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Original Research

Characterization of Natural Organic Matter Fractions by High Pressure Size-Exclusion Chromatography, Specific UV Absorbance and Total Luminescence Spectroscopy

J. Świetlik[1]*, E. Sikorska[2]

[1]Department of Water Treatment Technology, Adam Mickiewicz University, ul. Drzymały 24, 60-613 Poznań, Poland,
[2]Faculty of Commodity Science, Poznań University of Economics, al. Niepodległości 10, 60-967 Poznań, Poland

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Abstract

The composition of isolated natural organic matter (NOM) fractions was studied by means of high pressure size-exclusion chromatography (HP-SEC), specific ultraviolet absorbance (SUVA) at 254 nm, total luminescence spectroscopy (TLS) and synchronous scanning fluorescence measurements. The HP-SEC, SUVA and TLS studies revealed that humic acids (HA) and hydrophobic acids (HOA) are composed of complex, aromatic and high molecular weight subunits. Hydrophobic neutrals (HON) consist of few groups of compounds with relatively low molecular sizes and a degree of condensed aromatic moieties. Similar characteristics were observed for hydrophilic acids (HIA) and bases (HIB). Obtained results also demonstrated that hydrophilic neutrals (HIN) are made of non-humic, aliphatic and low molecular weight components. Furthermore, the synchronous excitation-emission spectra indicated the presence of protein-like constituents in HIB, HIN and HON fractions as well as lignin-derived constituents with relatively low molecular sizes in HIA.

Keywords: natural organic matter, fractions, HP-SEC, SUVA, fluorescence, synchronous spectra

Introduction

Natural organic matter (NOM) is a complex heterogeneous mixture of organic compounds, consisting of aromatic, aliphatic, phenolic, and quinonic structures with varying molecular sizes and properties. The complexity and heterogeneity of aquatic NOM have made its structural and functional characterisation extremely difficult. The fractionation of NOM into broad chemical classes becomes an established first step to examine its structure. The most frequently applied procedures for isolation/fractionation of NOM are based on non-ionic macroporous copolymers (such as XAD resins analogues) in conjunction with ion-exchange resins, which under the preadjusted condition (ca. pH 2) operationally classify organic solutes into different hydrophobic and hydrophilic fractions [1-5,25].

The ill-defined nature of aquatic organic matter means that there is no single analytical method which can provide definitive structural or functional information about NOM. Among non-destructive analytical methods applied for the characterisation of NOM fractions, high pressure size-exclusion chromatography (HP-SEC), fluorescence spectroscopy and specific ultraviolet absorbance (SUVA) at 254 nm appear to be useful.

HP-SEC is commonly implemented to determine the molecular weight distribution (MWD) of NOM from a

*Corresponding author; e-mail: askas@amu.edu.pl

variety of aquatic and terrestrial environments [6, 7]. The advantages of HP-SEC over other analytical techniques comes from small sample volumes, a minimal amount of pre-treatment, ease and speed of analysis and the ability of application of several detectors, i.e. UV-Vis, fluorescence and dissolved organic carbon (DOC) in-line analyzers [6]. The results obtained with HP-SEC strongly depend on applied column type and eluent. The proper selection of HP-SEC stationary phase ensures high resolution, low-column backpressure and good mechanical, chemical and thermal stability of the column. The best results are obtained on the columns made of bonded silica and polymeric gels. The eluent composition, particularly pH and ionic strength, can significantly impact the separation of NOM components by HP-SEC, as well. The surface charge characteristic of the gel, the NOM charge and structure as well as the gel-NOM interactions are also affected by the eluent [8,9]. However, there is no general agreement about what kind of eluent is the best applicable for studying aquatic NOM. The exclusion and adsorption effects in the column can be minimized by the use of mobile phase at pH=7. Depending on NOM characteristics, the widely used mobile phases in HP-SEC analysis of NOM, especially with UV detection, are phosphate buffer and sodium acetate [10].

In the last three decades fluorescence techniques, i.e. conventional emission, excitation measurements as well as multidimensional techniques: total luminescence spectroscopy (TLS) and synchronous scanning fluorescence have been successfully used as well, mainly to characterize and/or discriminate humic substances of different origins and naturally occurring organic matter [11-14]. The fluorescence techniques are mostly applied to the structural studies of unconcentrated and unfractionated NOM, although the fractionation of the NOM into a number of well-defined subcomponents offers advantages in characterising NOM and providing improved understanding of its structural and functional characteristic [15-17].

TLS is a simple, rapid and extremely sensitive method, requiring only a small volume of aqueous sample at a low concentration (usually < 20 mg/l) [14, 16]. Fluorescence, however, can be affected by many factors such as solution type, pH, ionic strength, temperature, redox potential of the medium and interaction with metal ions and organic substances [13,14,18,19]. Moreover, fluorescence intensity is strongly influenced by the molecular structure of NOM, i.e. molecular weight (MW) [16, 19], degree of condensed aromatic moieties (e.g. aromatic rings and conjugated unsaturated bonds [13, 18]) and electron-donating and electron-withdrawing groups presence [15, 16, 18, 20]. Electron-donating groups containing oxygen or nitrogen atoms (OH, NH_2, OCH_3) increase and electron-withdrawing groups, such as COOH, decrease intensity of fluorescence in aromatic compounds [15, 16, 18]. According to Senesi [20] carbonyl-containing substituents, hydroxyl, alkoxyl and amino groups tend to shift fluorescence to longer wavelengths. The same tendency is observed for HA molecules with a greater degree of aromaticity and polycondensation [13, 18]. However, fluorescence studies of NOM and identification of particular fluorophores remains difficult and contradictory owing to its complex chemical structure and to the spectral overlapping and peak shifting and broadening [15, 16].

Another practical parameter that provides insight into the nature of NOM and its fractions is SUVA. Specific UV absorbance is the ratio of ultraviolet light absorbance of wavelength λ (usually 254 nm) to the concentration of DOC in the water, i.e. UV (m^{-1})/DOC (mg L^{-1}). SUVA provides a quantitative measure of aromatic content per unit concentration of organic carbon [21]. According to Johnson et al. [22], SUVA value is correlated with molecular weight of NOM, i.e. increases with increasing MW. Within the past two decades SUVA has received increasing attention among drinking water researchers since it indicates the amenability of DOC removal during water treatment. Moreover, SUVA is a valuable characterization parameter for the assessment of NOM reactivity. Strong correlations have been reported between SUVA and DBP formation [21, and references therein].

In this work, NOM samples were concentrated and fractionated using XAD and ion-exchange resin adsorption methods into six fractions: humic acids (HA), hydrophobic acids (HOA), hydrophobic neutrals (HON), hydrophilic acids (HIA), hydrophilic bases (HIB) and hydrophilic neutrals (HIN). The composition of the isolated NOM fractions was studied by means of HP-SEC, fluorescence spectroscopy and SUVA. The main purposes of the study were to characterize individual NOM fractions as well as to compare their spectroscopic properties.

Materials and Methods

Materials

Natural water samples, after a treatment process consisting of aeration and sand filtration, were collected from a water treatment plant (Poznań Water Treatment and Sewage Co., treatment plant in Mosina) supplied mostly by underground water from the Mosina Water Intake (MWI). The raw water is aerated and filtered through the sand filters to remove excess iron and manganese. The pre-treated water at MWI usually reveals the following characteristic: 3.8–6.5 mg L^{-1} of total organic carbon (TOC), pH in the range of 7.06-7.48, total alkalinity – 3.35-4.30 mval L^{-1}, colour – 10-20 °Pt, Ca^{2+} - 90-140 mg L^{-1}, Mg^{2+} - 9-17 mg L^{-1} [23,24].

Fractionation Procedure

Original samples of MWI water, each of 3000 L volume, were pre-concentrated with the Membrane System 3XS28 (OBR Pleszew (Poland)). The NOM concentrate was enriched with molecules with molecular sizes ≥ 1 kDa. The obtained NOM concentrate accounted for ca.

Table 1. DOC, SUVA at 254 nm, UV absorbance and average MWs of NOM fractions.

Sample	DOC [mg L^{-1}]	SUVA at 254 nm [L mg^{-1} m^{-1}]	Absorbance 254 nm	M_W 254 nm	M_N 254 nm	ρ 254 nm	M_W 220 nm	M_N 220 nm	ρ 220 nm
HA	5,81	3.975	0.231	1943	945	2.05	1950	895	2.17
HOA	4.81	3.368	0.162	1796	972	1.84	1764	957	1.84
HON	5.54	3.068	0.170	1173	590	1.98	1166	516	2.25
HIA	4.98	3.092	0.154	1655	1048	1.57	1597	905	1.76
HIB	5.02	3.307	0.166	1600	883	1.81	1561	873	1.78
HIN	4.84	2.355	0.114	1645	1056	1.55	986	374	2.63

$$M_n = \frac{\sum_{i=1}^{N} h_i}{\sum_{i=1}^{N} h_i / M_i} \qquad M_w = \frac{\sum_{i=1}^{N} h_i (M_i)}{\sum_{i=1}^{N} h_i} \qquad r = \frac{M_W}{M_N},$$

- h_i is the height (detector response after baseline correction) of the sample HPSEC curve eluted at volume i;
- M_i is the molecular weight at eluted volume i determined from the standard calibration curve [9].

65% of the original DOC. As the cut-off of ceramic membranes is not sharp, in this step the small molecules were removed only to some extent. The 35% of DOC remaining in permeate was composed of molecules with lower molecular sizes (1200-300 Da), enriched with molecules with MW <1 kDa (Figure 1).

The fractionation of NOM concentrate (~170 mg DOC L^{-1}) was carried out using a modified resin isolation and fractionation procedure. Amberlite resins XAD-8 and XAD-4 (Supelco Bellefonte, PA USA, AG-MP-50, a strong acid, sulfonated, polystyrene macroporous resin (BioRad, Hercules, CA USA) and Duolite A7, a weak base, phenol-formaldehyde condensation macroporous resin (Supelco Bellefonte, PA USA) were all purified by Soxhlet extraction prior to being used in the process [1]. The condensed samples were pumped through subsequent columns filed with XAD-8 and XAD-4 resins. The fractionation procedure was described in details elsewhere [25]. As a result of the fractionation techniques, six fractions of the NOM were isolated. Prior to further analysis the model NOM fraction solutions were prepared. The individual NOM fractions were dissolved in MilliQ-water at a DOC-concentration of about 5 mg L^{-1} (Table 1). The pH value of all model NOM fraction solutions was 7.

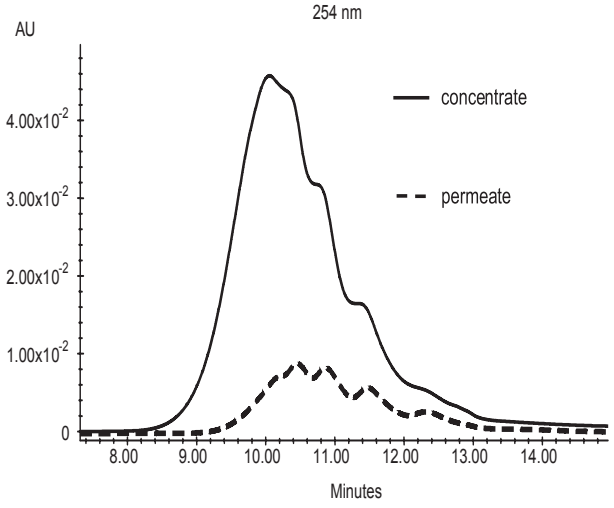

Fig. 1. The MWD of NOM in concentrate (M_w = 1685, M_n = 1009) and permeate (M_w = 982, M_n = 514) determined by HP-SEC at 254 nm

Fluorescence Spectroscopy

Fluorescence spectra were recorded on a Fluorolog 3-11 spectrofluorometer (Jobin Yvon – Spex Instruments S.A. Inc., USA). A 450 W Xenon lamp was used for excitation. Excitation and emission slit widths of 2 nm were used. The acquisition interval and the integration time were maintained at 1 nm and 0.1 s, respectively. A reference photodiode detector at the excitation monochromator stage compensated for the source intensity fluctuations. The individual spectra were not corrected for the wavelength response of the system. Right-angle geometry was used for water samples in a 10 mm fused-quartz cuvette. 3D-EEM spectra were obtained by measuring the emission spectra in the range from 250 to 700 nm repeatedly, at the excitation wavelengths from 240 to 500 nm, spaced by 10 nm intervals in the excitation domain. Spectra were then concatenated into an excitation-emission matrix (EEM). The 3D plots and contour maps of TLS were produced using DataMax Grams/32 program. Each set of contour maps was plotted using the same scale range of fluorescence intensities, and number of contours. Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromator in the

Table 2. Percentage of natural organic matter fractions isolated from MWI water compared to other water sources.

Fraction	NOM from MWI water	Marhaba et al. [4]	Korshin et al. [26]
HA	19%	-	-
HOB	traces	0-6%	0-22%
HOA	54%	8-12%	19-68%
HON	12%	13-22%	0-25%
HIA	7%	44-55%	8-50%
HIB	5%	4-6%	1.5-10%
HIN	3%	9-25%	1-35%

240–600 nm range, with constant wavelength differences Δλ between them. Six spectra were recorded for each sample, with Δλ values of: 21 nm, 32 nm, 44 nm, 66 nm, 77 nm, 89 nm. In order to reduce primary and secondary inner filer effects and concurrently obtained required signal-to-noise ratio we used a low concentration of solution (of order of 5 mg/L). No further absorbance correction was applied to EEMs and synchronous fluorescence spectra.

HP-SEC Analysis

Chromatographic characterization and MWD of NOM was determined by HP-SEC with UV-detection at 254 and 220 nm (AD 25 detector, Dionex, USA) on a DIONEX DX-500 Chromatography System with TosoHaas TSK gel G3000 SWXL column and TosoHaas TSK gel SW guard column (Tosoh Corporation, Japan). The eluent was a 0.01 M phosphate buffer, pH 7.00±0.05, while the samples were injected after filtration through 0.45 μm membrane filters without the addition of buffer. The flow rate was 1.0 mL min^{-1} and analysis time was 20 min. The injection volume was 100 μL. All analyses were performed at 30°C. The column was calibrated with sodium polystyrene sulphonate standards (PSS Polymer Standards, Germany) [23,24].

UV_{254}-Absorbance and $SUVA_{254}$ Measurement

UV_{254}-absorbance was measured with DR/4000U spectrophotometer (HACH, 254 nm, 1 cm quartz cell). Analytical variance of the method: ±0.001 (n = 6).

DOC Analysis

Dissolved organic carbon (DOC) was measured using a LABTOC system (Pollution and Process Monitoring Ltd., England) total organic carbon analyser using the method of sodium peroxydisulfate/orthophosphoric acid wet oxidation and UV radiation. Analytical variance of the method was ±0.01 mg DOC L^{-1} (n = 6).

Results

Natural Organic Matter Composition

In the present study, fractionation techniques based on non-ionic and ionic resins made it possible to obtain six different fractions of the NOM. Table 2 presents the results of NOM fractionation compared to the literature data. In most cases, achieved fractionation efficiencies for particular NOM fractions were similar to those obtained by Korshin et al. [26] and Marhaba et al. [4]. The overwhelmingly dominant fraction of NOM were hydrophobic acids (54%). However, Marhaba et al. [4], reported that a percentage of HOA in NOM does not exceed 12%. Humic acids and hydrophobic neutrals represented 19 and 12% of NOM, respectively. The absence of hydrophobic bases is frequent in natural waters, as described by Leenheer [1]. The total amount of hydrophilic fractions was low and did not exceed 15%, whereas Korshin et al. [26] and Marhaba et al. [4] revealed considerably higher percentages of hydrophilic components in total NOM.

HP-SEC Studies

The MWDs of all isolated fractions were determined by HP-SEC with UV detection at 254 and 220 nm (Figure 2). Weight-average (M_w), number-average (M_n) and polydispersity (ρ) of individual NOM fractions are presented in Table 1. Chromatographic studies reveal all NOM fractions to be composed of molecules with relatively small molecular sizes. Thus, the second wavelength (220 nm) was chosen to observe the MWD of low molecular weight NOM fractions due to their relatively higher absorptivity at 220 nm, that was stated by Egeberg et al. [7], O'Loughlin and Chin [6] and Świetlik et al. [25]. Despite similarity in terms of MWD among particular NOM fractions, HA and HOA reveal a superior content of molecules with the highest molecular weights and aromaticity, whereas HON consists of a few groups of compounds with lower molecular sizes and a low degree of condensed aromatic moieties. All by HIN hydrophilic fractions possess much the same structural composition as HON. HIN exhibits lower UV absorbance

at 254 nm, compared to other fractions. According to the literature data [1, 4, 5, 25], HIN fraction is composed of different classes of compounds with relatively small molecular sizes, mainly amines, amides, carbohydrates and polysaccharides. As stated by Leenheer and Croue [27], proteins, sugars, amino sugars, and aliphatic acids, which are ubiquitous components of aquatic NOM, are characterized by low-UV_{254} absorptivities. Hence, results presented in Figure 2 indicate high abundance of carbohydrates and polysaccharides in HIN. Moreover, the HIN chromatogram registered at 220 nm exhibits a very intense peak at t_R=12-13 min, corresponding to MW of ~300 Da. This confirms that HIN is a mixture of low molecular weight, aliphatic components with functional groups, strongly absorbing UV light at 220 nm.

$SUVA_{254}$ Studies

According to Karanfil et al. [21], specific ultraviolet absorbance (SUVA) at 254 nm provides a quantitative measure of aromatic content per unit concentration of carbon. Natural waters with high $SUVA_{254}$ values, e.g., ≥ 4 L $mg^{-1}m^{-1}$ have a relatively high content of hydrophobic, aromatic, and high molecular weight NOM fractions, whereas waters with $SUVA_{254}$ of ≤ 3 L $mg^{-1}m^{-1}$ contain largely non-humic, hydrophilic, and low molecular weight materials [21]. The UV_{254} absorbance and $SUVA_{254}$ values of all examined NOM fractions (Table 1) confirm the high content of complex, aromatic and high molecular weight subunits in HA and HOA fractions. The high $SUVA_{254}$ value of HIB is inconsistent with our HP-SEC findings as well as UV_{254} properties of major HIB components (i.e. proteins and amino sugars) reported by Leenheer and Croue [27]. Lower $SUVA_{254}$ values obtained for HON and HIA indicate the presence of aromatic components with low MW and/or low degree of condensed aromatic moieties in both fractions. The lowest $SUVA_{254}$ value obtained for HIN confirms HP-SEC findings for this fraction, i.e. the presence of non-humic, aliphatic and low MW components.

Fluorescence Spectroscopy Analysis

The contour maps of EEMs of NOM fractions (Figure 3), reveal two well resolved fluorescence maxima with Ex at 250-265 nm, and at 300-336 nm, respectively. Both bands have emission maximum between 415 and 452 nm. According to authors of papers [11, 12, 17, 28-30] the peaks with Ex/Em of 250-260/380-420 nm correspond to UV humic-like fluorophores, while the peaks with Ex/Em of 330-350/420-480 correspond to visible humic-like and/or fulvic fluorophores. Our results may indicate the presence of humic- and fulvic-like constituents in all examined NOM fractions. For HIA and HIB a third peak with very low intensity at Ex/Em of 250-300/350 nm is observed. The fluorophores with excitation at 220-280 nm and emission at 300-305, 340-350 nm are commonly ascribed to protein-like fluorescence, arising from aromatic

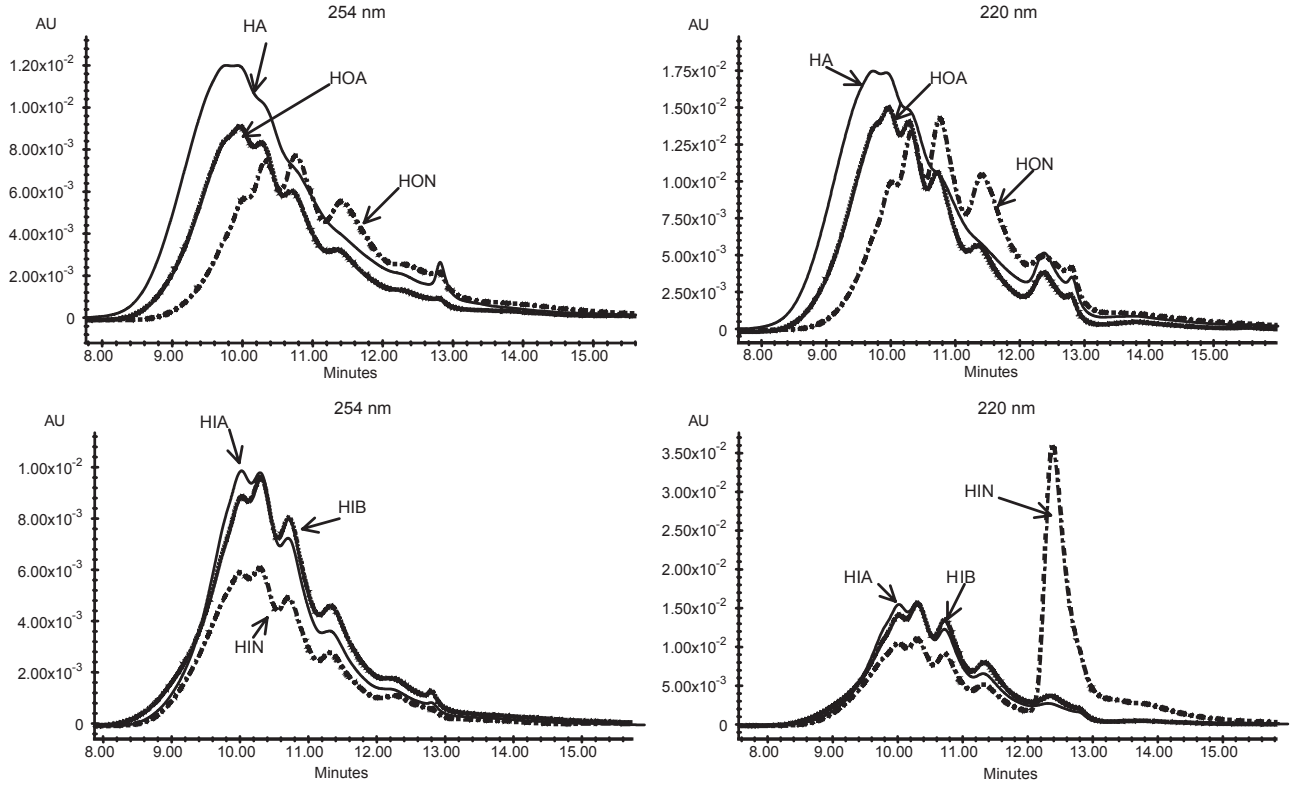

Fig. 2. The MWD of all isolated fractions determined by HP-SEC with UV detection at 254 and 220 nm.

amino acids, either free or as protein constituents [12, 27, 28, 30, 31].

The emission spectra extracted from EEMs (Figure 4) reveal more pronounced differences between individual NOM fractions. The emission bands' maxima (λ_{Em}) and specific fluorescence intensities (SI - intensity/mgC_{org}) for all NOM fractions are compiled in Table 3. According to Chen et al. [16], the decrease of fluorescence intensity normalized to organic carbon content as well as shift of λ_{Em} to longer wavelengths are related to the increase of molecular sizes and aromatic content in NOM. Figure 4 shows that the emission maxima of HA and HOA are red-shifted in comparison with the other NOM fractions. This result indicates the high aromatic content and the presence of high MW components in HA and HOA. Peaks' intensities for these fractions are also very low, which suggests the presence of condensed aromatic rings and other unsaturated bond systems capable of a high degree of conjugations, and electron-withdrawing groups, such as carbonyl and carboxyl [13, 15, 16, 18, 19]. The relatively low fluorescence intensity measured for HIN can be ascribed to the aliphatic structure of its constituents and the high degree of non-fluorescent carbohydrates [16]. The blue-shift of emission wavelengths and higher intensities measured for HIA, HIB and HON can be attributed to the low aromatic content, low MWs and the presence of electron-donating groups such as hydroxyls and metoxyls [15, 16]. According to Coble [12] the blue-shift in emission maximum can be caused by a decrease in the number of aromatic rings, reduction of conjugated bonds in a chain structure, or conversion of a linear ring system to a non-linear one. These effects were especially distinct for HON.

The excitation spectra of all NOM fractions, normalized by their respective maximum emission intensities, reveal that the excitation wavelength for HIA, HIB and HON is shifted towards shorter wavelengths (Figure 5). The blue-shift of excitation wavelength indicates that HIA, HIB and HON fractions are composed of relatively small molecular weight molecules [25].

The synchronous spectra (SF) presented in Figure 6 provide an improved peak resolution and increased selectivity in structural differences among NOM fractions. All the spectra were corrected by spectral substraction of the Raman spectrum of pure water. The location of peaks' maxima and specific fluorescence intensities are presented in Table 3. Two well-separated peaks of different intensities are observed for all spectra recorded with $\Delta\lambda$=44 nm (Figure 6a), while at $\Delta\lambda$=77 nm only one broad band appears (Figure 6b). The first band at Ex~280 nm ($\Delta\lambda$=44 nm) corresponds to the presence of amino-acids, such as tryptophan and tyrosine [28,32]. However, Peuravuori et al. [19] have suggested that fluorescence at Ex~280 can be assigned mainly to aromatic amino acids and some other volatile acids, containing conjugated aliphatic structures. Further, Duarte et al. [33] have reported that the signal at Ex~280 nm in humic and fulvic acid fractions spectra can be related to lignin-derived structural moieties, and the intensity of the band increases along with decrease of MW.

The protein-like band with the maximum at Ex~280 nm (Figure 6a) exhibits very low intensity for HA and HOA fractions, which confirms the EEM results showing the absence of protein-like constituents in these fractions. Higher fluorescence intensities at Ex~280 nm are ob-

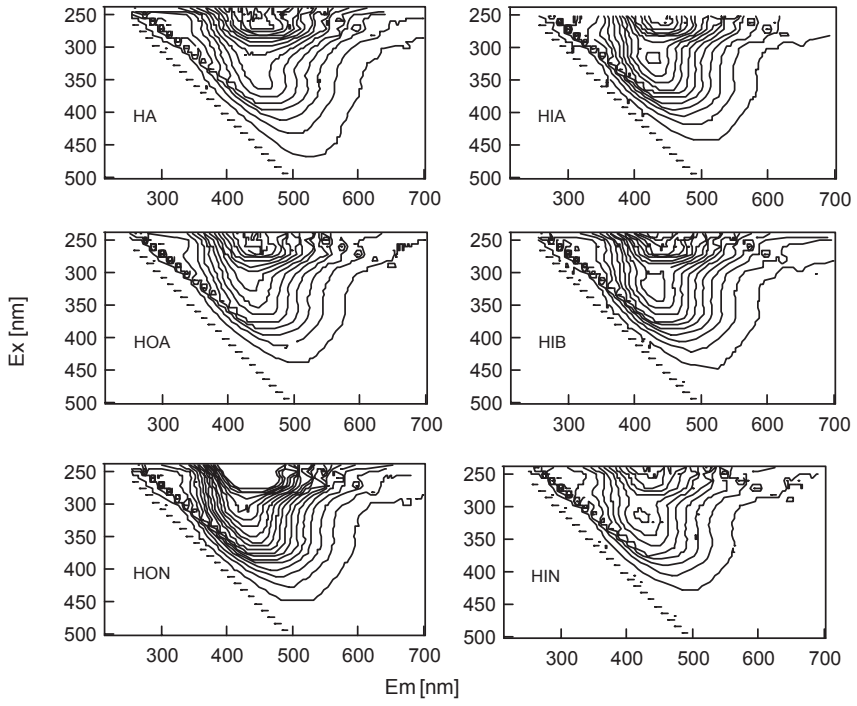

Fig. 3. Contour maps of NOM fractions.

Table 3. Fluorescence characteristics of NOM fractions.

Fraction	λ_{Em1} [nm] (SI_{Em1} [cps L mg^{-1}]) λ_{Ex1}=280 nm	λ_{Em2} [nm] (SI_{Em2} [cps L mg^{-1}]) λ_{Ex2}=320 nm	λ_{Em3} [nm] (SI_{Em3} [cps L mg^{-1}]) λ_{Ex3}=385 nm	SI_{Em1}: SI_{Em2}: SI_{Em3}	λ_{S1} [nm] (SI_{S1} [cps L mg^{-1}]), λ_{S2} [nm] (SI_{S2} [cps L mg^{-1}]) Δλ=44 nm	SI_{S1}: SI_{S2}	λ_{S3} [nm] (SI_{S3} [cps L mg^{-1}]) Δλ=77 nm
HA	459 (141739)	444 (85338)	474 (54704)	1:0.60:0.38	279 (15253) 370 (39987)	0.38:1	362 (76692)
HOA	446 (154915)	440 (108240)	470 (51769)	1:0.70:0.33	279 (15071) 370 (45383)	0.33:1	346 (92412)
HON	433 (209910)	425 (163757)	461 (71586)	1:0.78:0.34	279 (21708) 359 (69949)	0.31:1	341 (133969)
HIA	437 (168358)	430 (140621)	466 (61796)	1:0.83:0.37	281 (28878) 359 (61755)	0.46:1	339 (120946)
HIB	440 (163029)	431 (135495)	457 (65709)	1:0.83:0.40	283 (19231) 360 (64416)	0.30:1	338 (119109)
HIN	436 (110435)	427 (105238)	464 (42127)	1:0.95:0.38	286 (19946) 357 (45647)	0.44:1	333 (92912)

cps – count per second

served for HIA, HIB, HIN and HON. The HIB, HON and HIN fractions are known to consist of aromatic and aliphatic amines, amino acids and other molecules with smaller molecular sizes [25]. In the case of HIA, made up mainly of carboxylic acids [1,2,4,5,25], the high intensity of the band at Ex~280 nm can be presumably attributed to the presence of lignin-derived constituents with relatively low molecular sizes [33].

The other, broader band observed for all NOM fractions at Δλ=44 nm and Δλ=77 nm can be related to the humic-like and/or fulvic fluorophores [11,12,28]. The analysis of the SF spectra (Figure 6) together with specific peak intensities (Table 3) confirms the structural complexity and the high aromatic content in HA and HOA, and indicates the presence of low molecular sizes, more aliphatic constituents in HON, HIA and HIB as well as the carbohydrate-rich structural composition of HIN.

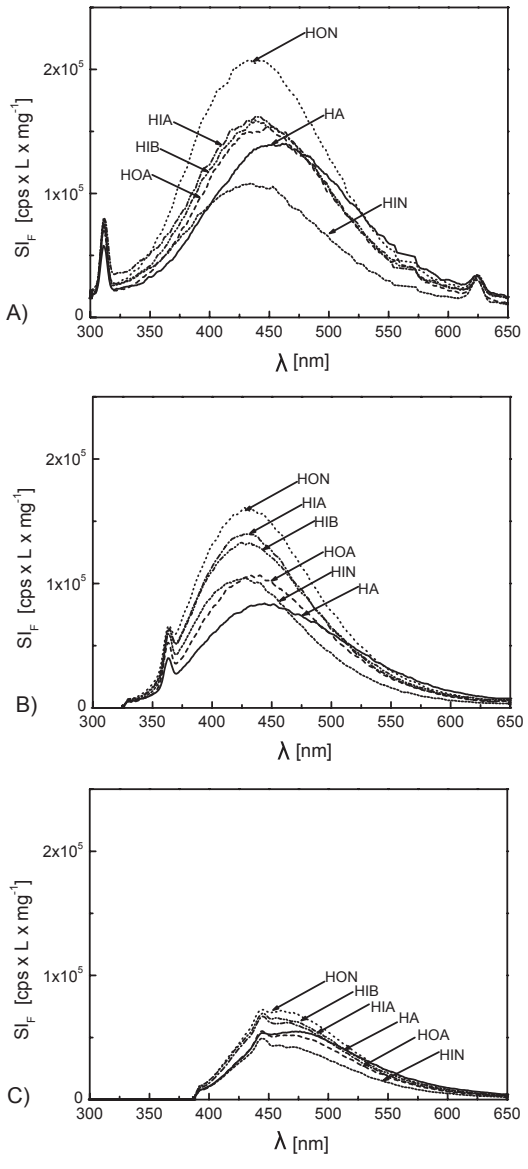

Fig. 4. Emission spectra of NOM fractions: A) λ_{ex}=280 nm, B) λ_{ex}=320 nm, C) λ_{ex}=385 nm.

Conclusions

The HP-SEC and SUVA studies reveal that HA and HOA are characterized by a high content of complex, aromatic, high molecular weight subunits, whereas hydrophobic neutrals consist of a few groups of compounds with lower molecular sizes and the lower degree of condensed aromatic moieties. Similar features are observed for hydrophilic acids and bases. The results also demonstrate that hydrophilic neutrals are presumably made of non-humic, aliphatic and low molecular weight components.

The EEM, emission, excitation and synchronous fluorescence spectra studies confirm the HP-SEC and SUVA results on the spectroscopic properties of individual NOM fractions. Additionally, the synchronous spectra reveal the presence of protein-like constituents in HIB, HIN and HON fractions. However, the higher intensity of the band at Ex~280 nm on HIA spectra can be attributed to the presence of low molecular size lignin-derived constituents, as well.

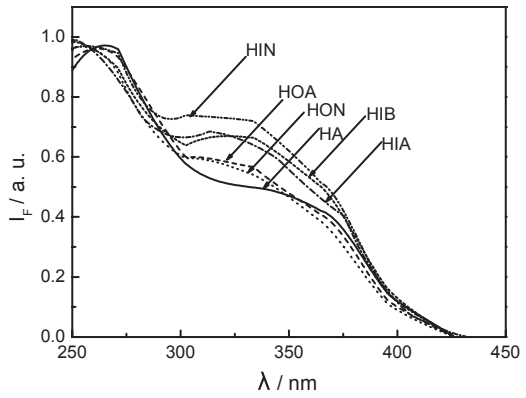

Fig. 5. Normalized excitation spectra of NOM fractions, extracted from EEM matrixes for λ_{em}=430 nm.

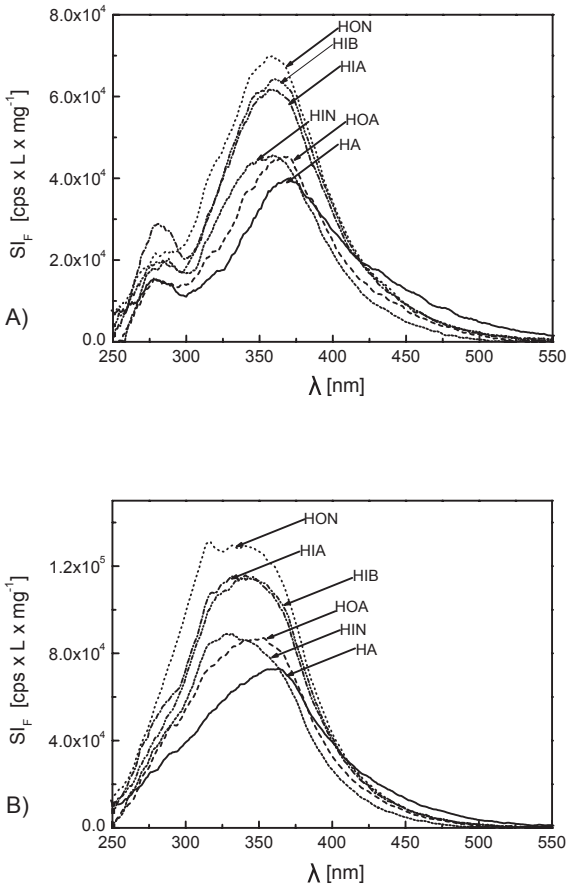

Fig. 6. Synchronous fluorescence spectra of NOM fractions recorded at: A) Δλ= 44 nm, B) Δλ= 77 nm.

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