RESEARCH ARTICLE

COLOR | WILEY
RESEARCH AND APPLICATION

Characterization of the evolution of indigo blue by multispectral imaging

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Funding information
FEDER/Junta de Andalucía-Consejería de Economía y Conocimiento, Grant/Award Number: A-HUM-164-UGR18; Ministry of Economy and Competitiveness of Spain, Grant/Award Number: DPI2015-64571-R; Spanish Ministry of Economy and Competitiveness, Grant/Award Number: Ramón y Cajal program; Ministry of Economy and Competitiveness; Junta de Andalucía; FEDER (European Regional Development Fund).

Abstract

Indigo blue is a natural organic color from the *Indigofera tinctoria* plant. It is insoluble in water and does not require mordant to fix the color; therefore, due to its nature it can be considered as a pigment or dye. The identification of indigo in cultural heritage is very difficult due to the complexity of its chemical formula and the changes that it can undergo in certain conservation and exposition conditions. For this reason, characterization of possible degradation, transformation, and reaction processes is essential for its identification. In this study, multispectral imaging has been used for the first time as noninvasive technique to characterize the aging state of indigo blue using samples aged in a controlled climate chamber under extreme conditions of humidity, temperature, and light. The technique has proven to be very promising for this application, together with classification techniques based on machine learning approaches.

KEYWORDS

artificial aging, capillary electrophoresis, indigo, multispectral imaging, principal components

1 | INTRODUCTION

Indigo blue is a material that has been very intensively studied by conservators and chemists for decades.[1-3] As it is a natural organic color extracted from the *Indigofera tinctoria* plant, indigo has been used as dye or pigment for the manufacture of many historical and artistic pieces as well as pictorial layers of mural and panel painting, as a colorant for textiles and for the tinting of paper.[4-6] It is

considered an organic dye insoluble in water, which does not require mordant to fix the color because it settles as microscopic particles between the fibers. Due to its nature, it can be considered as a pigment or dye.

Indigo is the result of a complex procedure of extraction and treatment of the *I. tinctoria* plant.[7] Indican glucoside is the dye precursor and is located in the plant leaves. For its correct extraction, the plants are cut at sunrise and immediately immersed in big barrels with lime

water in which they are soaked for 24 hours. The mixture is then energetically beaten to oxygenate it and release the maximum amount of dye. Afterward, it is left to set until the following day when the water is removed. The result is a dark blue paste which is made into balls and tablets, which when dry, can be preserved until their use.[8] As the leaves are treated by steeping them in water and leaving them to ferment, the precursor of indigo (glucoside indican), which is colorless and soluble in water, transforms, producing glucose and indoxyl, which exists in leuco- and keto- tautomeric forms.[9] When oxidized, two indoxyl molecules join with oxygen to form indigo blue or indigotine (insoluble in water).

This oxidizing procedure also results in the formation of indigo red or indirubin, the red dye that when mixed with indigo, gives way to violet tones, very characteristic of this natural indigo that differentiates it from indigos resulting from other plants or from synthetic indigo. The main component of indigo is indigotin but it also contains indirubin or indigo red, indium or dun indigo, nitrogenous components and mineral salts, silicate, calcium, potassium, magnesium, iron, and other lesser components.[10]

There is evidence of its existence and use as a textile dye since 6000 BC. That being said, however, its identification in artwork has only been carried out on rare occasions due to the complexity of its chemical formula and the changes that it can undergo in certain conservation conditions[11-13] or when affected by environmental factors.[14]

Only few studies have focused on the characterization of degradation products of indigo blue. Some have been realized by Sousa et al by high-performance liquid chromatography with diode-array detection,[15] Witkos et al by mass spectrometry,[16] Rondao et al by nuclear magnetic resonance (NMR) and single oxygen quantum yield[17] and Novotna et al for the photodegradation studies of indigo.[18]

In 2020, Tello et al characterized the degradation pathways of indigo blue dye and the detection of degradation products by capillary electrophoresis with diode-array detection (CE-DAD), NMR, and fluorescence spectrometry.[19] Based on the identification of degradation products of indigo blue even when it is present in a degraded state by using several analytical techniques, the main aim of this study is to investigate the possibilities and advantages of multispectral imaging as a noninvasive technique for the characterization and identification of the aging process of indigo blue.

Multispectral imaging is a noninvasive technique[20,21] that has been recently applied to characterize the state of preservation of artworks, because of the possibility of using spectral bands beyond the visible range as well (in the ultraviolet [UV] and near infrared [NIR] regions),

which is helpful for detecting cracks and the repainting of the artwork.[22,23] However, to the best of our knowledge, there are no previous studies in which this technique has been used to obtain information about the aging state of the pigments used in painting or artwork in general.

Conventional color images are usually captured with camera sensors that record information in three channels or spectral bands, with spectral sensitivities in the red, green, and blue regions of the visible spectral range. In contrast, when a multispectral imaging device is used to capture the same scene, it is possible to have access to a higher number of channels, and so potentially to narrower regions of the spectrum of visible light. It therefore follows that it is possible to see how this technique is able to sample the spectral range covered by the visible light much more finely, and give access to a greater amount of information for each pixel of the captured scene.

UV-vis spectrometry and CE-DAD have previously been used on laboratory samples in order to identify the degradation products and to classify the samples according to the aging state.[19] This information has been essential to adapt multispectral imaging for the characterization of the degradation of indigo blue. Multispectral imaging is a noninvasive and nondestructive technique which allows high-speed in situ analysis to be carried out in contrast to UV-vis spectrometry and CE-DAD which are invasive and destructive techniques. A machine-learning-based classifier has been implemented to demonstrate the potential of multispectral imaging in the characterization of the aging state of indigo. Using the results from the spectrometry and CE-DAD as reference, the multispectral image data is able to determine the aging state with high accuracy.

2 | METHODS

2.1 | Materials, reagents, and solvents

Indigo blue paste was obtained from its natural source (the *Isatis tinctoria* plant) of Indian origin that was supplied by Kremer-Pigmente (Germany). Reference standards of indigotin (INDt) and isatin (IS) were provided by Sigma Aldrich (St. Louis, Missouri).

The selected holder was handmade paper made from natural hemp fiber (*Cannabis sativa*) with no colorants or glue.

The solvent acetic acid (17.5 M) was obtained from Merck (Darmstadt, Germany). Sodium tetraborate decahydrate, sodium hydroxide, and sodium dodecyl sulphate (SDS) were provided by Sigma Aldrich (St. Louis, Missouri).

All the reagents were analytical grade, unless otherwise stated. Water was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA).

2.2 | Preparation and treatment of the samples

A prior study from Tello et al showed that the solution of acetic acid began to accelerate the process of degradation of indigo blue much more than when the indigo was simply applied to paper.[20] For this reason, both samples dissolved in acid and samples on paper have been used to classify the aging state of the dye in this study. The samples on paper simulate a historical real sample.

2.2.1 | Samples of indigo blue solution (vial samples)

Samples of indigo blue dissolved in acetic acid in vials, and 0.003 g of indigo blue was dissolved using 10 mL acetic acid (17.5 M). Dissolution was made in an ultrasonic bath for 45 minutes. A total of 100 viable samples were obtained. An ultrasonic bath from Selecta (Barcelona, Spain) was also used for the extraction and dissolution of the indigo blue dye.

2.2.2 | Samples of indigo blue on paper (paper samples)

Samples of indigo blue applied to a paper support; 1 g of ground indigo blue was prepared in distilled water. The color layer was applied twice with a soft brush on different directions in order to obtain a homogeneous layer and to ensure complete coverage of the paper. A total of 90 viable samples of 25 × 25 mm were obtained.

For the analysis by UV-vis spectrophotometry and CE-DAD the extract of indigo blue from paper was carried out with acetic acid in an ultrasonic bath for 45 minutes. The solution was filtered with a nylon filter of 0.2 μm (pore diameter), evaporated to dryness and dissolved again in 0.1 M SDS.

2.3 | Accelerated aging process

The aging process in a controlled climate chamber under extreme conditions of humidity, temperature, and light was carried out to provoke the degradation of the indigo blue in the prepared samples. A comparative study between the reference samples (without aging) and aged samples was performed to evaluate this degradation.

Accelerated aging tests were made in a SOLARBOX 3000eRH chamber equipped with xenon lamps with irradiation bandwidth control sensor (in the 300-400 nm band) and an indoor filter (S208/S408), controlled and monitored irradiance up to 1000 W/m^2 (in the 300-800 nm band). Parameter control inside the chamber was recorded using the XEN 32 Report Level software. The selected conditions for the climatic chamber followed the ISO regulations 5630-3:1996 and ISO 11341:2004. Vial samples and paper samples were placed in the aging chamber exposed to 80°C, 65% relative humidity (RH) and 550 Wm^{-2} irradiance with an indoor filter.

Based on our preliminary studies,[19] the gradual sequence of the degradation of indigo blue in dissolution occurs in the first aging interval (24 hours) so the total duration of the tests carried out in the aging chamber was 24 hours for the vial samples, sampling at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours.

For the paper samples, the duration of the aging process was higher amounting to 720 hours with sampling at 0, 24, 48, 72, 144, 288, 432, 576, and 720 hours, since the indigo compounds on paper are more resistant to aging.[19]

2.4 | Analytical methodology

The analytical techniques used for the identification and characterization of the principal degradation products of indigo blue were the UV-visible spectrophotometry, the CE-DAD, and multispectral imaging.

An Agilent 8453E (Waldbronn, Germany) UV-Vis spectrophotometer was used to record the absorbance spectra of indigo and the data were analyzed using the Agilent ChemStation software package.

CE separation was performed with an Agilent HP^{3D} CE instrument equipped with diode-array detector, a thermostated column cartridge, a high-voltage built-in power supply and an autosampler (Agilent Technologies, Germany). ChemStation v. A.0901 software package was used for acquisition and processing of electropherograms.

Multispectral images were captured with a Pixelteq VIS SpectroCam camera (Halma, UK), with 16 channels in the VIS and NIR range.

2.4.1 | UV-Visible spectrophotometry

After each aging session, 3 mL of dissolution from vial samples was extracted to be introduced in the quartz cell

TABLE 1 list of available filters including filter index, central wavelength in nanometers and band width (BW) in nanometers

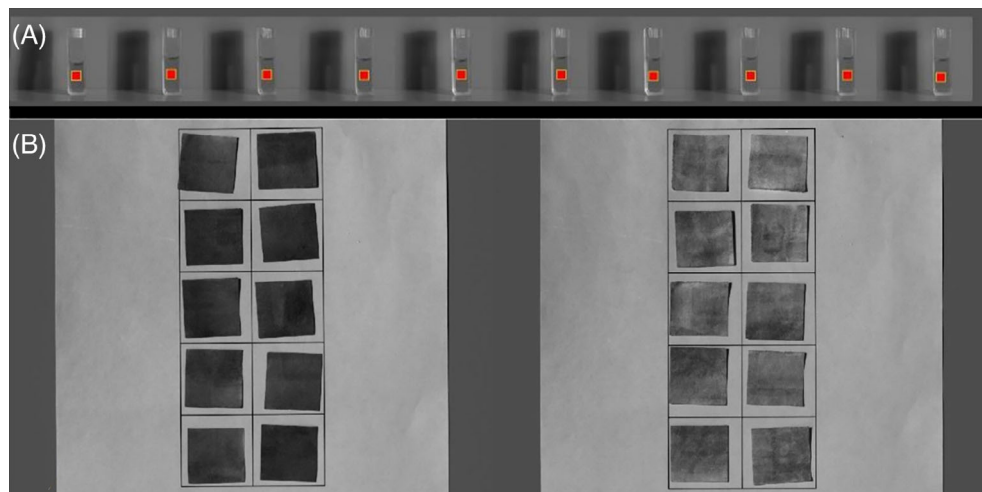| Idx	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
λ	425	460	482	509	530	555	570	581	615	650	680	704	770	833	875	950
BW	50	20	56	20	24	50	50	20	100	100	50	100	102	125	50	100

FIGURE 1 A, Image composition of the vial samples for the group labeled as "6 hours" and the 650 nm band. All extracted regions (in red) were selected avoiding any shadow effects. B, 550 nm band for the paper sample group corresponding (left) to the "0 h" reference condition, and (right) to the group corresponding to the 720 hours most aged samples

which was later returned to the vial in order to continue with the aging process. The absorption spectra of samples were recorded in the 250 to 950 nm range. For the paper samples, the indigo blue was extracted as described above.

2.4.2 | Capillary electrophoresis with a diode array detector (CE-DAD)

CE-DAD separation was carried out in fused-silica capillaries (72 cm × 75 μm id) with 56 cm inlet-to-detector distance. The samples were injected hydrodynamically into the anodic end of the capillary with a pressure mode of 50 mbar for 13 seconds. Electrophoretic separation was performed at 25 kV for 25 minutes, resulting in a current of about 117 μA. The temperature of the capillary was constant at 25°C. The capillary was equilibrated with the running buffer (40 mM tetraborate pH 9.25) for 5 minutes before each sample injection. After each analysis, the capillary was rinsed for 3 minutes with deionized water.[24]

2.4.3 | Multispectral imaging

Multispectral imaging in the visible and NIR spectral ranges was used because it is a noninvasive and nondestructive analysis technique to identify the evolution of degradation. The multispectral imaging data were comparatively analyzed with the results of the other techniques described above (ie, UV-vis spectroscopy and CE-DAD).

The spectral images were captured with a Pixelteq Spectrocam VIS camera (Halma, UK) which is a filter-wheel-based spectral camera with 16 (8 + 8) channels. The peak wavelengths of the filters used ranged from 425 nm to 950 nm, with bandwidths between 20 and 100 nm, as indicated in Table 1.

The camera was equipped with a monochrome sensor and the filter wheel rotated between the lens and the sensor so as to place a colored filter in the path of light reaching the sensor. This rotation was synchronized with the image capture and the exposure time was determined independently for each band. The camera obtains images in the visible and near-infrared regions within the 370 to 1100 nm spectral range.[25] The light source used as illumination to capture the images was a LED-based light (Nanguang CN600CSA, China).

The camera was placed in front of the samples with a Light Emitting Diode LED-based light source as illumination. The captured images were processed to extract a 50 × 50 pixels region from each vial. The vials were captured one by one, and any influence of shadowing effects were discounted by the use of a 45/0 geometry of illumination/observation. The images of the indigo samples deposited on a paper substrate were also captured (Figure 1).
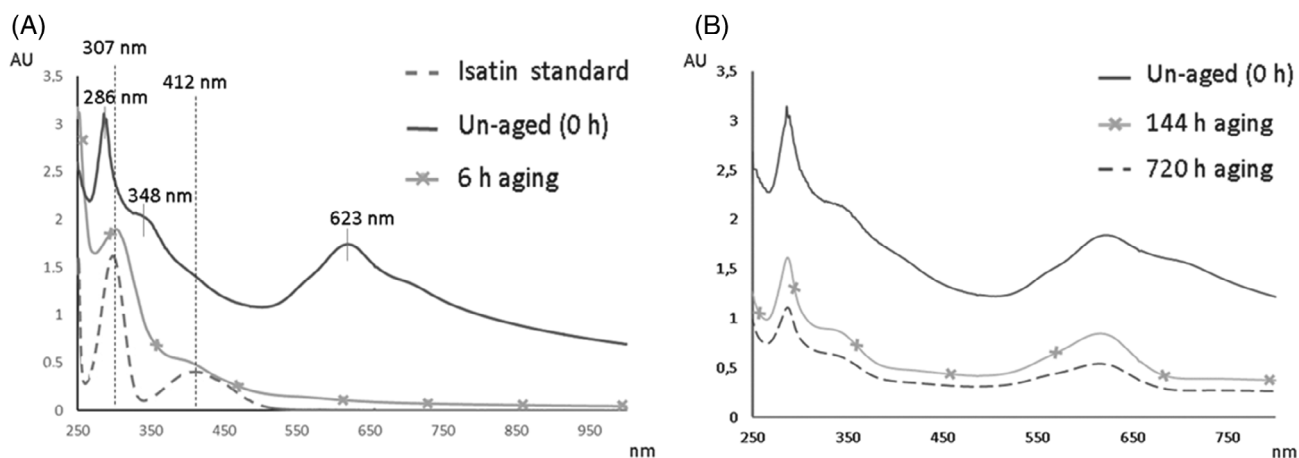
FIGURE 2 Absorption spectra of sample aging: A, Vial sample comparison between sample Un-aged (0 hour aging), isatin reference standard and sample at 6 hours aging. B, Paper samples comparison between sample Un-aged, sample at 144 hours aging and sample at 720 hours aging

3 | RESULTS AND DISCUSSION

3.1 | UV-visible spectrophotometry

A UV-visible spectrophotometer was used for the analysis of vial and paper samples in order to obtain the data of characteristic spectra for the different degradation products during the aging process.

The study of vial samples and paper samples shows the existence of two different spectra. Based on the bibliography[19] indigo is oxidized to dehydroindigo and then to isatin.

The first spectra were obtained before the sequence of aging in all kinds of samples corresponding to indigo without degradation (λ_{max}: 286, 348 and 623 nm). The same spectra as the one obtained from the reference standard of indigotin. In vial samples, these spectra were maintained until 6 hours aging. After 6 hours in the same condition, a second absorption spectrum was obtained (λ_{max}: 252, 307, and 412 nm) and was maintained until the end of the aging process. The comparison between this second spectra and the one of the reference standards of isatin confirmed the formation and conversion of indigo blue (blue in color) into isatin (yellow) with corresponds to a degradation product from the oxidation of indigo blue (Figure 2A). On paper samples, the spectrophotometric analysis revealed no significant changes, the first spectrum was maintained throughout the complete aging process, but there was a clear intensity decrease (Figure 2B).

UV-vis spectrophotometry does not allow us to distinguish between different degradation products (dehydroindigo and isatin) for indigo blue in dissolution and it does not allow the identification of any degraded compound of indigo blue as a layer on paper. Therefore, this technique has been completed with a composition study by CE-DAD.

3.2 | Capillary electrophoresis analysis

CE-DAD analysis was used in vial and paper samples for the identification of the principal degradation products: dehydroindigo and isatin.[24,26-28]

In vial samples, the principal compound of indigo blue, indigotin, was present up to 6 hours (indigotin: t_m 17.14 minutes), from that interval the first degradation product of indigo blue appeared (dehydroindigo: t_m 17.83 minutes) that completely replaced the indigo after 12 hours aging. From 12 hours a second degradation product appeared (isatin t_m 8.02 minutes), both degradation products remained until the end of the aging process (Figure 3). In order to distinguish between dehydroindigo and isatin (which have different t_m), it was tested the CE-DAD study of a reference standard of isatin that revealed a peak with t_m 8.027 minutes (Figure 3C).[19]

In the paper samples, indigo is present throughout the aging process. From 24 hours, the two degradation products appear (dehydroindigo and isatin) that are maintained together with the indigo until the end of the aging process (Figure 3E). It is necessary to point out that although the analysis is able to identify indigotin from 24 hours on, the degradation products still increase compared to the indigotin at the end of aging process. These results have permitted us to classify the samples according to the aging state as shown in (Figure 3).

3.3 | Multispectral imaging and support vector machine analysis

After the images of the indigo samples were captured with the multispectral camera, a dimensionality reduction technique called principal component analysis
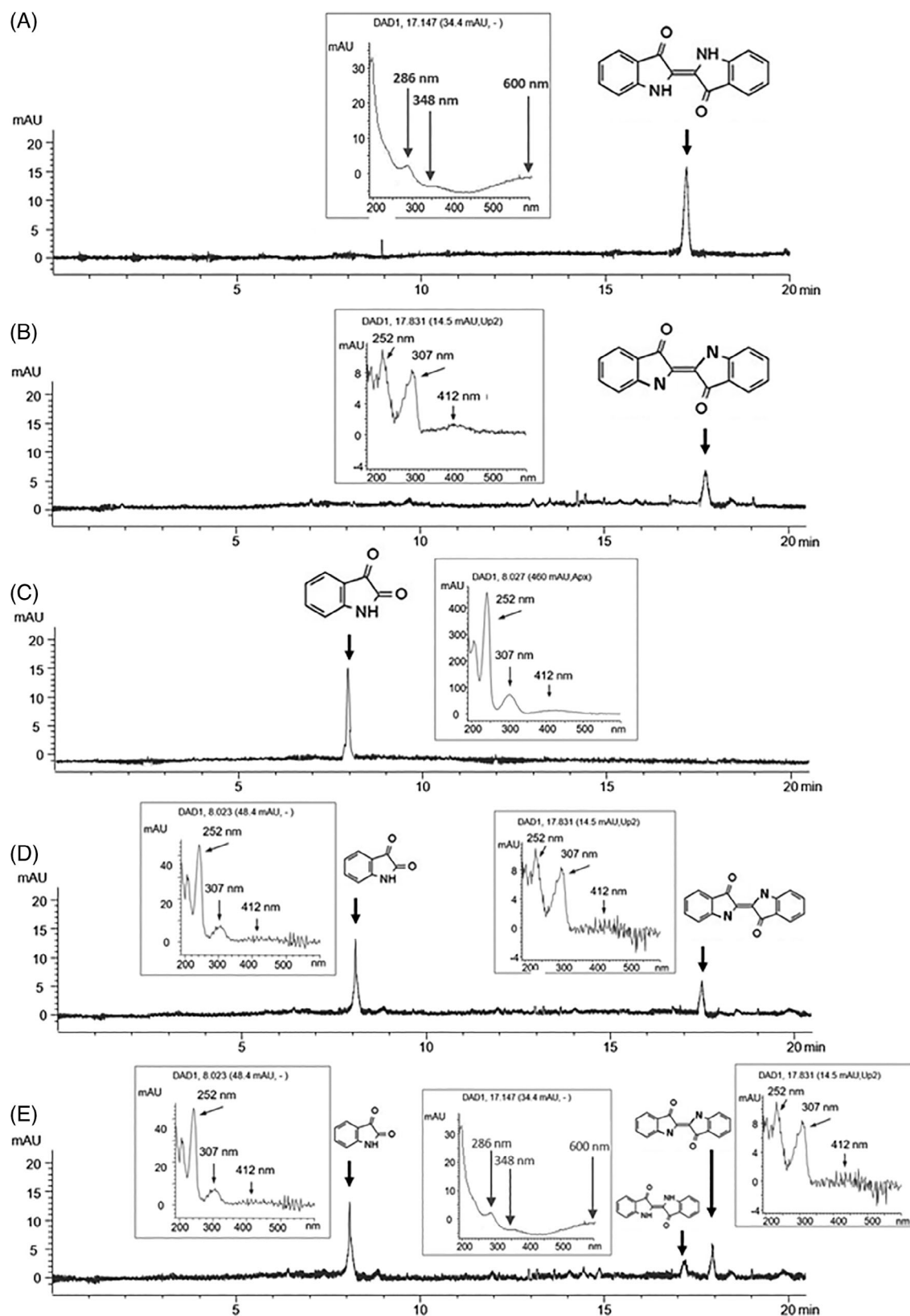
FIGURE 3 Electropherogram of vial samples and degradation products at different aging states. A, vial sample Un-aged 0 hour; B, vial sample at 6 hours aging; C, isatin reference standard; D, vial sample at 12 hours aging; E, paper sample at 720 hours aging
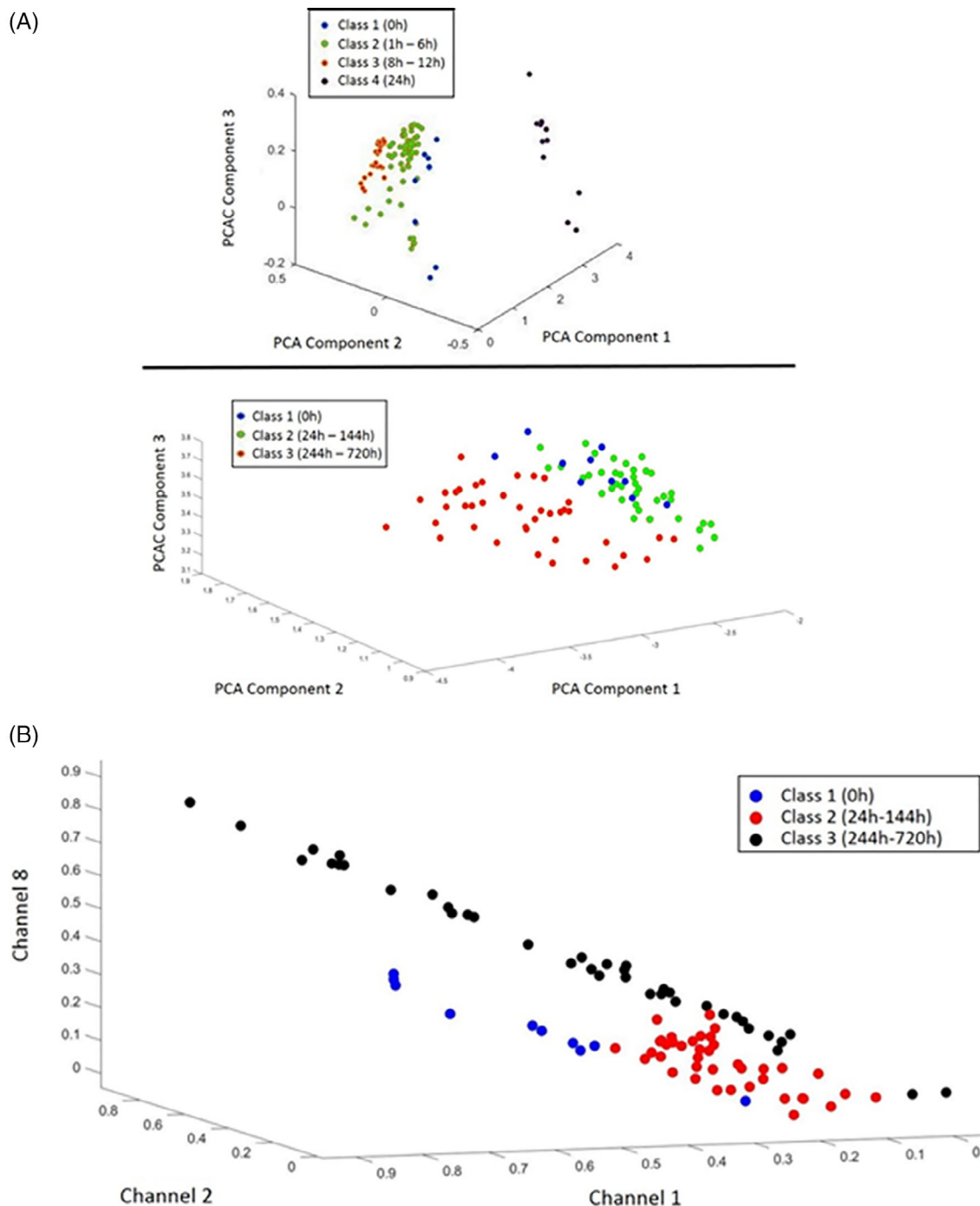
(A)

(B)

FIGURE 4 A, PCA first three components for the vial samples (plot above), and paper samples (plot below); B, Three selected channels for classification of paper samples

(PCA) was used to allow for a better visualization of the samples.[29] This technique allowed us to observe whether the different aging states were potentially easy to separate (formed well defined clusters in the principal components space) based on the information provided by the camera responses. In Figure 4A), preliminary analysis of the projections of the sample data for vials and paper in the first three principal components are shown. The

samples were divided into the corresponding aging classes based the spectrophotometric data and the onset of three compounds (IND, DHI and IS) according to the CE-DAD results (see previous sections). The vial samples posed a less complicated problem than the paper samples (see Figure 4A), because the aging classes identified were more clearly defined and easier to separate. The aging process was completed in 24 hours for the vial

samples, and four different aging stages were identified: class 1 for 0 hour (un-aged, no degradation product); class 2 for 1 to 6 hours (no degradation and moderate degradation state, DHI compound presence); class 3 for 8 to 12 hours (severe degradation state, DHI, and IS compounds presence); and class 4 for 24 hours (extremely severe degradation state, only IS compound presence). For the paper samples, even after 720 hours the aging process was not completed, so only three different aging states were identified in this case (see also Figure 3): class 1 for 0 hour (un-aged), class 2 for 24 to 144 hours (severe degradation state), and class 3 for 288 to 720 hours (severe degradation state). The appearance of the third class in the multispectral imaging analysis on the paper samples represents a subdivision of a moderate to severe aging state, as although three compounds can be chemically identified (IND, DHI, and IS), the multispectral imaging is able to differentiate between a sample with a greater quantity of the degradation product (DHI and IS). Thus, from 288 hours onward three compounds co-exist, but the electrophoretic peak for DHI and IS is higher due to the fact that there is a greater quantity (DHI+ e IS+). It would seem that a moderate to extreme degradation occurs, without

reaching an extreme level, where there is only one super-oxidized product.

Based on the above preliminary analysis, a machine learning algorithm—the support vector machine (SVM)—was used to determine if the aging class of a sample could be predicted using the information provided by the Pixelteq Spectrocam VIS multispectral camera. The SVM belongs to a group of techniques called wide-margin classifiers, which try to find a set of directions which maximizes the distance between representative instances of the different classes, using some previously classified samples as training data.[30,31] The samples were divided into training and testing groups. The training group was used by the SVM to learn the best support vectors for the classification, and the testing group, composed of different samples, was used to measure the accuracy (ie, true positive rate) of the SVM. Given the restricted number of samples, a cross-validation technique[31] was used to divide the samples into training and testing groups. A 10-fold cross validation was implemented, so in each of the 10 iterations, 10% of the samples were used as a test and 90% as training. The SVM included a Gaussian kernel, and the two parameters (the width of the kernel function and the regularization parameter) were optimized using an inner loop of 10-fold cross

TABLE 2 Aging state classification of vial samples and paper samples according to the results obtained by UV–vis and CE-DAD

Vial samples

t (h)	IND	DHI	IS	Aging state	SVM aging class
0	√	—	—	No degradation	1
1	√	—	—		2
2	√	—	—		
3	√	—	—		
4	√	—	—		
5	√	—	—		
6	—	√	—	Severe degradation	
8	—	√	—		3
10	—	√	—		
12	—	—	√	Extremely severe degradation	
24	—	—	√		4

Paper samples

t (h)	IND	DHI	IS	Aging state	SVM aging class
0	√	—	—	No degradation	1
24-144	√	√	√	Severe degradation	2
288-720	√	√	√	Severe degradation	3

Abbreviations: DHI, dehydroindigo; IND, indigotin; IS, isatin; SVM, support vector machine.

TABLE 3 Results of TS method and the corresponding channels for vial and paper samples separately

Sample	SVM	Channel set	Accuracy	Precision	Recall	Aging state
Vials	Class 1 (0 h)	2, 7, 10	0.99	1.00	1.00	No degradation
	Class 2 (1-6 h)	2, 7, 10	1.00	1.00	1.00	
	Class 3 (8-12 h)	2, 7, 10	0.99	1.00	0.96	Severe degradation
	Class 4 (24 h)	2, 7, 10	1.00	1.00	1.00	Extremely severe degradation
Paper	Class 1 (0 h)	2, 3, 8	0.99	1.00	0.90	No degradation
	Class 2 (24-144 h)	2, 3, 8	0.97	0.97	0.95	Severe degradation
	Class 3 (288-720 h)	2, 7, 8	0.98	0.95	1.00	

Abbreviations: TS, Thornton's separability; SVM, support vector machine.

validation within the training group. The accuracy results were obtained after the double-loop 10-fold cross validation was executed for each SVM. One SVM was trained for each class. As explained above, we have added two additional classes (denoted as SVM aging class in Table 2) over the CE-DAD data, to test if SVM was able to provide more sensitivity in the classification. The SVM output for a given sample was positive if the sample belonged to the aging class, or negative if it did not belong to the aging class as defined in Table 2.

Table 3 shows the accuracy, precision, and recall results obtained with the SVM when all the 16 channels are used as input for the classifier. The accuracy is defined as the ratio of correctly classified samples (between 0 and 1). The precision is defined as the number of true positives (TP, correctly classified samples with a positive +1 label) divided by the sum of TP and false positives (FP, samples that were wrongly identified as belonging to the class). And the recall is defined as the number of TP divided by the sum of TP and false negatives (FN, samples that were wrongly identified as not belonging to the class). A low precision result would indicate that the classifier is prone to false alarms (wrongly identifying samples as members of a given class); a low recall result would indicate that the classifier is prone to produce misses (failing to recognize the samples belonging to a given class). A classifier working perfectly would have an accuracy of 1, and also precision and recall values of 1.

Our results show that the multispectral imaging data coupled with the SVM were able to detect correctly the aging state of the indigo samples in both formats (vial and paper). The best results corresponded to the vial samples, as expected, given the preliminary analysis regarding the different complexity of the two sample groups using PCA, as shown in Figure 4. For the paper samples, the most difficult group to identify was the unaged (class 1), because the aging process was considerably slower due to the protecting effect of the paper substrate, which made

it more difficult for the system to detect any difference between the unaged samples (SVM class 1) and some of the early-stage samples of the first aging interval (SVM class 2).

In addition, the possibility of reducing the number of channels used as input for the SVM while still maintaining acceptable results in the classification was also investigated. So, a feature selection method based on the Thornton's separability index (TS) was implemented, which computes the accuracy of a Nearest-Neighbor classifier using the ground-truth for a given set of samples.[32] The better the samples are separated in the feature subset, the higher is the separability index value.

The TS values for all the combinations of three different channels extracted from the group of 16 channels were computed for vial and paper samples separately. The channel combinations that gave the maximum TS value were used as input for a newly trained SVM, and the accuracy, precision and recall values were obtained. The results and the corresponding channels are shown in Table 3.

For the vial group, the three channels that offered the best results had peak wavelengths of 460, 570, and 650 nm, while for the paper samples, the best results were obtained with channels 2, 3, and 8 (peak wavelengths of 460, 482, and 581 nm), or else 2, 7, and 8 (460, 570, and 581 nm). The SVM generally performed better with the reduced feature space of three channels as input than with all 16 channels as input, so some of the initial sets of channels were not providing adequate information for the classification of the aging state. The bandwidth of the selected channels varied between 20 and 100 nm.

Aiming for a simplified capture device that could be used for both tasks (detection of aging state of indigo in either vial or paper), the following three channels (2, 3 and 8) were selected, and the performance of the SVM was tested for both sample groups and all classes (Table 4).

TABLE 4 Results of TS method of the selected three channels (2, 3 and 8)

Sample	SVM	Channel set	Accuracy	Precision	Recall	Aging state
Vials	Class 1 (0 h)	2, 3, 8	0.97	0.87	0.80	No degradation
	Class 2 (1-6 h)	2, 3, 8	0.98	0.98	0.98	
	Class 3 (8-12 h)	2, 3, 8	0.99	0.96	1.00	Severe degradation
	Class 4 (24 h)	2, 3, 8	1.00	1.00	1.00	Extremely severe degradation
Paper	Class 1 (0 h)	2, 3, 8	0.99	1.00	0.90	No degradation
	Class 2 (24-144 h)	2, 3, 8	0.97	0.97	0.95	Severe degradation
	Class 3 (288-720 h)	2, 3, 8	0.98	1.00	0.98	

Abbreviations: TS, Thornton's separability; SVM, support vector machine.

We were able to achieve satisfactory results with this group of three channels for the two different sample formats tested. In Figure 4B), a plot of the paper sample group in the subspace formed by the three selected channels (channels 2, 3 and 8, see Table 1) is presented. The different classes were much better separated much better in this subspace than in the PCA components space shown in Figure 4c. The task for the classifier was then rendered easier using this subset of channels.

This result opens up the possibility of designing a simplified capture device for performing the task of detecting the aging state of indigo in the context of cultural heritage preservation.

4 | CONCLUSIONS

The results of all experiments suggest that it is possible to achieve very high accuracy in the automatic classification of the aging state of indigo samples, both in dissolution form and in solid form deposited on paper. Our naked eye is not able to perform this task (especially for the paper samples), but the multispectral camera can carry it out thanks to the higher amount of information acquired and to the machine learning algorithms used to process the data. It is also possible to operate with a reduced set of three channels to obtain very good results with the SVM classifier.

The main drawback of the proposed framework is that we need to have some knowledge of the aging stage of our training samples for the training stage of the SVM to work. But the SVM would only need to be trained once, and afterward it would perform well for samples acquired in similar experimental conditions. Nevertheless, we are aware that the approach proposed might not work well if the test samples are quite different from the training samples, and would require retraining in that case.

Comparing the results of analytical techniques and multispectral imaging, we have found that there is a good correlation between the classification based on multispectral data and the electrophoretic and spectroscopic data. The results obtained using multispectral imaging combined with SVM-based classification supports the feasibility of using a spectral camera as a noninvasive and nondestructive technique for characterizing the aging state of indigo samples. This technique can be realized in situ and with higher speed than other analytical techniques (processing times of less than 30 seconds in a laptop equipped with a standard A12 processor, once the SVM has been trained).

The proposed SVM approach is able to distinguish between a new (un-aged) color and one that has begun to age. In practice, the method would be able to identify a repainting or a recent restoration with indigo in an area that was previously covered with the same pigment. The ability to differentiate between unaged indigo and slightly aged indigo is a very useful advantage compared to other analytical techniques habitually used for conservation and analysis of cultural heritage.

DATA AVAILABILITY STATEMENT
The authors declare that they have no known competing financial interests or personal relationships that could

have appeared to influence the work reported in this paper.

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