



Determination of the molar mass of the humic substances of the Chubut River (Argentina) by electrospray ionization mass spectrometry[☆]



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ABSTRACT

Here, we studied the number-average molar mass of the humic substances of the Chubut River (Argentina) in order to progress in their characterization. We used electrospray ionization mass spectrometry in positive mode because of its high accuracy and non-destructive nature. The result obtained (818 Da) is a relatively low value, typical of fulvic acids.

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1. Introduction

Humic substances (HSs) constitute the majority fraction of dissolved organic matter in aquatic environments. Aquatic HSs have been classified in humic acids (HAs) which are soluble in alkali but insoluble in acid (pH < 2) and fulvic acids (FAs) which are soluble in water under all pH conditions [1]. HSs may significantly affect the environment [2] by forming complexes which influence transport and bioavailability of metals; by associating with organic pollutants such as pesticides and polynuclear aromatic hydrocarbons, by affecting the growth of algae and bacteria, and by generating carcinogenic disinfection products in the chlorination of drinking water, and affecting the stability of colloids. These properties of HSs can affect the uses of the Chubut River, the main source of fresh water of Chubut province (Argentina).

Elemental composition, spectroscopic characteristics (IR, NMR, UV–Visible, Fluorescence) [3] and relations with limnological parameters [4] have revealed that the HSs present in the Chubut River, in its Lower Valley, are essentially composed of aquagenic fulvic acids (FAs), which are highly aliphatic and have low concentration in the river. However, its molar mass (M) has not yet been determined.

As for the rest of the organic compounds, the molar mass M is a relevant fact in the study of HSs; however, since HSs are not well-defined compounds, M is determined as an average property, such as number-average (Mn) or weight-average (Mw) molar mass depending on the nature of the measurements made. Mn values are determined by vapor-pressure osmometry (VPO), whereas Mw values are obtained from the sedimentation rate [5]. Electrospray ionization mass spectrometry (ESI-MS), mainly used to analyze polar non-volatile macromolecules without their fragmentation, is becoming an important procedure. This methodology is less invasive and does not modify the properties of the analyte [6]. ESI has been used to characterize natural organic mixtures such as HSs [7–9] because other methods, such as Pyrolysis Gas-chromatography/mass spectrometry (PyGC-MS), are destructive and restricted to the analysis of derivatized or degraded products [10].

The aim of this work was to determine the Mn of the FAs of the Chubut River through the application of ESI-MS in order to progress in their structural characterization.

2. Material and methods

2.1. Samples

We used samples of HSs previously isolated and purified [3] applying the method of Mantoura and Riley [11], with modifications. This consisted in adsorption–desorption (1:1 methanol–2 mol L⁻¹ ammonium hydroxide) through Amberlite XAD-7 resin (previous

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filtration through GF/F and acidification to pH 2), concentration (35 °C, rotary evaporator), ion exchange (Amberlite IRA-120, H form), concentration (35 °C, rotary evaporator) and lyophilization. This methodology avoids the presence of salts that interfere in the analyses by ESI-MS [12].

The samples for the described isolation were taken (380 L) from the Chubut River in Dolavon City (43°21.1'S, 65°43.9'W), located in the lower section (semi-arid regions) of this Patagonian river, upstream of the anthropic activities. These waters are free of contamination, low in salinity, slightly alkaline, saturated in oxygen and poor in pigments, nutrients and organic matter [4].

2.2. Electrospray ionization mass spectrometry

ESI is a soft ionization technique that introduces the sample into a fine stainless steel capillary, which has a high voltage applied to its tip. This produces an aerosol of small droplets (solvent molecules and analytes), whose size decreases by solvent evaporation. This, in turn, increases charge repulsion in the droplets, generating charged molecular ions, generally protonated in positive mode and deprotonated in negative ionization mode. Desolvated ions can be moved to a quadrupole-time of flight (QqTOF) mass spectrometer, which is a combination of quadrupole mass filters and a time of flight (TOF) mass analyzer, to obtain their molar mass [6].

As Kim [13], the ESI-MS mass spectrum of the FAs of Chubut River was acquired on a quadrupole-time of flight mass analyzer (Q-TOF, Micromass) (capillary voltage of 2.5–3 kV and 50 V of cone voltage), by injecting a solution of 1 mg mL⁻¹ of FAs in water:methanol (80:20). Mn was calculated by using the expression (1):

$$M_n = \frac{\sum I_i M_i}{\sum I_i} \quad (1)$$

where M_n is the number-average molar mass, I_i is the intensity of each peak and M_i the molar mass of each peak obtained from the ESI-MS mass spectrum.

In this technique, a molecule of molar mass M does not give a single signal to m/z but a set of signals with different m/z where z varies between 1 and n , allowing more accurate information. The mass spectrum normally shows a great number of peaks with an assembly of peaks in each nominal mass region. Being HSs a complex mixture, each of its components would generate this set of signals, complicating the interpretation of the spectrum. However, the use of ultra-high resolution ESI has allowed different authors to determine that these ions are singly charged in the fulvic and humic acids [7–9].

3. Results and discussion

The positive mode ESI-MS mass spectrum obtained from the Chubut River FAs (Fig. 1) shows a distribution between 100 and 2000 m/z , concentrating the signals in the region less than 1000 m/z , with maximum intensities between 400 and 800 m/z . This suggests the predominance of components with relatively low Mn values. This is consistent with distributions of ESI-MS spectra previously reported [8,9].

Based on this spectrum, we calculated the Mn (13,852 intensity data) and obtained a value of 818 Da. This relatively low value, which is indicative of fulvic acids, is slightly higher than that obtained by Hatcher et al. (Mn = 680 Da) for the Suwannee River FAs [14], with the same procedure. In the cited work, the authors confirmed the results obtained by ESI through other methods (desorption ionization on silicon chip mass spectrometry (DIOS-MS) and diffusion ordered spectroscopy (DOSY)), thus highlighting the consistency among them. The authors also highlighted the difference with the value of 1330 Da obtained by Chin et al. [15] by high-pressure size exclusion chromatography (HPSEC), and pointed out that the latter method shows higher values because the parameter determined is volume, which is influenced by the solvating water molecules. Chin et al. [15] found a similar order between Mn and Mw values and found a relationship between the two (Mw/Mn) for different rivers that ranged from 1.5 to 2.5 (Mw/Mn of Suwannee River = 1.7). Chin's work also shows a good correlation between Mw and

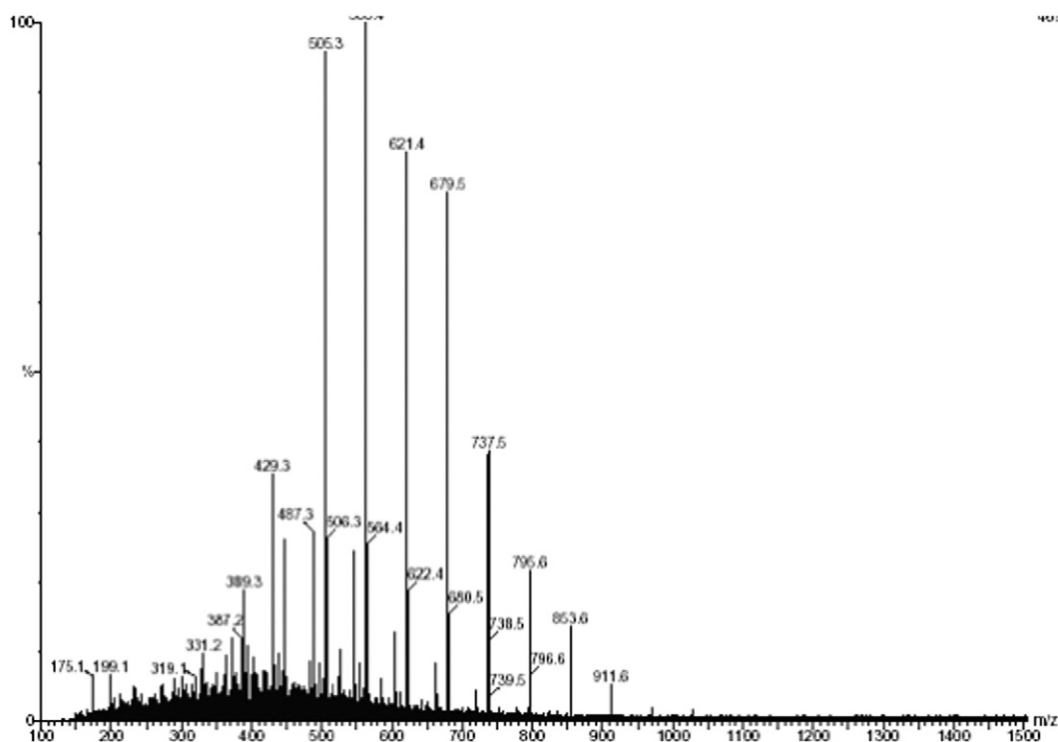


Fig. 1. Electrospray ionization mass spectrometry positive ion mode spectrum of fulvic acids of the Chubut River, Chubut Province, Argentina.

number-average molar absorptivity (ϵ) at 280 nm in units of organic carbon (OC) ($\text{mol of OC}^{-1} \text{ cm}^{-1}$).

$$Mw = 3.99\epsilon + 490 \quad (2)$$

$$r^2 = 0.97.$$

Here we applied Eq. (2), using a value of $\epsilon = 316 \text{ L cm}^{-1} \text{ mol}^{-1}$ of OC [16], to calculate the Mw of the Chubut River FAs. The value obtained was 1750 Da and then the Mw/Mn ratio was 2.1 (included within the aforementioned range).

Both Mn and ESI-MS spectrum distributions are indicative of compounds of low Mn, in contrast to traditional references at high molar mass for HSs with FAs included. The results suggest concordance with the hypothesis that HSs consist of assemblages of building blocks with similar structural features [17].

Our findings are further proof for the fulvic nature of the Chubut River HSs, because it is known that HAs have higher molar mass than fulvic acids [3,5].

The present work provides new information about the structural properties of HSs in this aquatic system, supports and complements results obtained previously, and contributes to the general knowledge of structural aspects of HSs.

4. Conclusions

ESI-MS indicated a Mn value of 818 Da for the HSs in the Chubut River, with a distribution concentrating in the region between 400 and 800 m/z . This relatively low value is indicative of fulvic acids, and similar to those obtained for the FAs of the Suwannee River. This methodology is appropriate to determine the molar mass of these complex compounds, because of its high degree of accuracy and non-invasive nature.

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