a controlled laboratory environment (20 °C, 65% relative humidity).

![Formation of indigo from its precursors Indican and Isatan B](image)

2.2 Infrared

The IR spectrum of the indigo carmine extracted from the Isatis plant was recorded using the Nicolet IS10 FTIR-ATR spectrophotometer by recording the scans in transmission mode (%) in the range.
from 4000 to 1000 cm\(^{-1}\). FTIR spectroscopy was used to determine and confirm the functions present in the extracted indigo molecule.

### 2.3 Visible UV spectroscopy

The spectrophotometer used in the laboratory is of the Thermo Evolution 300 brand, Variable bandwidth from 0.2 to 4nm, Dual-beam technology Autonomous or controllable by PC, Photodiodes detector or photomultiplier.

Using the absorption spectrum of the coloring solution \(A = f (\lambda)\), we started by identifying the peak of its maximum absorbance; the wavelength for which the absorbance of the solution is maximum \(\lambda_{\text{max}}\).

### 2.4 Dyeing conditions

The wool fibres are dyed on a Datacolor "AHIBA NUANCE" programmable laboratory dyeing machine. The procedures for dyeing the wool fibres followed by rinsing, oxidation, neutralization and drying [5] are described below in the diagram (figure 2), we have tested two samples of dyed wool fibres under one condition (ph=10,T=50°C), the first in the absence of NaCl and the second in the presence of 5% NaCl.

![Figure 2. Wool dyeing process by indigo](image)

### 2.5 Color fastness

The quality controls of the wool samples dyed with natural indigo were carried out according to international standards. For the washing and friction tests, the degrees of solidity are indicated by a gray scale numbered from 1 to 5 showing disgorgement and degradation: “1” representing the lowest values in quality and “5” the highest. These standards meet strict and well-defined criteria: color fastness to washing: ISO 105-C06, and stain resistance to friction: ISO 105-X12. [6].

#### 2.5.1 Washing test

1 g of dyed wool fiber is brought into contact with a specified control fabric and washed under appropriate conditions (concentration of detergent 5 g / l and temperature 60 °C in a container for 1 hour, once the sample has been recovered, it is rinsed and dried. After the 12 wash cycle, the degradation of the coloration of the dyed sample and its disgorgement on the control fabric are evaluated using gray levels (see table 2).

#### 2.5.2 Friction test

The friction tests consists in subjecting a 100 x 25 mm test piece to friction (from front to back) under constant pressure with a dry or wet standardized felt for 1 hour. Degradation and disgorgement are then evaluated using gray scales. This test makes it possible to check whether, by repeated rubbing, the color of the dyed wool can on the one hand deteriorate and on the other hand if it can degrade (transfer) on the control supports. (Table 2).

### 3. Results and discussion
3.1 Optimization of the extraction

Although of natural origin, indigo is not found as such in plants but in the form of precursors, the two main ones being Indican and Isatan B [3]. The maximum yield of indigo depends on the complete release of the Indican precursor from the plant biomass during fermentation (figure 1). We evaluated the role of sugar on the yield of indigo dye during biomass fermentation. The yield of indigo with 10% sugar during 24-hour fermentation is 0.9%, these parameters were adequate to increase the yield by 0.5% (figure 3). This result can be explained by the fact that during the fermentation period the bacteria feed on the sugar and better allow to predigest and transform all the precursors of the plant, the sugar then influences the fermentation kinetics of the plant. After fermentation, as indigo is insoluble in water, the centrifugation technique was adequate for the separation of the blue pigment from the water, the centrifuge with a rotation speed of 7000 rpm for 5 minutes separated the mixture well according to their difference in density, easily recovering the indigo blue as sediment after emptying the supernatant of clear water at the top of the tubes. It was found that this method had a higher efficiency compared to the conventional filtration technique which caused a large loss of pigment in the filter papers.

![Figure 3. Indigo yield obtained as a function of sugar concentration in fermented baths](image)

3.2 Characterization

3.2.1 Infrared IR

The infrared spectrum of the extracted indigo dye is shown in the figure 4, the values of the IR vibration bands found in this case are in agreement with previous studies carried out on the indigo molecule [7]. The latter comprises three main groups whose bands characteristic of the different vibration modes are easily identifiable. 3600-3150 cm\(^{-1}\) very wide peak bands of high intensity indicate the presence of elongation vibrations of the associated O-H bonds. 3280 cm\(^{-1}\) very thin peak bands indicate elongation vibrations of N-H bonds noted with high intensity. 3950-2800 cm\(^{-1}\)st bands of several very thin peaks indicate elongation vibrations of Csp\(^2\)-H bonds noted with variable intensities. 1700 cm\(^{-1}\) several modes of C = C bond elongations, they are found here at Medium intensity. 1650 cm\(^{-1}\)st band of a single low intensity peak, corresponds to elongations of the links C = O. 1490 cm\(^{-1}\) bands of very fine average intensity, corresponds to the presence of deformations in the plane of the links N-H. 1480 cm\(^{-1}\) bands of very fine average intensity, of high intensity, corresponds to the presence of elongation vibrations S = O. 1400-1200 cm\(^{-1}\) bands of very fine average intensity, corresponds to the presence of elongation vibrations C-N. 1050 cm\(^{-1}\) bands of very fine average intensity, corresponds to the presence of elongation vibrations S-O. It is concluded that the product
obtained is exactly indigo carmine (or sodium sulfoindigotate) $\text{C}_{16}\text{H}_{10}\text{N}_{2}\text{Na}_{2}\text{O}_{8}\text{S}_2$, its structure is almost identical to that of indigo $\text{C}_{16}\text{H}_{10}\text{N}_{2}\text{O}_{2}$, only two sulphate groups, in positions 5 and 5’ differentiate them [7].

![Infrared spectrum of indigo carmine extracted from the plant Isatis](image)

**Figure 4.** Infrared spectrum of indigo carmine extracted from the plant Isatis

### 3.2.2 UV-visible spectrophotometer

The measurement of the absorbance of an indigo carmine solution, as a function of wavelength leads to the spectrum below (figure 5); we place ourselves at the wavelength $\lambda_{\text{max}}$ corresponding to the maximum absorption in order to obtain the greatest precision for the dosage. The maximum absorption is around 598 nm which explains that indigo is blue and that the indigo molecule absorbs mainly in the orange-yellow, this result was confirmed by the literature and previous studies. [7]. The 600 nm band, present in a highly diluted medium, is attributed to the indigo carmine monomer. [7]

![Absorption spectra for carmine indigo](image)

**Figure 5:** Absorption spectra for carmine indigo
3.3 Influence of dye parameters

3.3.1 Temperature
For this dye, we avoided working at high temperatures to avoid oxidation of the reduced dye and the temperature of 50 °C is sufficient to saturate the wool fibers with dye provided that the soaking time of 35min is respected.

3.3.2 pH
The indigo carmine dye being very slightly soluble in water, the variation in pH slightly improves the solubility of the dye and the existence of several acid functions in the dye molecule results in a change in color of the solution as a function of pH
- In the range of pH from 0 to 10, the solution is blue and insoluble.
- In the range of pH from 9 to 12.6, the solution is green and soluble.
- pH greater than 12.6, the solution becomes yellow but the color is unstable and discoloration occurs quickly even in the dark.

pH to be chosen for dyeing the wool fiber must therefore be between 9 and 12.6 or the predominant leuco-indigo carmine molecule is soluble in water and capable of incorporating wool fiber [8].

3.3.3 Contact time
The contact time is the time needed to dye the wool fiber, depending on the optimum conditions (pH = 10, T = 50 °C). Samples of the dye bath were taken every 5 minutes and the absorbance measurement was carried out at $\text{max} = 598 \text{ nm}$, in order to follow the kinetics of the dye, it can be noted that the duration of 35 min of dyeing allowed the maximum absorbance of the dye on the wool fiber to be reached (Figure 6), it can be concluded that this time is sufficient to saturate the fiber with indigo blue dye and beyond 35 min the dye bath is saturated, in fact, the dye adsorbs on the easily accessible sites on the fiber surface. Moreover, the diffusion of the dye towards the less accessible sites is favored by a regular agitation until saturation.

![Figure 6](image_url)

**Figure 6.** Monitoring the variation of absorbance as a function of time in the optimal dye bath (pH=10, T=50°C)
3.3.4 Dye concentration (light, medium, dark)
1 g of pastel dye was used to dye 1 g of wool with a bath ratio Rb: 1/100 in order to obtain a dark shade for 40 min at 50 °C. The amount of dye used corresponds to 100% relative to with wool fibers. However, to obtain other shades with this pigment, only 40% is required for the medium shade, and 20% of the dye is required for a light shade. (Figure 7).

![Figure 7. The shade of the wool fiber color (light, medium and dark) as a function of the concentration of the blue pigment.](image)

3.3.5 Influence of salt NaCl on dye fixation
By using grey scales in the wash test, it was found that the indigo dye disintegrated on the cotton and wool multifiber giving a violet color for the first sample dyed in the absence of NaCl in the dye bath, which explains the poor fixation of the dye on the surface dyed fiber (table 2, sample 1'). However, the wool fabric dyed under these same conditions (T=50°C ,pH=10) by adding 5% NaCl to the dye baths (sample 2) did not bleed in different control samples (acetate, cotton, nylon and wool) keeping their initial colors, the dye that was not eliminated in the washing stage depends on the dyeing process in the presence of salt. In fact, the NaCl acting as a fixative converts the dye into an insoluble molecule within the fiber and offers increased resistance properties in the dyeing process compared to the other test on fabric dyed in the absence of NaCl; the salt has the function of catalyzing the reaction between the dye and the salt on the fiber to permanently fix the color. The rubbing test then confirmed that the salt fixed the indigo color very well on the surface of the fiber (sample 2), which not leave traces of blue in the control sampled while being rub-resistant.

Table 2. Evaluation of indigo dye fixation by washing and rubbing tests

| Test            | Disgorgement(sample 1) | Evaluation | Disgorgement(sample 2) | Evaluation |
|-----------------|------------------------|------------|------------------------|------------|
| Washing test    | Acetate                | 4          | Acetate                | 5          |
| 105-C06         | Cotton                 | 2          | Cotton                 | 4          |
|                 | Nylon                  | 5          | Nylon                  | 5          |
|                 | Wool                   | 3          | Wool                   | 5          |
| Friction test   | dry friction           | 2/3        | dry friction           | 3          |
| 105-X12         | wet friction           | 3          | wet friction           | 3/4        |
4. Conclusion

In summary, the yield of indigo is increased by 0.4% by extracting the leaves of Isatis by adding 10% sugar during fermentation, as indigo is insoluble in the extraction water. The centrifugation method had also reduced the separation time of the mixture: organic matter (indigo) and extraction water compared to conventional filtration, which caused a large loss of traces of indigo in the filter papers. Once the pigment was obtained, the factors influencing its dyeing by the wool fiber were temperature and Ph in an alkaline medium, the rinsing strength of the dyed wool fibers was considerably improved on the surface of the dyed fibers in the presence of NaCl. Natural and traditional dyeing can fundamentally change the current situation of severe wastewater pollution for denim production, we can also say that this dyeing technology will greatly expand the imagination of cowboy product designers for environmental protection and ecology.

Reference

[1] Angelini L G, Tozzi S, and Nassi o Di Nasso N 2004 Environmental factors affecting productivity, indican content, and indigo yield in Polygonum tinctorium Ait., a subtropical crop grown under temperate conditions *Journal of agricultural and food chemistry* 52(25) 7541-7547
[2] Comlekcioglu N, Efe L, and Karaman S 2015 Extraction of indigo from some Isatis species and dyeing standardization using low-technology methods *Brazilian Archives of Biology and Technology* 58(1) 96-102
[3] Vuorema A, John P, Keskitalo M, Mahon M F, Kulandainathan M A and Marken F 2009 Anthraquinone catalysis in the glucose-driven reduction of indigo to leuco-indigo *Physical Chemistry Chemical Physics* 11(11) 1816-1824
[4] Park S, Ryu J Y, Seo J, and Hur H G 2012 Isolation and characterization of alkaliphilic and thermotolerant bacteria that reduce insoluble indigo to soluble leuco-indigo from indigo dye vat *Journal of the Korean Society for Applied Biological Chemistry* 55(1) 83-88
[5] Luo Y, Pei L, and Wang J 2020 Sustainable indigo dyeing and improvement of rubbing fastness of dyed cotton fiber using different fixing agents for obtaining eco-friendly cowboy products *Journal of Cleaner Production* 251 119728
[6] Mongkolrattanasit R, Kryštůfek J and Wiener J 2010 Dyeing and fastness properties of natural dyes extracted from eucalyptus leaves using padding techniques Fibers and Polymers 11(3) 346-350
[7] Komorsky-Lovrič Š, Mirčeski V, and Scholz F 1999 Voltammetry of organic microparticles *Microchimica Acta* 132(1) 67-77
[8] Venil C K, Zakaria Z A and Ahmad W A 2013 Bacterial pigments and their applications *Process Biochemistry* 48(7) 1065-1079