



# Determination of humic substances in sediments by focused ultrasound extraction and ultraviolet visible spectroscopy



Juan C. Raposo\*, Unai Villanueva, Maitane Olivares, Juan M. Madariaga

Analytical Chemistry Department, University of the Basque Country (UPV/EHU), Leioa, Spain

## ARTICLE INFO

### Article history:

Received 8 January 2016

Received in revised form 5 April 2016

Accepted 5 April 2016

Available online 8 April 2016

### Keywords:

Humic acid

Fulvic acid

Sediment and focussed ultrasound extraction

## ABSTRACT

The development of a new/fast methodology in order to determine the amount of organic matter (HSs) present in sediment samples was deeply optimized in this work. Emphasis was performed on alkaline extraction because traditional methods required high volumes and long extraction times. Focused ultrasound solid liquid extraction (FUSLE) followed by separation of HSs in acidic media and determination by means of UV–Vis turned out to be a very suitable analytical procedure to speed up HSs extraction, specifically humic acid (HA) and fulvic acid (FA). All variables affecting the extraction process (sodium hydroxide concentration and volume, ultrasound power, cycles and time) were screened by using the analysis of variance and multivariate design. These variables led optimum values of 29 min, continuous application power of 95% (10 cycles) and 15 ml of NaOH 1 mol l<sup>-1</sup>. This methodology was found precise (RSD of 3.3% for HA and 3.7% for FA, for 5 determinations in 3 different days) and sensitive (LODs were 0.1 µg g<sup>-1</sup> and 0.3 µg g<sup>-1</sup> for HA and FA, respectively). Finally, the addition of Mn(II) was proven to be a good alternative to prevent the degradation of HSs in the extraction step.

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## 1. Introduction

Even though it is not possible to accurately detail the structure of a typical humic substance (HS), it is widely accepted that HSs show a great complexing ability of trace metals [1]. This property is attributed to the different oxygen-containing functional groups they have, such as carboxylic, phenolic and carbonyl groups, which can solubilise metal ions from sediments and, therefore, affect their mobilization. Therefore, knowing, not only the amount of hazardous substances (trace metals) but also the amount of HSs that can make them available, allow us in assessing with better accuracy the real danger of contaminated places.

In general terms, a substantial proportion of carbon-containing substances in the environment can be referred to as HSs. In that sense, HSs can be generally described as naturally occurring, highly decomposed, oxidized and polymerized organic substances with very complex structures [2–4]. They are derived from plant and animal residues (any kind of organic molecule including carbohydrates, lipids, terpenoids, tannins, pigments, and amino acids) and are usually dark in colour as well as coal, leonardite and peat. The final compound of all of these C/H non-biological molecules after their deposition and sedimentation for years is kerogen [5,6]. The basic characteristics, physicochemical properties and reactivity of HSs are widely described in many classical monographs and review works [7–9].

HSs are classified depending on their solubility [7] and are divided into three groups trying to classify them or sorting them somehow despite their heterogeneity. On the one hand, *humic acid* (HA) is not soluble under acid conditions (pH < 2) but is soluble at higher pH values. It is often referred to as the highest molecular weight fraction (1500–5000 Da and 50,000–500,000 Da in streams and sediments, respectively). On the other hand, *fulvic acid* (FA) is soluble in water at any pH conditions, contains more acidic functional groups and is ranged from 600 to 1000 Da in streams and a little bit higher in soils (1000 to 5000 Da). Finally, the fraction not soluble in water at any pH is known as humin (HN). Its molecular weight is considerably higher than the rest, and due to its deposition tendencies and ground accumulation and sedimentation, this molecule became the main kerogen precursor [10].

Several methods have been already used to isolate and characterize HSs and have been reviewed in some outstanding works [11,12]. Due to the heterogeneity of HSs, there are many research works trying to improve the selectivity and sensitivity of the developed methods in an attempt to unravel the complex properties of these substances. Until now, there is no standardized analytical approach or combination of methods that provide accurate data about the structure of HSs, and so, many efforts are being conducted to this end. Determination of elemental composition, acidic functional group content and UV–Vis absorbance or average molecular weight are some of the most bulk parameters measured to characterize HSs [12]. Currently, there are more proposals that combine spectroscopic, chromatographic and electrophoretic hyphenated techniques [11,12], and even computational analytical approaches [13]. Nuclear Magnetic Resonance (NMR) [14], Infrared spectroscopy (IR) [15], Surface Enhanced Raman Spectroscopy (SERS)

\* Corresponding author at: Analytical Chemistry Department, UPV/EHU, Barrio Sarriena s/n, Leioa, 48940, Spain.

E-mail addresses: [juancarlos.raposo@ehu.es](mailto:juancarlos.raposo@ehu.es) (J.C. Raposo), [juanmanuel.madariaga@ehu.es](mailto:juanmanuel.madariaga@ehu.es) (J.M. Madariaga).

[16], Fluorescence spectroscopy [17], Pyrolysis–Gas Chromatography coupled to mass spectrometry (Py-GC–MS) [18] and Infrared detectors (Py-GC–IR) [19,20] have been widely used for the characterization and quantification of HSs.

Prior to the detection of HSs, the isolation of HA and FA from sediments must be conducted. The published methods often require various and tedious steps with lots of solutions and with a final column (XAD-8, cation exchange phase) purification. Most of the extraction methods use aqueous sodium/potassium hydroxide and sodium pyrophosphate as extracting solutions, but other saline solutions, chelating agents and organic solvents have been also used [11]. Among them, sodium hydroxide solution has been recommended by the International Humic Substances Society (IHSS) for HSs extraction from organic sediments and soils [21].

Regarding the extraction mechanism, mechanical stirring and shaking devices are the most common ones, regardless of the long extraction periods (often 24 h) and poor reproducibility [7,22–25]. In this regard, Romaris-Hortas et al. [26] developed an extraction method based on microwave energy in order to accelerate the extraction process, which allows the simultaneous extraction of multiple samples. However, the stability of the HSs during the extraction step could not be assured.

As an important alternative, solid–liquid pretreatments based on ultrasound baths (USB) drive effective extraction processes in shorter time, with less analyte degradation during the sample pretreatment and using a safe, low-cost and eco-friendly methodology [27]. The first work dealing with the use of USB to accelerate extractable HSs extraction from marine sediments was published by Mecozzi et al. [28] and improved by Moreida-Piñeiro et al. later on [29]. However, in many applications, USB is replaced by focused ultrasound solid–liquid extraction (FUSLE) systems because of the higher reproducibility, efficiency and sonication power (100 times higher than USB). Although FUSLE has been satisfactorily used for the extraction of organic compounds from sediments samples [30], it has not still been applied to the extraction of HSs.

In this framework, the main objective of this work was to develop a fast, cost-effective, eco-friendly and sensitive analytical method based on FUSLE, which allow the isolation and separation of HA and FA from sediment samples to be afterwards quantified by means of UV–Vis. The developed method is based on the proposed method by the IHSS [21] and taking into account various published methods to extract HSs via different techniques [26,29]. As far as we know, most of the published works deal with the isolation of pure HS in order to elucidate their chemical structure. The present work, however, aims to extract HSs quantitatively joining the better steps from the previous works and deleting unnecessary steps for quantification in which HSs can be lost [22].

## 2. Experimental

### 2.1. Sampling procedure

The method development was carried out using real estuarine sediments collected in the Nerbioi-Ibaizabal estuary (Metropolitan Bilbao, Bay of Biscay, Spain). The sediment sample used for optimization purposes was collected in *Gobela river* (Metropolitan Bilbao, Bay of Biscay, Spain, N 43°19'4.5"; W 2°59'35.1"). Other two additional sampling points in the Nerbioi-Ibaizabal estuary were selected for application purposes: *Arriluze* (N 43°20'15.1"; W 3°00'37.2") and *Galindo* (N 43°18'14.7"; W 2°59'01.9").

Sediment sample was collected manually from the top sediments with 2 cm of depth assuring that only fresh sediment was gathered. The sample was stored in sealed plastic containers, transported to the laboratory in cold boxes (4 °C) and frozen until freeze-drying. The sediments were freeze-dried at –52 °C and 10<sup>–1</sup> bar during 24 h using a Cryodos 50 freeze-drier. The lyophilized samples were sieved

through a 63 µm sieve, homogenized and then stored in glass bottles at –20 °C until analysis.

### 2.2. Reagents

All the laboratory material was carefully cleaned with abundant pure water (Elix® quality, Millipore™, Bedford, MA, USA) and without using detergent to avoid possible interferences produced by detergent residues. Afterwards, the material was maintained in a clean 10% nitric acid (Merck, Darmstadt, Germany) bath overnight and then, it was thoroughly rinsed with pure water and with ultrapure water (Milli-Q® quality, Millipore™, Bedford, MA, USA).

HA (technical grade) was obtained from Fluka (Barcelona, Spain) and FA was purchased from the International Humic Substances Society (IHSS, Saint Paul, Minnesota, USA). Individual stock solutions from each standard were dissolved to prepare an ≈1000 µg g<sup>–1</sup> stock solutions in water at pH = 10.0 for HA and pH = 1.0 for FA, which were stored at 4.0 °C. Mixed fresh solutions at lower concentrations were daily prepared according to the experimentation.

Hydrochloric acid (37%, tracepur), nitric acid (69%, tracepur) and manganese sulfate (MnSO<sub>4</sub>·H<sub>2</sub>O, puriss. p.a.) were supplied by Merck (Darmstadt, Germany). Sodium hydroxide (NaOH pellets, 98% puriss) and ammonia (NH<sub>3</sub> solution, 28%) were purchased from Fluka (Barcelona, Spain).

### 2.3. Apparatus and instruments

The FUSLE extractions were carried out using an HD 2070 Sonoplus Ultrasonic Homogenizer (Bandelin, Germany) equipped with a GM 2070 generator (70 W, 20 kHz), an UW 2070 ultrasonic converter and either a SH 70G horn or MS 73 titanium probe (3 mm). A variable power setting (0–100%) allows controlling amplitude of the delivered ultrasound. A Labquake Tube Shaker Rotator from Thermo Fisher (Massachusetts, USA) was used for the extraction of HA and FA when conventional shaking extraction was assessed. Also, after FUSLE extraction, the same Labquake Tube Rotator was used for HA precipitation and separation from FA. The separation of liquid and solid extracts was performed using a Centromix centrifuge from Selecta (Barcelona, Spain).

The analysis of the HA and FA extracts was performed using a Jasco v-670 double-beam UV–vis–NIR spectrophotometer (Jasco, Tokyo, Japan) equipped with 10 mm quartz cells with a single monochromatic design covering a wavelength range from 190 to 2700 nm. The monochromator features dual gratings (automatically exchanged): 1200 grooves/mm for the UV–Vis region and 300 grooves/mm for the NIR region. A PMT detector is provided for the UV–Vis region and a Peltier-cooled PbS detector is used for the NIR region.

The solid residues of the extracts were also characterized by means of infrared spectroscopy using a Jasco-6300 spectrometer (Jasco, Tokyo, Japan). Unlike measures performed in transmittance mode, the analyses carried out in reflectance mode allow the direct analysis of the solid samples without any pretreatment. Hence, the infrared spectra were acquired in reflectance mode by attaching an attenuated total reflectance (ATR–IR) module (Single reflection ATR, Miracle, Pike, USA). The measurements were performed using a single-reflection sampling plate with a 1.8 mm round crystal (diamond/ZnSe) surface allowing reliable analysis of small samples. The module is provided with a high-pressure rotating clamp which is calibrated to deliver over 10,000 psi. All the measurements were carried out in the same pressure conditions which were controlled by the specifications of the equipment.

### 2.4. HSs extraction method

The IHSS method to isolate extractable HSs (i.e., HA and FA) from soil samples [21] was adapted to estuarine sediments. Briefly, this method

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