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Fractionation and spectroscopic properties of fulvic acid and its extract

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ABSTRACT

Novel results were obtained when a fulvic acid was isolated from Acros humic acid and fractionated by traditional preparative thin-layer chromatography. Eight colorful bands were directly viewed and analyzed showing very different fluorescence and absorption properties. The fluorescence quantum yield of the bands ranged from 2% to 9.4%, significantly higher than that of natural humic substances. An aqueous fulvic acid solution was also extracted with methylene chloride (CH_2Cl_2) by continuous liquid–liquid extraction. The CH_2Cl_2 extract was further fractionated by thin-layer chromatography. Eleven highly fluorescent colorful bands and six weakly fluorescent bands were observed and examined. UV–vis absorption and fluorescence (including 3D matrix) spectra and fluorescence quantum yields revealed that each band still represented a mixture of compounds. Moreover, substantial differences in optical properties were observed among bands. A single band possessed the highest fluorescence quantum yield (6%) and highest specific fluorescence (fluorescence/mass), and accounted for 21% of the total fluorescence of the extract. The mass of individual bands varied from 1.6% to 14.1% of the total materials recovered. Components of all fractions were grouped into 11 fluorophore families according to their maxima on 3D matrix fluorescence spectra. No component is dominant in the whole fulvic acid or extracted portion in terms of optical properties. Over 40 natural products are proposed for model chromophores.

1. Introduction

The importance of humic substances to the natural world is well documented (Zepp and Schlotzhauer, 1981; Blough and Green, 1995; Vodacek et al., 1997). Many experimental attempts (Khairy, 1980; Leenheer, 1981; Thurman and Malcolm, 1981; Santos et al., 1994; Busseler et al., 1996; Wander and Traina, 1996; Trubetskoj et al., 1997; Wu and Tanoue, 2001) including PARAFAC analysis (Stedmon et al., 2007) have been made to fractionate these extremely complicated mixtures. XAD resin series have been widely used to extract humic acid (HA) or fulvic acid (FA) as a whole from ocean, river or lake water (Leenheer, 1981; Thurman and Malcolm, 1981; Malcolm et al., 1994; Santos et al., 1994) or soil (Wander and Traina, 1996). Cross-flow filtration has been employed to concentrate organic carbon and to fractionate them by molecular weight (Busseler et al., 1996). Reverse osmosis was able to separate DOM from water by removing solvent and thus leaving the DOM unfractionated (Serkiz and Perdue, 1990). These three methods were not able to further fractionate HA or FA into subfractions of specific characteristics. Preparative and non-preparative thin-layer chromatography (TLC) methods have been applied to fractionate FA or HA into 2, 3 or 4 portions (Khairy, 1980; Andres and Romero, 1988; Khairy, 1990). Reverse phase HPLC (Saleh and Ong, 1989; Lombardi et al., 1994; Woelki et al., 1997), size-exclusion chromatography (SEC) (Lin et al., 1995; Trubetskoj et al., 1997; Franke et al., 2004), ultrafiltration (Trubetskoj et al., 1997), flow field-flow fractionation (Zanardi-Lamardo et al., 2002), capillary electrophoresis (Pompe et al., 1996), CE/MS (de Lourdes Pacheco and Havel, 2004) and LC/MS (Zwiener and Frimmel, 2004) have been employed to fractionate HA or FA successfully into 4–7 peaks as determined by UV–vis absorption or fluorescence. Nevertheless, because of lacking narrow-band separation of individual fractions, the numerous components of HA or FA with broad affinity to the chromatographic column produced a continuous chromatogram in the spectrophotometric detector and overwhelmed the spectrum details of different fractions. Moreover, the limited number of absorption and fluorescence spectra collected on HPLC is insufficient for detailed optical analysis, especially fluorescence matrix scans which are essential for multi-fluorophore molecules. Therefore, the chemical and photochemical properties of individual fractions are hard to evaluate.

In this study, liquid–liquid extraction was employed to extract the most hydrophobic (partitioned in CH_2Cl_2) organic materials from a FA isolated from an Acros HA. Preparative TLC was used to fractionate this FA organic extract as well as the methanol-soluble portion of FA. As the modern instruments, like GC/MS and

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LC/MS, are developed, traditional separation method like preparative TLC are used less and less due to the labor skill requirement and lacking automation and computerization. However, preparative TLC method has advantages over the modern instruments in terms of large sampling and fractions amount, versatile mobile phase, unmatched direct 2-dimensional image, simple equipment, easy to perform and low cost. In this study, a non-fluorescent TLC plate was adopted to prevent interference from the fluorescent indicator. The developing reagent was selected from tens of tests on small TLC strips by varying the kind, polarity and ratio of many organic solvents (e.g., methanol, acetone, methylene chloride, ethanol, chloroform, propane, hexane and water) to optimize the separation.

The mass, UV–vis absorption and fluorescence (including 3D matrix) spectra of the separated fractions and extracted materials were examined, from which fluorescence quantum yield (QY) were computed for each fraction. The goal of this work was to separate specific less polar portions of FA from the continuous distribution and to study the relationship between the spectroscopic properties of the extract and that of the identifiable sub-fractions of FA.

2. Experimental

FA (110 mg) were isolated from 20 g of a commercial humic acid (Acros Organics) at pH 2.0. The dried FA was dissolved into 0.6 ml methanol to form a brown solution which was applied onto a preparative TLC plate (Fisherbrand, 250 μm, 20 × 20 cm^2, Redi/Plate, Silica Gel G, without fluorescence indicator) using a capillary tube (~10 μl). The material formed a dark brown straight line of 16 cm × 0.5 cm and was 2 cm from the bottom of the plate. After the methanol evaporated (over night), the TLC plate was developed in a mixed solvent of methanol and ethyl acetate (2:1) for 50 min

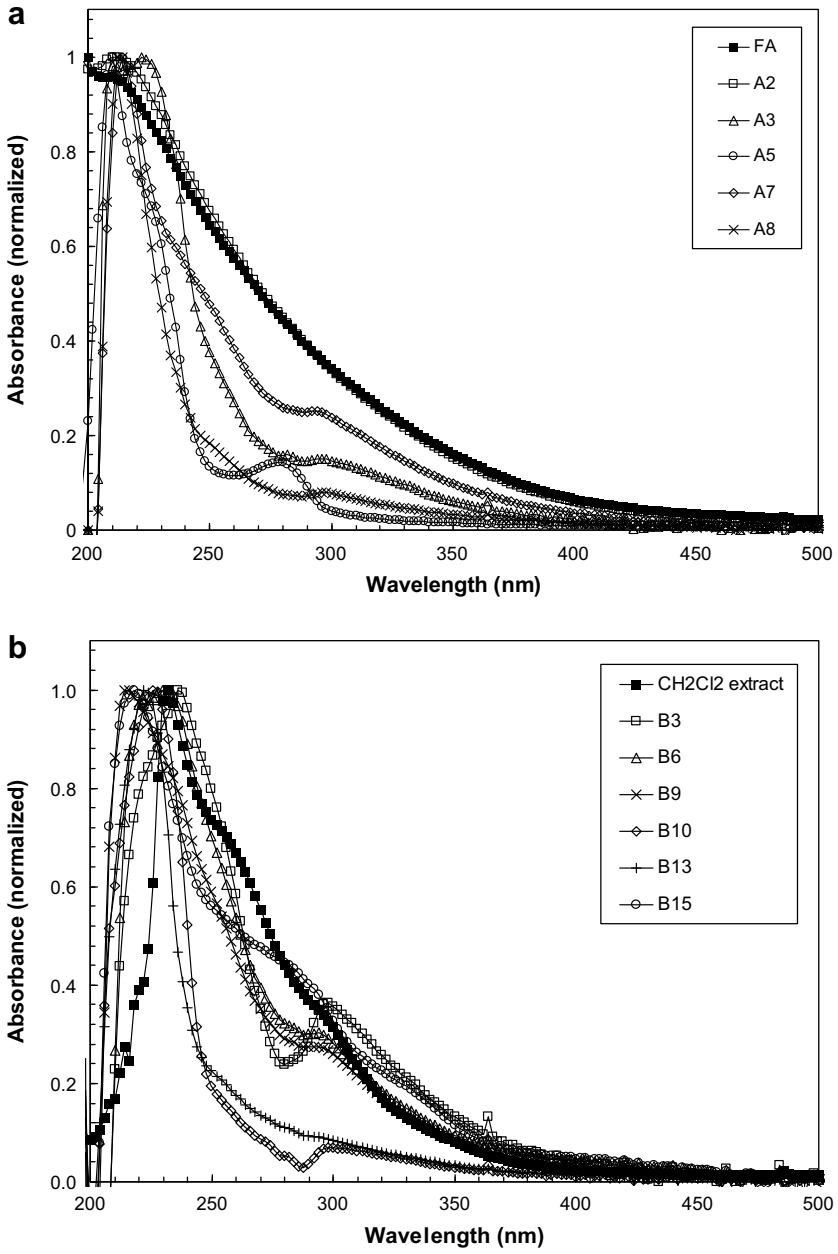

Fig. 1. Selected UV–vis spectra of: (a) fractionated FA bands; and (b) fractionated B bands. All spectra were normalized to 1.000 at their peak wavelength. FA was measured in aqueous phase, CH_2Cl_2 extracted material was measured in CH_2Cl_2. All others were measured in methanol.