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Photochemical properties of purpurin and its implications for capillary electrophoresis with laser induced fluorescence detection

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ABSTRACT

Purpurin is an anthraquinone dye that is found in plant material such as Madder root. The separation of purpurin using capillary electrophoresis under alkaline condition can be complicated by reproducibility in peak height and peak shape. In this study the poor peak reproducibility of capillary electrophoresis separations was attributed to photochemical properties of purpurin. Experiments were carried out using UV–visible and fluorescence spectrophotometry to measure the 1st order rate constant for the photochemical fading of purpurin. At pH = 9.24 the rate constant was 4.5×10^{-3} s^{-1} and at pH = 7.0 the rate constant was determined to be 5.0×10^{-5} s^{-1}. Proton NMR, thin layer chromatography, gas chromatography and UV–visible spectrophotometry techniques were also employed to identify the photodegraded products of purpurin.

1. Introduction

The anthraquinone dyes have been used for centuries as coloring agents in textiles. Historically an important source of red dye was the common madder root (*Rubia tinctorum*). The compounds in madder that are responsible for color are primarily alizarin and purpurin. The structure of purpurin is shown in Fig. 1(a). For comparison, other dyes used in this study such as carmine (Fig. 1(c)) and morin (Fig. 1(d)), flavone, are also shown. Purpurin (Fig. 1(a)) and alizarin (Fig. 1(b)) are very similar structurally with purpurin having one additional OH group on the aromatic ring structure. It is also well known that the color of these dyes can be modified with changes in pH [1]. Under acidic conditions, an aqueous solution containing purpurin has a yellow appearance and under basic conditions it is a deep red, depending on concentration.

Detecting these dyes in historical objects (textiles, paintings) has been achieved using a number of separation methods including high performance liquid chromatography (HPLC) [2,3] and gas chromatography (GC) [4]. These instruments can be equipped with different detection methods such as UV–visible and fluorescence detection, photodiode array (DAD) and mass spectrometry (MS).

Separations of anthraquinone dyes with HPLC have been carried out using methanol/H_2O, trifluoroacetic acid and acetonitrile [5,6]. Other studies using HPLC have described the use of phosphate buffer under acidic conditions (pH = 2.3–2.9) [7,8]. Generally, the chromatograms with phosphate buffers provided well-shaped peaks for the anthraquinone dyes including purpurin. Fewer manuscripts have been published on the use of GC for studying dyes since these dyes are rather large polar molecules which need to be derivatized with N,O-bis (trimethylsilyl) trifluoroacetamide prior to analysis [9].

Capillary electrophoresis (CE) is also an attractive separation technique that has been used for identifying anthraquinone dyes. For CE, good separations of anthraquinones are commonly achieved when the target species are negatively charged using a phosphate or borate buffer under alkaline conditions (pH = 9.24).

Puchalska [10] used CE coupled with diode-array detection (DAD) and electrospray mass spectrometric (ESI-MS) detection to isolate and identify a number of dyes in cochineal, lac dye and

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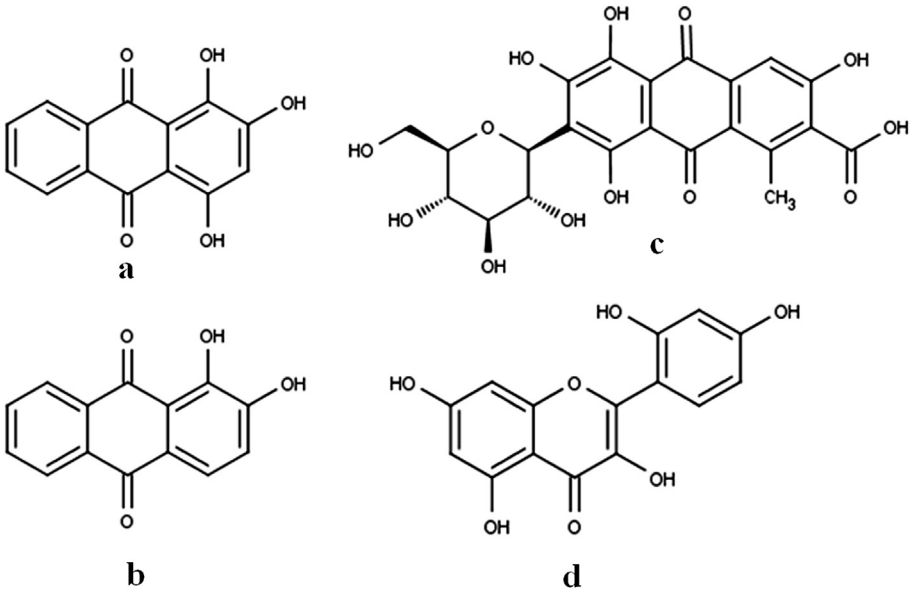

Fig. 1. Structure of (a) purpurin, (b) alizarin, (c) carmine and (d) morin.

madder. In this study alizarin and purpurin were detected in madder lake extract. In both DAD and ESI-MS a detectable peak for purpurin was obtained. The running buffer for these CE separations with DAD detection was 5 mM phosphate at pH = 8.5, and with MS detection it was a 20 mM carbonate buffer at pH = 9.0. The separation of anthraquinones and flavonoids using CE with MS detection has also been described by Surowiec [11]. A well-shaped peak for purpurin was achieved using a running buffer of 40 mM ammonium acetate at pH = 9.5. The reproducibility of the peak area for purpurin had the highest value among the other dyes (14.3% vs. 2.0% for emodin) under these conditions. Micellar electrokinetic chromatography (MEKC) has also been described for the separation of anthraquinones and flavonoids in Coptic textiles from the National Museum in Warsaw, Poland. In this study a mixture of dyes was separated using borate buffer at pH = 8.5 which resulted in well-resolved peaks [12]. Maguregui [13] reported the extraction and separation of alizarin, quinizarin and purpurin in a 17th century oil painting using MEKC equipped with DAD. The separation was carried out using sodium tetraborate buffer at pH = 9.0 with 20 mM SDS. The separation of a variety of natural dyes including purpurin has been obtained using MEKC with UV-DAD detection [14]. In this study the dyes were separated using 40 mM sodium tetraborate buffer solution at pH = 9.25 and this methodology was used for identifying the dyes in a collection of drawing and maps from the Royal Chancellery Archives in Granada.

Using laser induced fluorescence (LIF) detection schemes with CE can result in significant improvements in detection limits over light absorption methods which makes this technique very attractive for work on valuable samples such as historical objects. Therefore CE-LIF would be considered as an ideal technique to study the historical objects. Previous work in our laboratory has focused on the use of MEKC with LIF for the separation of selected anthraquinone and flavonoid dyes under alkaline conditions [15,16]. Separations were carried out using 20 mM borate buffer with 20 mM SDS under alkaline conditions and in these studies pH was found to have a pronounced effect on the fluorescence and separation properties of purpurin. It appeared that both time and pH were extremely important for peak shape and reproducibility of peak height. Therefore, in this study the spectroscopic properties of purpurin are reported in an effort to explain our observations for purpurin analysis using CE.

2. Experimental section

Separations were carried out on a laboratory-built CE instrument equipped with a post-column laser-induced fluorescence detection system [17]. The injection end of a 500 mm long, 10 μm inner diameter fused silica capillary (Polymicro Technologies, Inc., Phoenix, AZ, USA) along with a platinum electrode connected to a high voltage supply (Spellman model CZE 2000, Hauppauge, NY, USA) was placed into a buffer-filled vessel. The applied voltage across the capillary was typically 18–25 kV. The detection end of the capillary, from which a 1 mm length of the external polyimide coating was removed by flame, was inserted into a quartz sheath flow cuvette with a 250 by 250 μm inner bore (Hellma Ltd, Concord, ON, Canada). The system was grounded through the sheath flow buffer within the cuvette. A diode laser (Coherent, Inc., Santa Clara, CA, USA) with a 407 nm output (50 mW) was employed. Light from the laser was focused with a 6.3×, N.A. 0.2 microscope objective (Melles Griot, Irvine, CA, USA) approximately 10 μm below the detection end of the capillary. Emission was collected at 90° using a 60×, N.A. 0.7 microscope objective (Universe Kogaku, Inc., Oster Bay, NY, USA) passed through a 535AF45 optical filter (Omega Optical, Inc., Brattleboro, VT, USA) and a slit and then onto a photomultiplier tube (PMT, Hamamatsu model 1477, Middlesex, NJ, USA). The analog PMT signal was collected and digitized using a computer through a PCI-MIO-16XE I/O board utilizing LabVIEW® software (National Instruments™, Austin, TX, USA) at 10 Hz. The same board was used to control the electrophoresis voltage and PMT bias. Data was analyzed using IgorPro™ software (Instrutech, Port Washington, NY, USA).

A Varian Cary eclipse fluorescence spectrophotometer (Varian Inc., Palo Alto, CA, USA) was used to measure the spectra of purpurin. Measurements were carried out using a quartz cell with a 10 mm path length. The excitation and emission slits were set to 5 and 2.5 nm respectively. In addition to absorption spectra, emission spectra were also measured at a fixed excitation wavelength of 407 nm. The PMT was set at 1000 V for the samples in pH = 9.24 and 850 V for the samples in pH = 7.0. UV spectra were recorded on a Shimadzu UV 250 spectrophotometer (Shimadzu Co. Ltd., Kyoto, Japan).

Purpurin solutions of 5 μg mL^{-1} at pH = 9.24 and 7.0 were exposed to a Solux lamp (3500 K Color Temp, Color Rendering Index: CRI 99, 10° spread; Solux, Rochester, NY, USA) for 1 min to