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Direct inlet mass spectrometry for a rapid characterization of indigo in lipidic and proteinaceous matrices

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ABSTRACT

Direct inlet mass spectrometry (DI-MS) was examined for its suitability to quickly identify indigo in mixtures with lipidic and proteinaceous binders, such as those characteristic of paintings and polychromy. The technique was tested on naturally aged reference mixtures of indigo with linseed oil, rabbit skin glue, and whole egg. DI-MS with electron ionization at 70 eV served as an efficient tool for screening indigo in complex matrices and provided, at the same time, a mass spectral fingerprint of the organic binder in just a few minutes. The technique was applied to the identification of indigo in a blue sample from a mural painting dated from the 18th century and housed in an Andean church in Bolivia. At the same time, the acquired data revealed for the first time the use of an egg tempera binding medium in an Andean mural painting.

1. Introduction

Natural indigo has been widely used as a textile dye and as an oil and watercolor pigment [1]. Until the end of the nineteenth century, indigo was exclusively prepared from the fermentation in water of leaves of plants of the families Papilionaceae, Brassicaceae, and Polygonaceae. The most important sources of indigo are *Indigofera tinctoria* L., *Indigofera suffructicosa* Mill., *Isatis tinctoria* L., and *Polygonum tinctorium* [2]. Indigotin (**1**) (Fig. 1), the compound responsible for the blue colour in indigo, is formed by enzymatic hydrolysis of the precursor glycosides indican and isatan B to give indoxyl, which is subsequently oxidized by atmospheric oxygen to the colorless *leuco* indigotin and then to **1** [1]. If indoxyl is oxidized to isatin during the processing of indigo plants, the combination of this molecule with one molecule of indoxyl leads to indirubin (**2**) (Fig. 1), a red minor isomer of **1**. The commercial production of synthetic indigo by BASF in 1897 rapidly displaced the natural sources of the dye [1,2].

Indigo is one of the oldest natural blue dyestuffs and it has been identified in textiles from different places in the world [2–6]. As a pigment, it is suitable for watercolors and tempera painting and it has found application in glue medium and oil paints [7–10]. Indigo and Prussian blue, the latter not before 1777, seem to have been the blue pigments most employed in the workshops of Cuzco in the 17th and 18th centuries. Indigo was used in mixtures with other pigments, most commonly with lead white to obtain different shades of blue [11–13], and with orpiment (As_2S_3) to produce green [14].

Several analytical techniques have been applied for the detection of indigoids in artworks and archaeological objects. High-performance liquid chromatography (HPLC) coupled with diode-array (DAD) and mass spectrometer detectors is by far the most well-established technique for the identification of dyestuffs in archaeological and historical textiles [15]. Prior to HPLC analysis, indigo has to be extracted from the fibres. As a *vat* dye, it does not require a mordant metal to attach to the fibres and extraction with dimethyl sulfoxide (DMSO) is generally used [3,16]. Mass spectrometry techniques were also applied to the identification of indigoids in textiles. DMSO and dimethylformamide (DMF) extracts

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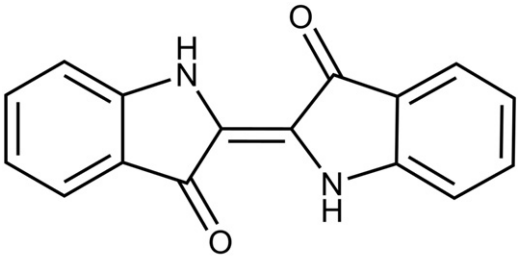

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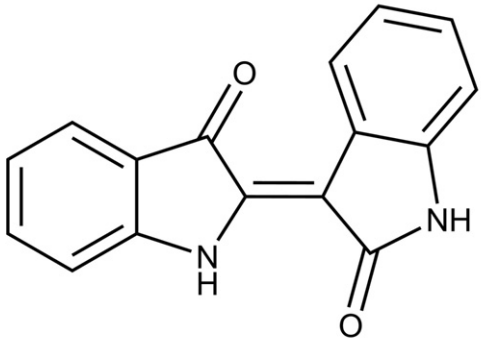

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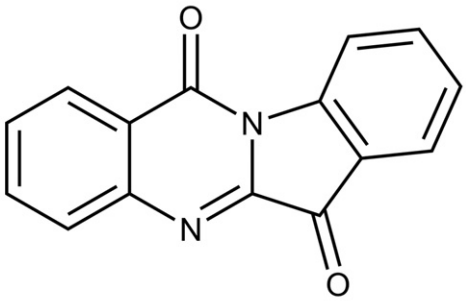

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Fig. 1. Chemical structures of indigotin (**1**), indirubin (**2**), and tryptanthrin (**3**).

of fibres dyed with moluscs containing indigotin, 6-bromoindigotin and 6,6′-dibromoindigotin were successfully analyzed by direct exposure mass spectrometry and negative ion pressure photoionization mass spectrometry [17–20]. Direct analysis of indigo dyed textiles was also assessed by time of flight secondary ion mass spectrometry (TOF-SIMS) [21,22], laser desorption mass spectrometry (LD-MS) [23] and fibre optics reflectance spectroscopy [24]. Recently, an analytical procedure based on pyridine extraction, silylation and further analysis by gas chromatography coupled to mass spectrometry (GC-MS) succeeded in the identification of indigo in two historical textiles [25].

Regarding the identification of indigo in paintings, micro-Raman spectroscopy is usually applied as a nondestructive technique, although it is often limited by the high fluorescence of aged binders in the complex painting matrix [10,26]. In the last decade, surface-enhanced Raman spectroscopy (SERS) has been increasingly applied to the identification of organic pigments in paintings and textiles [27]. Although this technique is very sensitive, SERS analysis of indigo in paintings requires a sample pretreatment, for example, with H_2SO_4 [28]. Recently, tip-enhanced Raman spectroscopy (TERS) has proved efficient for the identification of indigo on dyed rice paper references [29]. Finally, reactive pyrolysis under methylating or silylating conditions, in combination with mass spectrometry (Py-GC-MS), has been evaluated for the analysis of reference painting mixtures of indigo with linseed oil as a binder [7]. The main drawback of this methodology was the detection of various derivatives of indigo in low levels in comparison with the methyl esters of fatty acids from the binder.

With the aim of searching for a simple and rapid methodology for the identification of indigo in lipidic and proteinaceous matrices, such as those in paintings, the goal of the present study was to evaluate the application of DI-MS with electron ionization at 70 eV to the identification of indigo in naturally aged reference mixtures with linseed oil, rabbit skin glue and whole egg. At the same time, we wished to explore if the binder and its degradation products could be characterized in the same analysis. Finally, the technique was applied to the analysis of a blue sample from a mural painting dated from the 18th century and housed in an Andean church in Bolivia.

2. Experimental

2.1. Model samples

The model samples were prepared by applying synthetic indigo, mixed to a binder in a ratio binder/indigo = 1.0, on glass slides. Three different types of binder were used: linseed oil, rabbit skin glue, and whole egg. Each model sample had dimensions of 2.5 cm × 5.0 cm and a thickness of about 0.15 mm. The samples were naturally aged at room temperature for 13 years. Indigo was purchased from Allied Chemical & Dye Corporation, New York, USA. Linseed oil, rabbit glue, and egg were purchased from local suppliers.

2.2. Painting sample

One microsample (M6) was extracted with a scalpel from the blue area of the handle of a flower vase (Fig. 2) in a wall painting from the church of Copacabana de Andamarca in Bolivia before a restoration treatment. The church wall paintings, dated from the early 18th century, depict the escathological themes of Death, Judgment, Glory, and Hell. The pictures of the Last Four Things were vital for the Catholic belief and were part of the process of evangelization done under the Spanish domain [30]. The painting sample was analyzed by DI-MS in the same conditions as the model samples.

2.3. Instrumentation

Elemental chemical analyses of the blue layer and the ground layer of the mural painting microsample M6 were obtained by using a field environmental scanning electron microscope (FE-SEM) Zeiss:Supra 40 coupled with an energy-dispersive X-ray spectrometer (SEM-EDS) INCA X Sight (Oxford Instruments). DI-MS analyses were carried out on a ThermoScientific EM/DSQ II spectrometer with electron ionization at 70 eV in the positive ion mode. The samples (<0.5 mg) were placed in a small glass vial, which was inserted into the direct insertion probe and introduced into the ion source. The temperature was increased from 100 °C to 400 °C at a rate of 40 °C/min. The mass spectrometer was scanned over a m/z range of 40–1050. Mass spectral fingerprints were obtained by averaging the mass spectra in the indicated time range