

Quantification of humic acids in surface water: effects of divalent cations, pH, and filtration†

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Humic acids (HAs) content of raw water is an important analytical parameter in water treatment facilities because HAs in the presence of chlorine may lead to the formation of dangerous by-products (e.g., trihalomethanes). The concentration of HAs in water is not directly accessible by common analytical methods due to their heterogeneous chemical structure. The aim of this study was to compare two methods to assess humic acids (HAs) in surface water namely absorbance of ultraviolet light at 254 nm (UV₂₅₄) and total organic carbon (TOC), as well as to evaluate the effects of calcium and magnesium concentrations, pH and sample filtration on the methods' results. An aqueous solution of a commercial HA with 10 mg L⁻¹ was used in the present work. Quantification of the HA was carried out by both UV₂₅₄ and TOC (combustion-infrared method) measurements. UV₂₅₄ results were converted to TOC using a calibration curve. The effects of calcium (0–136.3 mg L⁻¹) and magnesium (0–34.5 mg L⁻¹) concentrations, pH (4.0, 7.0 and 9.0) and sample filtration on UV₂₅₄ and TOC measurements of the HA suspension were evaluated. More accurate TOC values of HA suspensions were obtained by the combustion-infrared method than by the UV₂₅₄ absorbance method. The higher differences of TOC values between unfiltered and filtered samples were detected in the presence of calcium at pH 9.0 using the spectrophotometric method.

Introduction

Natural organic matter (NOM) concentration in surface water typically ranges from 0.1 mg L⁻¹ to 20 mg L⁻¹ and is mainly composed of humic substances (HSs).¹ HSs composition varies from source to source as they result from microbiological, chemical and photochemical transformations of plant and animal residues.² The main constituents of HSs include aromatic and aliphatic structures as well as carboxylic, phenolic-OH, amino and quinone groups. These groups may be evaluated by nuclear magnetic resonance (NMR) spectroscopy which is by far the single most powerful tool for structural HSs studies.³ The fraction of HSs that is soluble at high pH but insoluble under acid conditions is denominated as humic acids (HAs).⁴ In general, HAs present in surface water carry an electric negative charge (zeta potential), associated to the dissociation of carboxylic and phenolic-OH groups, that can be determined by electrophoretic mobility.^{5,6} In the presence of divalent cations (e.g. calcium), HAs form intramolecular and intermolecular aggregates due to charge neutralization and functional group bridging.^{7,8}

The HSs content of raw water is an important analytical parameter in water treatment facilities since they have been associated with the formation of carcinogenic disinfection by-products (e.g. trihalomethanes) upon chlorination of drinking water.⁹ The concentration of HAs in water is not directly accessible to common analytical methods due to their heterogeneous chemical structure.^{10,11} Instead, total organic carbon (TOC) and absorbance of ultraviolet light at 254 nm (UV₂₅₄) are used as group parameters to represent the concentration of HSs in water.^{12,13} Spectrophotometry utilizes the capacity of HSs to absorb ultraviolet light at 254 nm, which increases with their content of aromatic rings, the ratio between aromatic and aliphatic carbon, the total carbon content in the water, and the molecular weights of the HSs.¹⁰ Given stable absorption characteristics, the UV light absorbance of HSs at a specific wavelength is proportional to their concentration according to Beer's law. TOC content has been widely used as a standard method for monitoring NOM in surface waters.¹⁴ This method is able to monitor organic carbon independently of their molecular configuration; therefore the chemical reaction between constituents has less influence in the accuracy of the results than the same results obtained by the spectrophotometric method. Comparing their practical application, the spectrophotometric method requires less expensive equipment than the TOC method (sample combustion followed by infrared CO₂ detection). It is also easier to maintain and operate, as the only sample pretreatment necessary is filtration and the time necessary to analyze each sample is approximately one minute.¹⁵ The main disadvantage of spectrophotometry, however, is its possible corruption by chemical reaction between constituents of the solution, namely molecular aggregations.

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Although several investigations have reported either on the quantification of HSs using ultraviolet UV and TOC methods or on the aggregation of HSs under certain environmental conditions, little attention has been given to the effect of divalent cations (namely calcium and magnesium), sample pre-treatment and pH on these methods' results.^{16–23} The aim of this study was to compare two methods to assess humic acids (HAs) in surface water, namely absorbance of ultraviolet light at 254 nm (UV₂₅₄) and total organic carbon (combustion-infrared method), as well as to evaluate the effects of calcium and magnesium concentrations, pH and sample filtration on the methods' results.

Materials and methods

Humic acid characterization

A commercial humic acid (HA) from Fluka (commercialized by Sigma Aldrich, 53680 Humic acid) was used to simulate humic substances (HSs) in surface water. Its elemental composition was determined with an elemental analyzer (Carlo Erba, model EA 1108). Functional groups were identified by ¹³C solid-state and ¹H solution nuclear magnetic resonance (NMR). ¹³C NMR was carried out using a Bruker MSL 400P operated at ¹³C frequency of 100.63 MHz and magic-angle spinning rate (MAS) of 6.0 kHz. The solid HA samples were filled into a 4 mm diameter ZrO₂ rotor with a Kel-F cap (1.2 s recycle time and 1 ms contact time). Each spectrum consisted of 2400 data points and the chemical shifts were referenced externally to glycine (176.03 ppm). ¹H-NMR was carried out using a Varian Unity Plus 300 spectrometer NMR with a 5 mm probe head. The solid HA, 20 mg, was dissolved in 1 mL of dimethylsulfoxide (DMSO-*d*₆) and deuterated sodium hydroxide solution (NaOD/D₂O) and was used for standard measurements (45° pulse acquisition, 1 s delay).

Experimental design

Total organic carbon concentration of HA solutions (10.0 mg L⁻¹ C) in the presence of different concentrations of calcium (0–136.3 mg L⁻¹) and magnesium (0–34.5 mg L⁻¹) ions, at three pH values (4.0, 7.0 and 9.0) was directly measured by the combustion-infrared method and indirectly determined by UV spectroscopy at 254 nm. Unfiltered and filtered samples were analyzed. Filtered samples were obtained using a 0.45 μm filter (514–4156 Membrane disc filters Supor-450, VWR). Experiments were performed in triplicate.

Humic acid solutions

A concentrated stock solution was prepared by dissolving 206.8 mg of humic acid (HA) in 1 L of an aqueous solution of 4.0 g L⁻¹ of NaOH. The stock solution was stored in the dark at 4 °C. A series of solutions were prepared by dissolving a weighed amount of CaCl₂·2H₂O or MgSO₄·7H₂O (Sigma Aldrich, p.a.) in a ten-fold dilution of the HA stock solution (20.7 mg L⁻¹ final concentration or 10.0 mg L⁻¹ carbon). Experiments were performed at three different pH values (4.0, 7.0 and 9.0) which were adjusted by addition of HCl (73.0 mg L⁻¹) or NaOH (80.0 g L⁻¹) concentrated solutions. Ultrapure water was used in the preparation of all solutions.

Total organic carbon

Total organic carbon was determined by sample combustion and infrared carbon dioxide detection (5310 B) according to Standard Methods using a Shimadzu TOC-5000A analyzer.²⁴

UV spectroscopy

UV absorbance of humic acid solutions was carried out at 254 nm using a single beam spectrophotometer UV-160A (model STR-458) and a 1 cm quartz cell and the respective values were converted to total organic carbon using a calibration curve. For that purpose, serial dilutions of the concentrated HA stock solution in the range 0–41.4 mg L⁻¹ (or 0–20.0 mg L⁻¹ of carbon, based on HA elemental composition) were prepared at three pH values namely 4.0, 7.0 and 9.0. pH adjustment was achieved as previously described. The mathematical relationship absorbance *versus* concentration is presented in Table 1.

Zeta potential of HA in the presence of divalent cations

The colloid's zeta potential of HA in the presence of calcium and magnesium was determined using a Malvern Zetasizer instrument. The zeta potential cell (DTS1060) was rinsed using a disposable syringe (DTS1060) with at least 20 mL of each sample solution before measuring the zeta potential of the HA in the test solution. All experiments were carried out at 20 °C using suspensions aged for 24 h. The zeta potential was derived from the electrophoretic mobility using the Smoluchowski approximation.²⁵

Statistical analyses

A *t*-test was used where specific means were being compared. Acceptance or rejection of the null hypothesis was based on an α-level of 0.05 in all cases.²⁶

Results and discussion

Humic acid characterization

The elemental composition of the Fluka humic acid (HA) determined in the present study was 48.36% of C, 26.91% of O, 4.24% of H, 0.78% of N and 0.78% of S. This result is in agreement with the one provided by Fluka (47.9% of C, 4.91% of H, 0.67% of N and 1.18% of S) but both differ from the one

Table 1 Linear regression equations from standard calibration^a

pH	Filtered sample	Unfiltered sample
4.0	$A = 4.69 \times 10^{-2} \text{ TOC} + 5.10 \times 10^{-3}$ $R^2 = 0.9995$	$A = 6.06 \times 10^{-2} \text{ TOC} - 4.00 \times 10^{-3}$ $R^2 = 0.9999$
7.0	$A = 5.48 \times 10^{-2} \text{ TOC} - 3.60 \times 10^{-3}$ $R^2 = 0.9998$	$A = 6.33 \times 10^{-2} \text{ TOC} - 4.40 \times 10^{-3}$ $R^2 = 0.9999$
9.0	$A = 5.64 \times 10^{-2} \text{ TOC} + 1.20 \times 10^{-3}$ $R^2 = 0.9999$	$A = 6.43 \times 10^{-2} \text{ TOC} + 1.00 \times 10^{-7}$ $R^2 = 1.0000$

^a A: absorbance; TOC: total organic carbon in mg L⁻¹.

published by Pluciński *et al.* (54.37% of C, 39.84% of O, 4.28% of H, 0.66% of N and 0.85% of S).²⁷

To obtain additional information regarding the structure of the HA, the atomic H/C, N/C, and O/C ratios has been determined. Previous studies suggest that the H/C ratio is an indicator of the amount of saturation of C atoms and/or branched structures within the molecule, the N/C ratio is related to the amount of proteinaceous compounds, and the O/C ratio is assumed to indicate the carbohydrate content and degree of oxidation.^{28–30} Literature studies reported H/C ratio for soil HAs in the range of 0.78–1.60 and for aquatic HAs between 1.23–1.46.^{31,32,28} Regarding the O/C ratio, values reported for soil HAs are in the range of 0.35–0.59 while for aquatic HAs the values are between 0.55–0.63, respectively. The N/C ratio presented values ranging from 0.021 to 0.080 for soil HAs and from 0.036 to 0.070 for aquatic HAs. The atomic ratios determined in the present study were 1.04 (H/C), 0.42 (O/C), and 0.012 (N/C) which are within the values reported in the literature for soil HAs, with the exception of the N/C ratio. This value is below the lower limit reported, and probably reflects a chemical composition with a poor content of amino acid units. Additional information on the structure of Fluka HA (Sigma–Aldrich) was obtained from FTIR and NMR results.

¹³C Solid-state NMR and ¹H solution NMR spectra are depicted in Fig. 1 and Fig. 2, respectively.

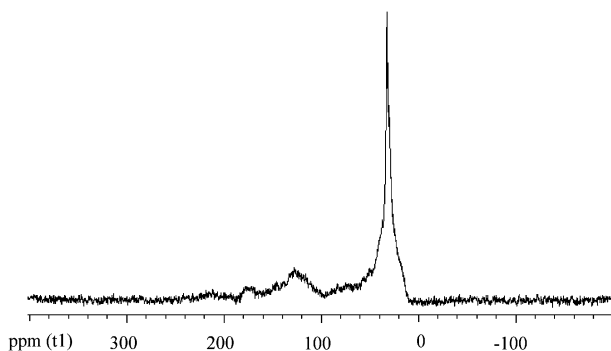


Fig. 1 Solid-state ¹³C NMR (¹³C-NMR-MAS) spectrum of the HA.

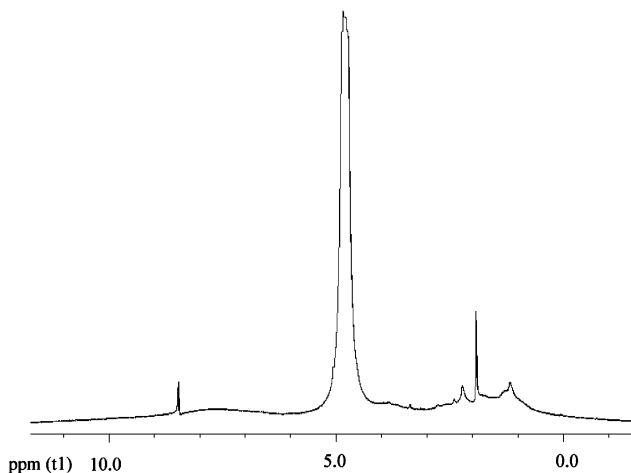


Fig. 2 Solution ¹H NMR spectrum of the HA.

The ¹³C NMR spectrum shows broad signals between δ 10 ppm and 225 ppm from which a sharp and intense singlet emerges at $\delta \approx 30$ ppm. This signal must be associated with an equally intense singlet in the ¹H NMR spectrum of the same sample at δ 1.93 ppm, typical of acetate methyl groups. Other sharp but less intense signals at δ 1.19 ppm and δ 2.23 ppm in the ¹H NMR spectrum may indicate that other methyl groups are present either as part of alkyl chains or linked to carbonyl, or to aromatic groups. They may be associated with two shoulders visible in the ¹³C NMR spectrum around δ 25 ppm and 35 ppm. The remaining signals in the ¹³C NMR also indicate the presence of non-polar aliphatic carbon atoms (δ 10–40 ppm, under the intense acetyl absorption) and O-alkyl/N-alkyl carbons (δ 40–90 ppm). A broad signal in the ¹³C NMR spectrum between δ 100–160 ppm indicates the presence of aromatic groups with both electron-donating (amino/alkoxy/hydroxy substituents) and electron withdrawing groups (carboxylic acids and derivatives). The presence of carbonyl groups was confirmed by the broad signal between δ 170–225 ppm. A similar pattern was reported in the literature for the ¹³C NMR spectrum of a solid purified Fluka HA sample.³³ In this case, five broad peaks were identified in the 0–50, 50–85, 85–105, 105–160 and 160–200 ppm regions, confirming the presence of the same type of functional groups. A notorious difference observed in the present study is the sharp and intense singlet at $\delta \approx 30$ ppm, assigned to the acetate methyl group. As a result, comparatively small signals are registered for the remaining regions. Broadening of this band as it reaches the baseline leads to partial overlapping with the 85–105 ppm band identified in the literature and assigned to C–O in carbohydrate compounds.

The signals in the ¹H NMR spectrum are spread between δ 0.5–10 ppm and are, in general, very broad. The intense band centered at δ 4.8 ppm was assigned to the water peak due to the presence of a large amount of water. In this case, hydration molecules were reinforced by water molecules formed by deuterium exchange of all the hydroxy/amino protons (of alcohols, phenols, carboxylic acids and amines) present in HA with D₂O used as co-solvent. This allowed us to calculate the molar ratio of protons on saturated and unsaturated carbon atoms (approximately 2.1 : 1), from the integration of the signals in the δ 0.5–4.2 ppm and of the δ 5.6–10 ppm regions respectively.

The FTIR spectrum (Fig. 3) shows an intense signal centered at ν 3429 cm⁻¹ assigned to the N–H/O–H stretching vibrations, confirming the presence of alcohols/phenols, amines/amides and possibly carboxylic acids. Two medium intensity peaks at ν 2922 and 2852 cm⁻¹ may be due to the C–H stretching vibration of alkyl chains. The stretching vibration of the aromatic C–H bonds, usually visible in the ν 3200–3000 cm⁻¹ region, may be masked by the broad N–H/O–H signal. An intense band at ν 1608 cm⁻¹ can be assigned to the C=O stretching vibration in the carboxylate function and also to the C=C stretching vibration in the aromatic ring and alkene groups. A shoulder around ν 1700 cm⁻¹, typical of the stretching vibration of carbonyl groups of ketones (including quinones) carboxylic acids and/or amides, confirms that these units are present in humic acid. The bending vibrations of methyl and methylene groups (ν 1387 cm⁻¹) and the stretching vibration of the C–O bond in alcohols, phenols and ethers (overlapped bands between ν 1000 and 1200 cm⁻¹) also support the presence of these functional groups. The main

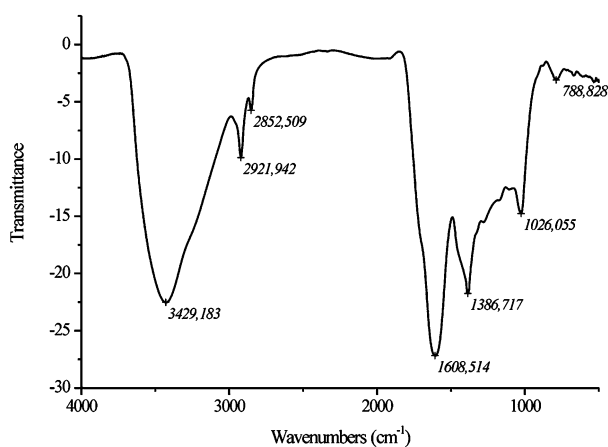


Fig. 3 FTIR spectrum of the HA.

absorbance bands and the corresponding assignments are in agreement with data reported in literature.^{33–41}

Zeta potential as a function of pH and concentration of divalent cations

Zeta potential (ZP) values became more negative, decreased from -38.4 ± 0.35 mV to -43.6 ± 0.30 mV, as pH was increased from 4.0 to 9.0. The variation of the colloid's zeta potential with pH reflects the ionization of the carboxylic and phenolic acidic groups.⁴² The charge, as well as intra- and intermolecular electrostatic repulsion, increases as acidic groups are ionized with increasing pH, restricting aggregation phenomena. The effect of the concentration of divalent cations on ZP values at different pH values is depicted in Fig. 4 for calcium and in Fig. 5 for magnesium.

The results show that ZP values increase (become less negative) with increasing calcium and magnesium concentrations, which can be explained by HA's charge reduction due to metal ions binding to the negatively charged carboxylic groups.^{43,44,23} The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. A dividing line between stable and unstable aqueous dispersions is generally taken at either +20 or -20 mV. Colloids with ZP more positive than +20

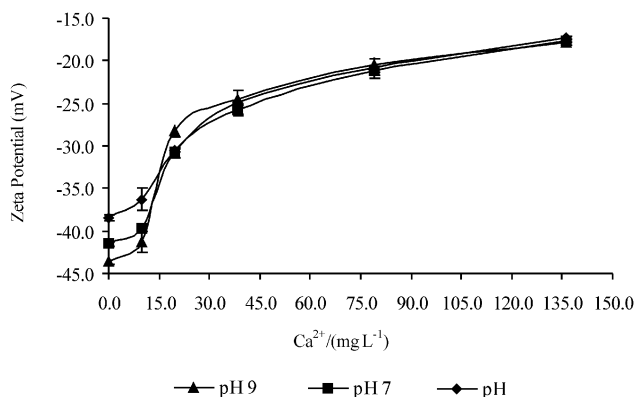


Fig. 4 Zeta potential of the HA suspension (10.0 mg L^{-1} carbon) as a function of pH at several calcium concentrations.

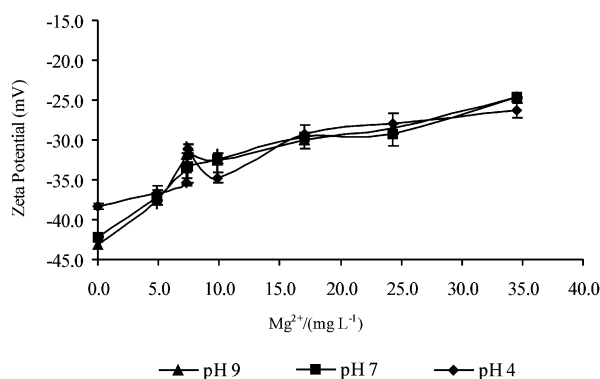


Fig. 5 Zeta potential of the HA suspension (10.0 mg L^{-1} carbon) as a function of pH at several magnesium concentrations.

mV or more negative than -20 mV are normally considered stable.⁴⁵ The DVLO theory says that the stability of a colloidal system is determined by the sum of the electrical double layer repulsive and van der Waals attractive forces which the particles experience as they approach one another.^{46,47} In practice, the repulsive forces can be greatly affected by changing the ionic strength of the dispersion medium. In this study, at a calcium concentration of 136.3 mg L^{-1} there was dispersion instability, the ZP was -17.3 ± 0.20 mV at pH 4.0, -17.6 ± 0.21 mV at pH 7.0 and -17.9 ± 0.25 mV at pH 9.0, and, therefore, the colloids in the dispersion adhered together and formed visible aggregates. A conclusion, which can be drawn from these results, is that the HA's zeta potential increases with increasing calcium and magnesium concentrations.

Monitoring humic material in the presence of divalent cations

Total organic carbon (TOC) values obtained directly by sample combustion and infrared CO₂ detection and indirectly by UV spectroscopy at 254 nm (as described in materials and methods: total organic carbon and UV spectroscopy, respectively) were used as surrogates for HA quantifications. The effect of pH, concentration of divalent cations and sample filtration on TOC values, determined by both methods, is depicted in Fig. 6 for calcium and in Fig. 7 for magnesium.

Control measurements carried out with pure solutions of calcium chloride and magnesium sulfate at the concentrations in question never yielded TOC values above 0.08 mg L^{-1} , so that direct influence of these substances in both methods was considered negligible. In most cases unfiltered samples presented considerably higher TOC concentrations than filtered ones independently of the method used, the concentration of divalent cations and the pH values. These results evidenced that a considerable portion of molecules were removed in the filtration process. Two hypotheses may explain the strong removal of organic carbon in the filtration process. The first hypothesis suggests that the presence of divalent cations enhances aggregation of HAs due to charge neutralization and functional group bridging; aggregates are more readily removed by filtration.⁸ This hypothesis is corroborated by the observed increase of the zeta potential (became less negative) with increasing concentrations of calcium and magnesium (Fig. 4, 5) that indicated suspension instability. The second hypothesis is based on the adsorption of

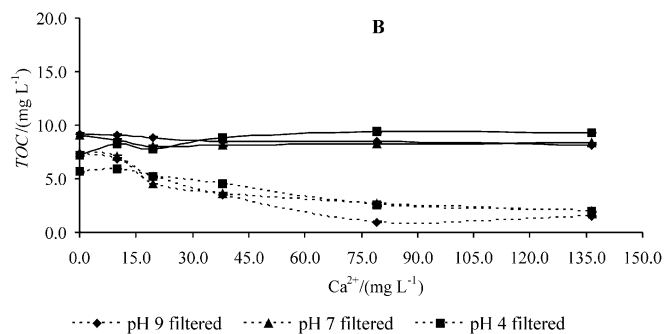
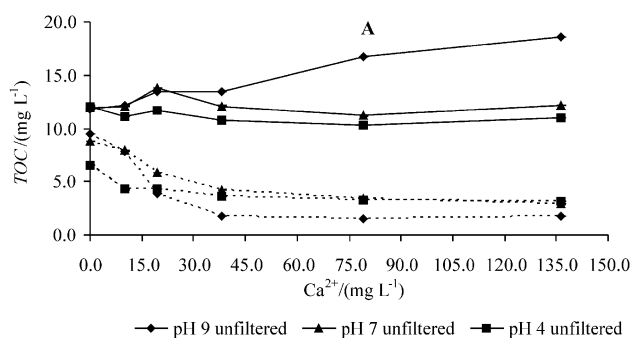


Fig. 6 Effect of calcium on total organic carbon concentration of the HA suspension (10.0 mg L⁻¹ carbon) determined by: (A) spectrophotometric method; (B) combustion-infrared method.

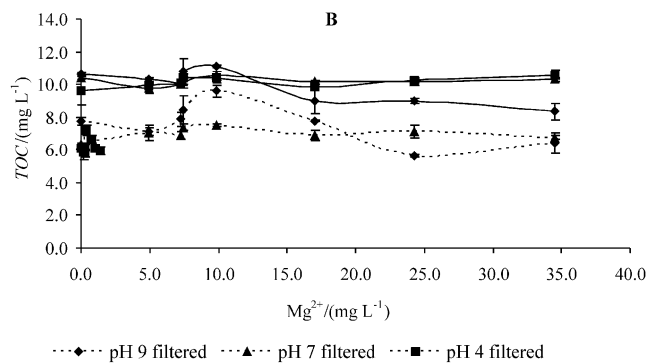
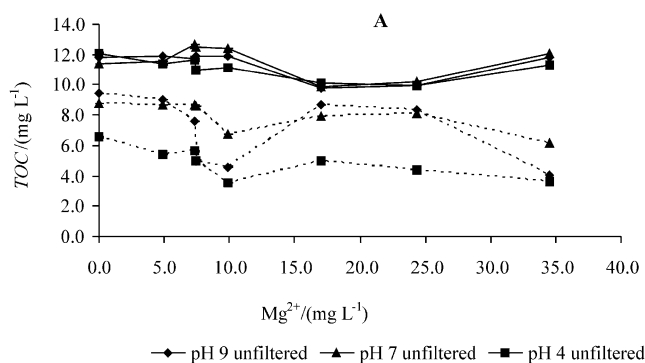


Fig. 7 Effect of magnesium on total organic carbon concentration of the HA suspension (10.0 mg L⁻¹ carbon) determined by: (A) spectrophotometric method; (B) combustion-infrared method.

HA molecules onto the filtration membrane. As pointed out in several studies the adsorption of HAs increases with increasing concentrations of the metal ions, which shield the electrostatic repulsion among HA molecules and thus facilitate their deposition on the membrane surface.^{48,49,16}

Differences in TOC results between spectrophotometric *versus* combustion-infrared methods were detected mainly for unfiltered samples, and specifically in the presence of calcium at both pH 7.0 and 9.0, which is in keeping with the different basic measurement principles. The principle of the spectrophotometer is based on Beer's law: the absorbance of a compound at a specific wavelength is proportional to the concentration of the compound.¹⁵ This law is only valid when all of the constituents in the sample have no chemical reaction between each other which was not the case in the present study. Calcium ions interacted with the HA and molecular aggregation was promoted with the consequent increase in turbidity, principally, at basic pH. The higher TOC results obtained by the spectrometric method might thus be explained by light scattering effects.⁹ The principle of the combustion-infrared method is the oxidation of organic carbon to CO₂ by combustion, therefore, the molecular structure as well as molecular interactions do not influence the TOC measurement.

It is important to stress that UV spectroscopy (254 nm) and combustion-infrared methods are based on different principles, while the first method measures the amount of aromatic structures,⁵⁰ the second method quantifies carbon independently of the molecule's chemical structure. The use of UV spectroscopy to

monitor natural organic matter in surface water is a fast and relatively inexpensive method that gives information about the aromatic content as well as the total organic content (when associated with a standard calibration curve). However, the results should be interpreted with care since the presence of calcium and magnesium ions and the pH interfere with the analysis' results.

To compare both analytical methods, the errors associated with the determination of TOC of an unfiltered aqueous suspension of HA with a carbon concentration of 10.0 mg L⁻¹ at pH 7.0 were compared. The results were 10.3 ± 2.0 mg L⁻¹ for the combustion-infrared method and 11.9 ± 0.1 mg L⁻¹ by the UV spectroscopy method. The combustion-infrared method gave the most accurate value although the precision was lower than the one obtained in the UV spectroscopy method. Moreover, the combustion-infrared method provided the least changes for unfiltered and filtered samples in the presence of divalent cations, at different pH values.

Surface water may present a highly variable composition regarding calcium (1.6–413.5 mg L⁻¹) and magnesium (1.6–259.2 mg L⁻¹) concentrations and pH values (2.5–8.2).^{51–54} Thus, the combustion-infrared method is recommended for HA quantification in surface water.

Conclusions

From this work's results can be concluded that humic acids monitoring with respect to its total organic carbon content

(TOC) by different methods leads to different results. Unfiltered samples presented considerably higher total organic carbon (TOC) values than filtered ones, independently of the method used. The higher differences of TOC values between unfiltered and filtered samples were detected in the presence of calcium at pH 9.0 using the spectrophotometric method. The UV spectroscopic method gives information about the aromatic content of the sample, unlike the combustion infrared method, but suffers from interferences resulting from molecular aggregation in the presence of divalent cations. The combustion-infrared method gave the most accurate values although the precision was lower than the one obtained in the UV spectrophotometric method. In this regard and due the fact that pH values and divalent cations concentration in surface water may vary considerably, the combustion-infrared method is recommended for HSs quantifications in surface water.

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