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Comparison between DAX-8 and C-18 solid phase extraction of rainwater dissolved organic matter

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ABSTRACT

Rainwater is a very low concentrated matrix and, for dissolved organic matter (DOM) characterization, an efficient extraction procedure is essential. Isolation procedures based on the adsorption onto XAD-8 and C-18 sorbents have been used in the literature for rainwater DOM isolation, but a comparison between these procedures is lacking. In this work, UV–visible and molecular fluorescence spectroscopies highlighted differences between rainwater DOM isolated by DAX-8 (replacement for XAD-8) and by C-18. It was possible to recover higher rainwater DOM percentage by the C-18 based procedure than by the DAX-8 one. Rainwater protein-like compounds were better concentrated by the C-18 procedure than by the DAX-8 one, while humic-like compounds were similarly concentrated by both procedures. Furthermore, rainwater DOM extracted by the C-18 procedure was more representative of the global matrix, while DAX-8 preferentially extracted humic-like compounds.

1. Introduction

Dissolved organic matter (DOM) is defined operationally, almost universal consensus, as the organic matter that passes through a filter of 0.45 μm pore diameter [1,2]. Although there are techniques that can be applied directly to rainwater samples for the characterization of DOM, such as fluorescence spectroscopy, a deeper knowledge of DOM requires a previous extraction from samples. Ultrafiltration or solid-phase extraction, using XAD or C-18 sorbents, are the main ways used to concentrate and isolate DOM from water samples for further analysis [3]. However, there is no single technique that can achieve quantitative isolation of all organic solutes from water [4] and properties of DOM isolated with different techniques may differ markedly [3,5–7]. Besides, some isolation techniques allow for the selective isolation of a certain DOM fraction, which may be advantageous when the aim is obtaining more homogeneous fractions for further characterization.

A vast literature exists on solid phase extraction of organic matter (OM) from matrixes such as soil, freshwater or marine water. Unlike soil and freshwater OM, there is neither a robust protocol for the quantitative isolation of marine DOM nor any commercially available marine reference sample with which to compare extraction efficacy or DOM characteristics of the isolate [8] so the debate on this topic remains. A similar situation has also been already created for water soluble organic matter (WSOM) from aerosols [9], although research on this OM is quite recent, compared with that on marine DOM. Regarding rainwater, the study of the dissolved organic fraction was especially pushed by Willey et al. [10] and most of the references on this matter have been published subsequently. Among these references, even when rainwater is a very low concentrated matrix and, thus, concentration is particularly relevant for the study of DOM, to our best knowledge, DOM extraction has only been referred in four published works [11–14].

Wang et al. [11], in a work not exclusively focussed in rainwater, applied the methodology used for the isolation of aquatic humic substances, which was based on the fractionation of the dissolved organic carbon (DOC) of the water samples into hydrophobic and hydrophilic fractions using Amberlite XAD-8 resin.

Kieber et al. [12] extracted chromophoric dissolved organic matter (CDOM) from rainwater using C-18 cartridges by a method previously described for the isolation of marine DOM [15]. Then, Miller et al. [13], carried out solid phase extraction of CDOM from rainwater by C-18 cartridges, stating that they were employing the extraction technique previously described by Kieber et al. [12]. Both works highlighted that C-18 was chosen because earlier studies had found that, relatively to XAD, C-18 was able to better retain the UV–visible and fluorescence characteristics of isolated chromophoric organic material, which was supported by referring the

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work by Amador et al. [15]. However, Amador et al. [15] is a work on extraction of humic substances (HSs) from seawater and the elution procedure was different from the one used by Kieber et al [12]. Furthermore, the International Humic Substance Society (IHSS) (http://www.ihss.gatech.edu) operationally defined dissolved HSs on the base of adsorption on XAD-8 [16]. Also, seawater and rainwater are very different matrixes. Thus, rigorously, conclusions on HSs from seawater by Amador et al. [15] should not be taken as valid for CDOM from rainwater.

Finally, the procedure used for the isolation and extraction of DOM from rainwater by Santos et al. [14] was adapted from the one used by Duarte and Duarte [17] for isolating WSOM from atmospheric aerosols. Santos et al. [14] highlighted that XAD-8 was able to isolate the most hydrophobic macromolecular rainwater organic solutes.

The present work aims at comparing DAX-8, the available replacement for XAD-8 [18,19], and C-18 procedures for the isolation of rainwater DOM. For this first comparison, UV–visible and molecular fluorescence spectroscopies were used because they can be applied to rainwater samples with low DOC concentrations, without pre-concentration, allowing to follow the DOM isolation process. Moreover, the techniques are rapid and non-destructive, and the molecular fluorescence spectroscopy is a very sensitive technique which detects subtle differences in properties and distribution of fluorophores. Thus, spectra were determined for rainwater and the effluents and eluates from each of the applied isolation procedures. Finally, since research on rainwater DOM is still progressing, this work will be a major contribution for choosing the adequate method of DOM extraction from rainwater.

2. Experimental

2.1. Rainwater sampling and sample preparation

Rainwater was collected at a sampling station (40°38′ N, 8°39′ W) located in the western part of the town of Aveiro, Portugal: one sample was collected in June of 2009 (J09) and two samples were collected in October of 2009 (O09a and O09b). Collection was carried out 70 cm above the ground, through glass funnels (30 cm diameter) into glass bottles (5 L). Sampling containers were left out open in order to collect both wet and dry depositions on a 24 h basis. Prior to use, all glass materials were immersed for 30 min, in a solution of NaOH (0.1 M), then rinsed with distilled water, followed by another immersion for 24 h in a solution of HNO_3 (4 M), and finally rinsed with ultrapure (Milli-Q) water. After collection, samples were transported to the laboratory where they were filtered through hydrophilic PVDF Millipore membrane filters (0.45 μm). In all cases, rainwater was dark stored in glass vials at 4 °C for a maximum of four days, which was verified not to alter the optical properties of samples.

2.2. Fulvic acids solution preparation

For further comparison of the DOM isolation procedures considered in this work, in what concerns their capacity to isolate the humic fraction from rainwater or from other aqueous samples, both procedures were also applied to a known and previously characterized sample of fulvic acids. Fulvic acids (FA) extracted [20] from river Vouga at Carvoeiro, Aveiro, Portugal, were used for this purpose. That sample of fulvic acids has been isolated using the XAD-8 procedure recommended by the IHSS. It is worth to notice that the DAX-8 isolation procedure used in the present work uses a different elution procedure, with methanol/water instead of NaOH 0.1 M. Solutions of 2 ppm of these FA were prepared in ultrapure water and three replicate extractions of DOM were carried out as described below, exactly in the same way as for rainwater.

2.3. DOM extraction

Rainwater samples (500 mL) and FA solutions (500 mL) were subjected to two different procedures for the isolation and extraction of DOM: one based on the use of Supelite^{TM} DAX-8 resin (considered the substitute of XAD-8 since the production of the latter stopped [18]), and another one based on the use of C-18 sorbent (Supelclean envi-18 cartridges, Supelco, 500 mg mass, volume size 6 mL).

The Supelite^{TM} DAX-8 resin is comprised of a poly(methyl methacrylate) resin (pore size = 225 Å; surface area = 160 m^2/g), which is slightly polar, while C-18 sorbent is constituted by alkyl chains of C-18 covalently bonded to a silica substrate (pore size = 60 Å; surface area = 475 m^2/g) being non polar (highly hydrophobic). DAX-8 and C-18 isolation procedures separate DOM into polar and non polar fractions taking into account the molecular size of the solutes and interactions between them and the sorbents.

The isolation procedure based on DAX-8 was adapted from the one described by Santos et al. [14] for the extraction of DOM from rainwater. Fig. 1(a) represents the schematic diagram of the exper-

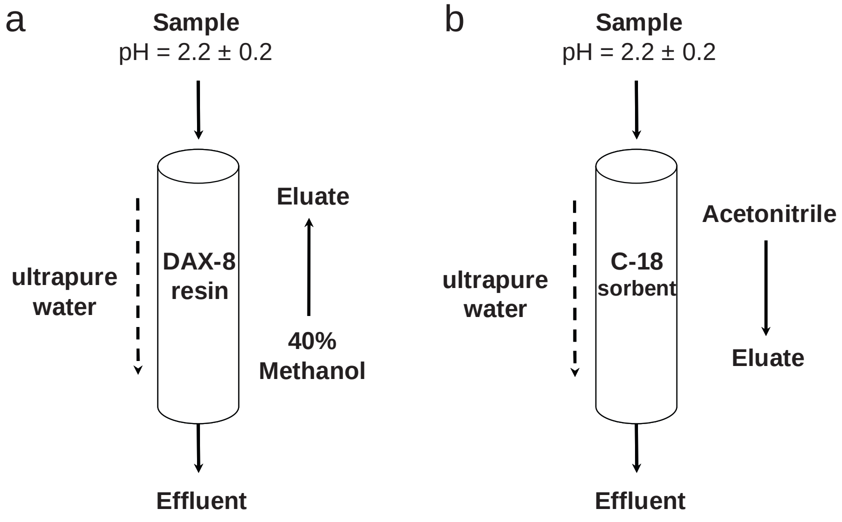

Fig. 1. Schematic diagram of the experimental procedures adopted for DOM isolation.

imental procedure adopted in the present work. A glass column containing DAX-8 resin (15 mL—bed volume) was prepared for adsorption of DOM. The resin was thoroughly cleaned by Soxhlet extraction as described elsewhere [21]. Former to usage, the resin column was rinsed, first with ultrapure water (≈100 bed volumes), then with 60 mL of a methanol solution (40%) and with ultrapure water (≈50 bed volumes), after that with 0.1 M NaOH (1 bed volume), followed by 0.1 M HCl (1 bed volume) and finally with 0.01 M HCl (1 bed volume). Rainwater and FA solutions were acidified to pH = 2.2 ± 0.2, with a solution of HCl 6 M, before being pumped to the DAX-8 column at a flow rate of ≈1.3 mL/min for DOM concentration. After pumping 500 mL of sample, the inorganic matter retained in the DAX-8 column void volume was down washed with ultrapure water (1 bed volume) pumped at ≈1.3 mL/min. The OM adsorbed in DAX-8 was then back eluted with 60 mL of a methanol solution (40%) at a flow rate of ≈0.4 mL/min (1/3 of the rate of fixation). Eluate was evaporated almost to dryness (volume ≤ 1 mL) in a rotary evaporator at 30 °C, and then transferred to a volumetric flask (20 mL). Ultrapure water was then used to make up the flask volume.

The isolation procedure based on C-18 was adapted from the one described by Kieber et al. [12] for the extraction of DOM from rainwater. Fig. 1(b) represents the schematic diagram of the experimental procedure adopted in the present work. As indicated by the manufacturer, C-18 cartridges were preconditioned by washing with methanol (6 mL) followed by ultrapure water (6 mL) and dried under N_2; cartridges were then washed with acetonitrile (2 × 5 mL) and by ultrapure water (2 × 5 mL). Then, 500 mL of acidified (pH = 2.2 ± 0.2) rainwater or FA solutions were pumped at a flow rate of 15–20 mL/min for DOM concentration. After the adsorption stage, the cartridge was washed with ultrapure water (2 × 5 mL) to remove salts and then dried under N_2. DOM was then eluted with acetonitrile (2 × 3 mL) into volumetric glass flasks (20 mL) and dried under N_2. Ultrapure water was then used to make up the flask volume.

For optical analysis, aliquots of rainwater, FA solutions and the corresponding DAX-8 and C-18 eluates and effluents were acidified to pH = 3.0 ± 0.2, with solutions of HCl 6 M or HCl 1 M or NaOH 1 M depending of the pH of the fraction.

For DAX-8 and C-18 extraction procedures, blanks (ultrapure water) were carried out and analysed in the same way than rainwater and FA solutions in order to be possible to subtract an averaged blank from each sample or solution spectrum.

Both the above procedures include few steps of sample handling, which constitutes an advantage for preventing possible modifications of samples chemical composition. However, with respect to operation time, the C-18 based procedure is less time-consuming than the DAX-8 one.

2.4. Optical analysis

UV–visible spectra (in the range of 200–600 nm) of rainwater samples, FA solutions and the corresponding effluents and eluates from DAX-8 and C-18 were recorded on a Shimadzu (Dusseldorf, Germany) Model UV 210PC spectrophotometer. Quartz cells of different path lengths were used depending on the observed absorbance: 5 cm (for the rainwater samples, FA solutions, effluents and rainwater eluates) and 1 cm (for the FA eluates). Ultrapure water was used as reference and to obtain the baseline.

The spectral slope coefficients (*S*) were inferred from the obtained UV–visible spectra. For comparison with *S* values published for rainwater DOM [12,14], S values (μm^{-1}) were calculated from non-linear least-square regressions of the absorption coefficients (a_λ) vs. wavelength for the range between 240 and 400 nm using the equation of Markager and Vincent [22]: $a_\lambda = a_{\lambda_0} e^{S(\lambda_0-\lambda)} + K$, where λ_0 is the reference wavelength (300 nm) and *K* is a background parameter to improve the goodness of fit. Absorption coefficients (a_λ, m^{-1}) at each wavelength (λ) were calculated as $a_\lambda = 2.303\,A_\lambda\ /l$, where A_λ is the corrected spectrophotometer absorbance reading at wavelength λ and l (m) is the optical pathlength. The maximum wavelength for spectral slope calculations was 400 nm because it was the highest wavelength where absorbance values were consistently above the detection limit.

The molecular fluorescence spectra corresponding to rainwater and FA fractions were obtained by a Fluoromax 3 (JobinYvon-Spex Instruments S.A., Inc., now HORIBA Jobin Yvon Inc, Edison, NJ, USA) spectrophotometer with a xenon lamp source. Fluorescence analyses were carried out under thermostated 20 °C, using a water bath connected to the fluorometer, which most approximates the room temperature in the laboratory. Spectra were recorded using 5 nm bandpasses on both the excitation and emission monochromators and 1 cm cells. Synchronous spectra ($\Delta\lambda = 70$ nm) were acquired using λ_{ex} from 240 to 400 nm (5 nm intervals). Scans were corrected for instrument configuration using factory supplied correction factors [23]. Data were normalized to a daily-determined water Raman intensity ($275_{ex}/303_{em}$, 5 nm bandpasses) and converted to Raman normalized quinine sulphate (QS) equivalents in ppb [24]. In all cases, replicate scans within 5% agreement in terms of intensity and within bandpass resolution in terms of band location were obtained.

The corresponding averaged blank spectra were subtracted from samples/solutions spectra.

3. Results and discussion

Fig. 2 shows the UV–visible spectra obtained for the FA solutions (average spectrum), for the rainwater samples J09, O09a and O09b, for the blank (average spectrum), together with the spectra corresponding to their DAX-8 and C-18 effluents and eluates. The absorbance values were normalized for a 1 cm pathlength. In all the spectra, the absorbance decreased with increasing wavelength, following a trend similar to that already described for rainwater samples [12,25]. The UV–visible spectra show that the isolation procedure based on C-18 is able to recover more DOM, either from FA solutions or rainwater, than the one based on DAX-8.

The values of spectral slope coefficients (*S*) determined for the different FA and rainwater samples and fractions, obtained by the DAX-8 and C-18 isolation procedures here used, are shown in Table 1. The S has been used as a proxy for molecular weight (MW) in a broad range of samples and it has been found that it is inversely related to the MW of the CDOM [26]. For rainwater samples, the S values are higher than for the river FA sample, which suggests that rainwater DOM had lower molecular weight. With respect to FA solutions, the S values of the eluates are very close to those obtained for the FA solutions, suggesting that the eluates are representative of the FA sample for both isolation procedures. In the case of rainwater eluates, with the unique exception of DAX-8 eluate of sample J09, *S* values are lower than those obtained for rainwater samples, which indicates that the higher molecular weight OM is preferentially extracted, either by DAX-8 or C-18. This is in agreement with the fact that the S values of the effluents are always higher than the *S* values of the samples suggesting that the effluents must be represented by compounds with lower molecular weight than those retained in DAX-8 and C-18 sorbents during the concentration stage.

The molecular fluorescence synchronous spectra ($\Delta\lambda = 70$ nm) obtained for the FA solutions (average spectrum), for the rainwater samples J09, O09a and O09b, for the blank (average spectrum) together with the spectra of their DAX-8 and C-18 eluates, are shown in Fig. 3. The $\Delta\lambda = 70$ nm for the synchronous spectra was chosen in order to highlight the protein-like and the humic-like

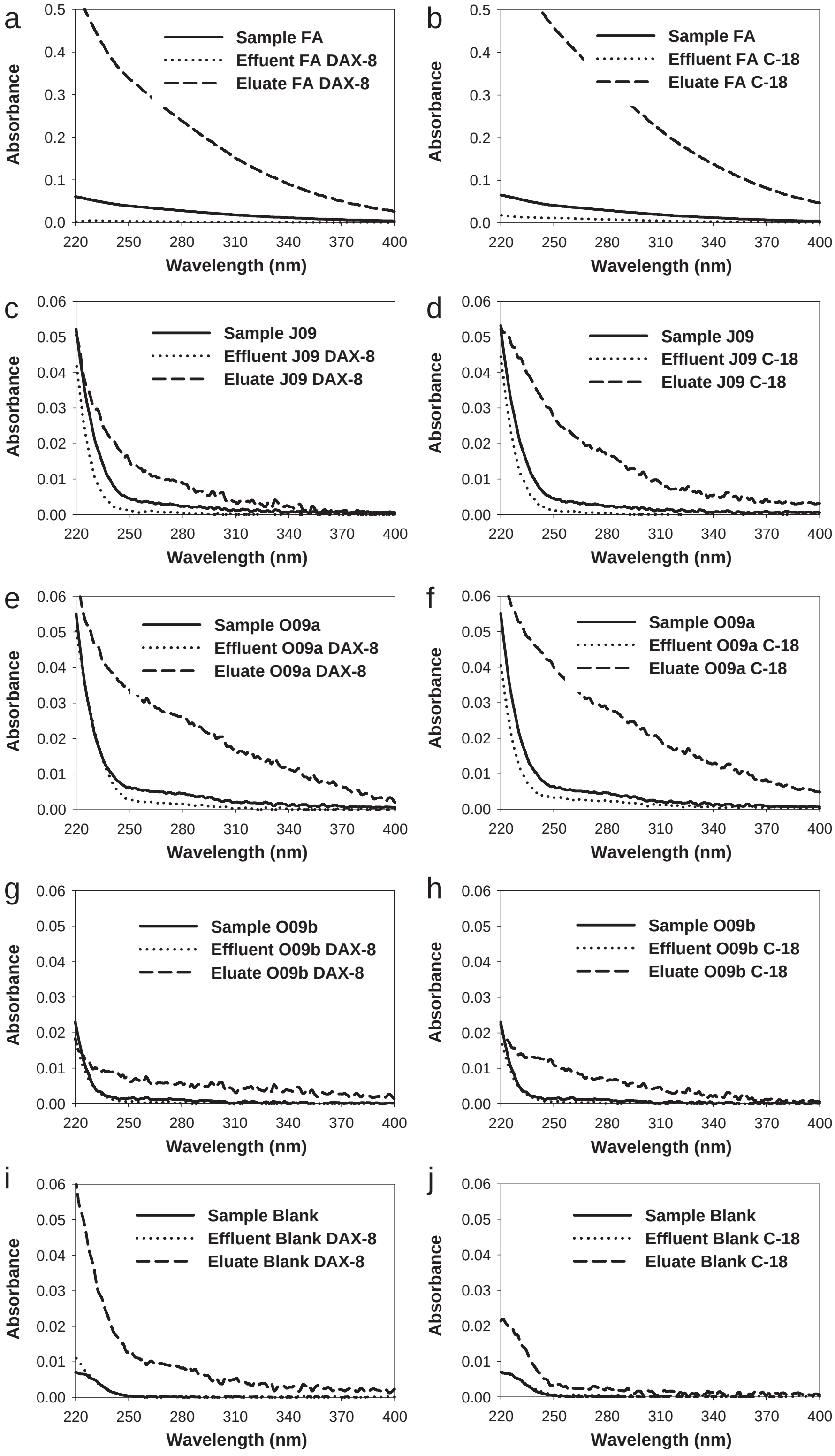

Fig. 2. UV–vis spectra, with blank subtraction, obtained for the different fractions of FA solution (averages spectrum; (a) and (b)) and rainwater (J09, (c) and (d); O09a, (e) and (f); O09b, (g) and (h)), by each of the isolation procedures considered. The blanks of the procedures (averages spectrum) are presented in the graphs (i) and (j).

fluorescence [25]. Fluorescence emission–excitation matrix (EEM) have been used to characterize DOM in rainwater [12,14,25,27] and may exhibit the presence of seven fluorescent bands, as described in Table 2. These fluorescence bands that may be present in rainwater have been found in most EEMs of aquatic samples, even though the limits for the ranges of their λ_{exc} and λ_{em} maxima can be slightly different from those presented in Table 2 for rainwater samples [28,29]. Bands A and C have been usually assigned to humic-like compounds, while the band M has been usually assigned to marine humic-like compounds. Bands at the same $\lambda_{ex}/\lambda_{em}$ than B_1, B_2 and T_1, T_2 are attributed to protein-like compounds, such as tyrosine (B_1, B_2) and tryptophan (T_1, T_2). Burdige et al. [29] used the nomen-

Table 1
Spectral slope coefficients (μm^{-1}) calculated for the different fractions of FA solution and rainwater, obtained by the DAX-8 and C-18 isolation procedures.

Matrixes	Phase solid	Sample name	Fractions					
			Sample	Sample average	Effluent	Effluent average	Eluate	Eluate average
FA solution	DAX-8 resin	FA1	13.5	13.5 (±0.1)	16.0	15.4 (±0.8)	13.9	13.8 (±0.1)
		FA2	13.4		14.5		13.7	
		FA3	13.6		15.8		13.8	
	C-18 sorbent	FA1	12.9	13.1 (±0.4)	17.4	15.5 (±1.9)	12.6	12.8 (±0.2)
		FA2	12.9		13.5		12.8	
		FA3	13.6		15.6		13.0	
Rainwater	DAX-8 resin	J09	19.2	–	25.3	–	22.3	–
		O09a	17.7		32.7		11.9	
		O09b	18.5		29.3		10.2	
	C-18 sorbent	J09	19.2	–	34.8	–	18.2	–
		O09a	17.7		19.4		12.1	
		O09b	18.5		26.8		15.1	

Note: Standard deviations are indicated between brackets.

Table 2
Band, excitation/emission wavelength maxima range and attribution of fluorescent DOM in rainwater based in previous works [12,14,25,27].

Band	λ_{ex} max (nm)	λ_{em} max (nm)	Attribution
A [12,14,25]	220–260	380–460	Humic-like
M [12,14,25]	280–310	370–420	Marine humic-like
C [12,14,25]	320–360	390–475	Humic-like
T_1 [14,25]	220–230	335–360	Tryptophan-like, protein-like
T_2 [12,14,25,27]	260–295	330–370	Tryptophan-like, protein-like
B_1 [14,25]	220–230	295–310	Tyrosine-like, protein-like
B_2 [27]	265–285	295–315	Tyrosine-like, protein-like

clature S and R for designating the bands B_1 and T_1, and B and T for designating B_2 and T_2.

Fluorescence spectra obtained for FA (Fig. 3(a) and (b)) contain three bands: one protein-like band, T_2 ($\lambda_{ex} \approx 270$ nm); and two overlapped humic-like bands at $\lambda_{ex} = 340$–370 nm, M ($\lambda_{ex} \approx 340$ nm) and C ($\lambda_{ex} \approx 370$ nm). Regarding fluorescence spectra obtained for rainwater (Fig. 3(c)–(h)), they contain two protein-like bands B_1 ($\lambda_{ex} \approx 240$ nm) and T_2 ($\lambda_{ex} \approx 270$ nm); and one humic-like band, M ($\lambda_{ex} \approx 325$ nm). The lower excitation wavelength of the marine humic-like band (M) in rainwater than in FA solutions suggest that rainwater samples contain a lower amount of conjugated aromatic π-bond systems with electron-withdrawing functional groups [30].

Synchronous spectra in Fig. 3(a) and(b) shows that both isolation procedures allow a similar recovery of protein-like and humic-like compounds from FA solutions, since the fluorescence intensities of the eluates are similar for both isolation procedures. In the case of rainwater, as it may be seen in Fig. 3(c)–(h), the C-18 based procedure allows a more efficient recovery of protein-like compounds than DAX-8 based procedure, while the humic-like compounds were similarly extracted by both procedures, except for O09b (Fig. 3(g) and (h)). The lower efficiency of recovery of the humic-like compounds by the DAX-8 based procedure observed for O09b, may be due to the influence of subtraction of blanks whose intensity are higher for DAX-8 than for C-18, affecting more significantly the samples like this one with less DOM.

Table 3 shows the percentages of retention of DOM from FA solutions and rainwater samples obtained by the DAX-8 and C-18 isolation procedures, expressed as percentages of UV absorbance at 250 nm ($UV_{250\,nm}$) and of fluorescence intensities (FI) at 270 and 325 or 340 nm ($FI_{270\,nm}$ and $FI_{325\,nm}$ or $FI_{340\,nm}$, respectively) of the original samples. $UV_{250\,nm}$ was chosen because it may be used as a way of assessing OM content [17]. On other hand, $FI_{270\,nm}$ corresponds to the maximum of protein-like fluorescence and $FI_{325\,nm}$ or $FI_{340\,nm}$, to the maximum of humic-like band (M) for rainwater or FA, respectively, as observed in Fig. 3. The recoveries after elution, also shown in Table 3, and expressed in terms of UV absorbance or of FI were calculated using the equation:

$$\%\,\text{Recovery} = \frac{(\text{UV or FI})_{el} \times V_{el}}{(\text{UV or FI})_{in} \times V_{in}} \times 100$$

where UV or FI are the UV absorbance at 250 nm or the fluorescence intensities at 270 and 325 or 340 nm, respectively. V_{in} is the initial volume of sample that passes through the DAX-8 and the C-18 columns, while V_{el} is the eluate volume. The percentages of retention and recovery of DOM from FA solutions and rainwater samples obtained by the DAX-8 and C-18 isolation procedures were compared recurring to the one sided students t-tests, with the significance level of 5%: t-test for means in the case of FA solutions and paired t-test for rainwater samples.

Results in Table 3 show that, in the case of FA, DAX-8 retained more DOM than C-18, as evaluated by $UV_{250\,nm}$ ($p = 0.002$) and by both protein-like ($p = 0.006$) and humic-like ($p < 0.001$) fluorescence. However, the recovery percentages depend not only on the retention capacity but also on the elution efficiency. As can be seen in Table 3, the recoveries of DOM from FA solutions, expressed as $UV_{250\,nm}$, are higher ($p = 0.001$) by the C-18 based isolation procedure than by the DAX-8 one. However, when expressed as FI ($FI_{270\,nm}$ or $FI_{340\,nm}$), similar recoveries were obtained by both the isolation procedures used ($p = 0.429$ for $FI_{270\,nm}$ and $p = 0.069$ for $FI_{340\,nm}$). These results suggest that fluorescent bands do not represent all the OM that absorbs.

Regarding rainwater, for each of the samples, and according with $UV_{250\,nm}$ values, DAX-8 retained more DOM than C-18 ($p = 0.016$), which is in agreement with results obtained for FA. However, according with $FI_{270\,nm}$ and $FI_{325\,nm}$, DAX-8 retained more protein-like ($p = 0.008$) but less humic-like ($p = 0.012$) fluorescence than C-18 for each of the separate samples, which evidence a different nature of rainwater humic matter relatively to the river FA, also suggested above by the lower MW of OM attributed by the spectral slope and the lower fluorescence excitation wavelength

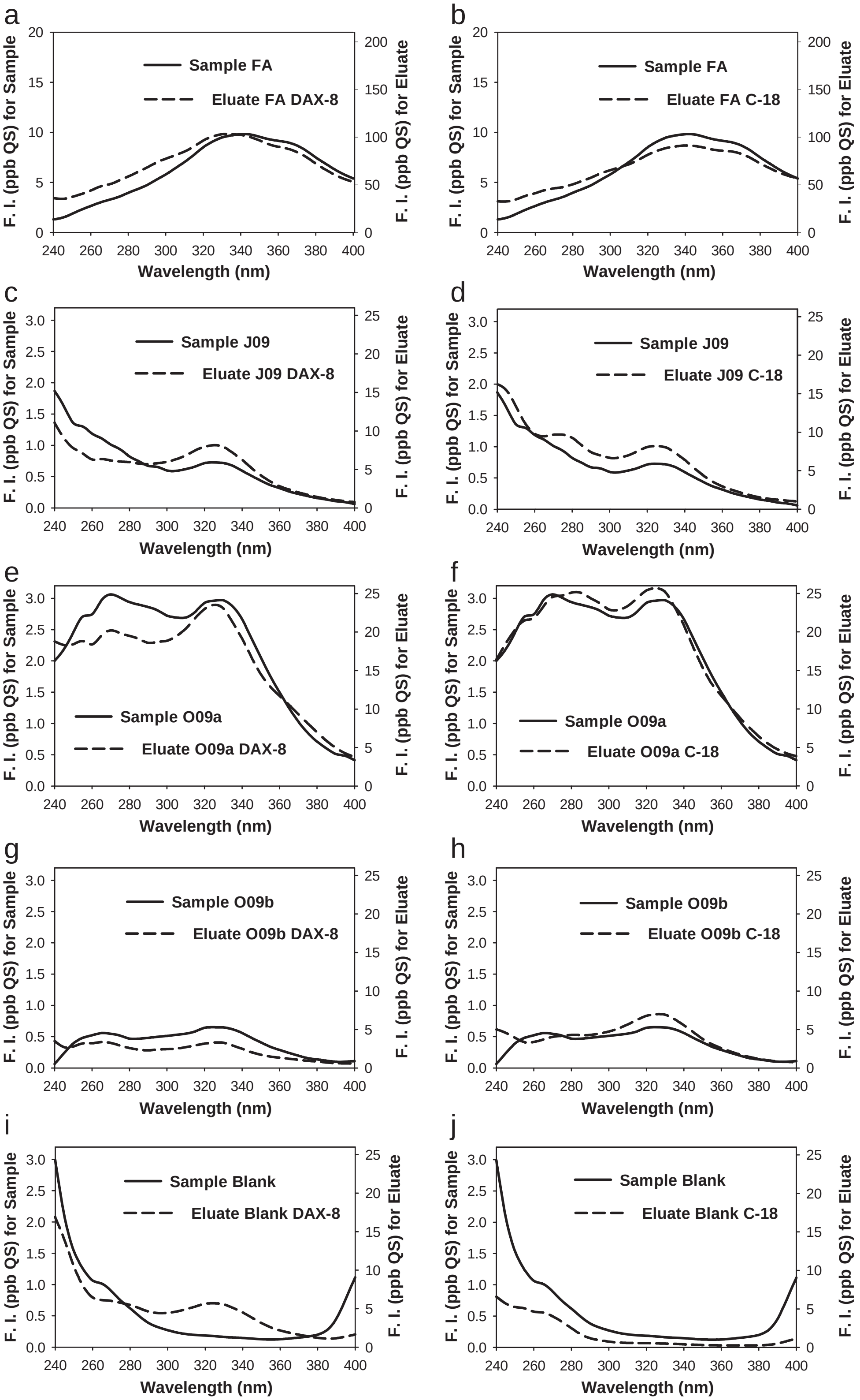

Fig. 3. Synchronous spectra of fluorescence, with blank subtraction, obtained for the different fractions of FA solutions (averages spectrum; (a) and (b)), rainwater (J09, (c) and (d); O09a, (e) and (f); O09b, (g) and (h)), by each of the isolation procedures considered. The blanks of the procedures (averages spectrum) are presented in the graphs (i) and (j).

of marine humic-like compounds for rainwater (M). Anyway, the percentages of DOM recovery from rainwater, either expressed by $UV_{250\,nm}$ or FI, were higher for the C-18 based isolation procedure than for the DAX-8 one, except for the humic-like band of the sample J09. Differences between both procedures recoveries showed to be significant for the $UV_{250\,nm}$ ($p = 0.035$) and the protein-like band ($FI_{270\,nm}$; $p = 0.041$) and not significant for the humic-like band ($FI_{325\,nm}$; $p = 0.201$). It must be pointed out that rainwater samples,

Table 3
Percentages of DOM retention and recovery obtained from the DAX-8 and C-18 isolation procedures.

Matrixes	Fractions	Sample name	(%)							
			$UV_{250\,nm}$	$UV_{250\,nm}$ average	$FI_{270\,nm}$	$FI_{270\,nm}$ average	$FI_{325\,nm}$	$FI_{325\,nm}$ average	$FI_{340\,nm}$	$FI_{340\,nm}$ average
FA solution	Retained in DAX-8 resin	FA1	93.9	93.3 (±0.6)	89.8	90.5 (±1.4)	–	–	87.4	87.6 (±0.8)
		FA2	92.8		89.5		–		86.9	
		FA3	93.3		92.1		–		88.5	
	Retained in C-18 sorbent	FA1	60.8	68.5 (±7.1)	72.6	67.6 (±9.1)	–	–	55.4	58.2 (±3.8)
		FA2	74.7		73.1		–		62.5	
		FA3	70.1		57.0		–		56.6	
	Recovery from DAX-8 resin	FA1	37.6	36.9 (±0.7)	54.1	58.1 (±4.6)	–	–	44.4	44.1 (±3.2)
		FA2	36.3		57.1		–		40.7	
		FA3	36.8		63.1		–		47.1	
	Recovery from C-18 sorbent	FA1	44.3	47.2 (±2.6)	52.0	59.0 (±7.1)	–	–	35.4	39.2 (±3.2)
		FA2	49.2		66.3		–		40.8	
		FA3	48.2		58.8		–		41.3	
Rainwater	Retained in DAX-8 resin	J09	78.7	–	76.4	–	69.8	–	–	–
		O09a	53.3		57.6		56.7		–	
		O09b	73.1		60.9		68.5		–	
	Retained in C-18 sorbent	J09	72.6	–	68.2	–	77.0	–	–	–
		O09a	47.3		51.9		67.3		–	
		O09b	63.0		52.1		74.9		–	
	Recovery from DAX-8 resin	J09	15.1	–	27.6	–	50.8	–	–	–
		O09a	21.6		26.9		32.4		–	
		O09b	18.9		25.2		21.5		–	
	Recovery from C-18 sorbent	J09	27.4	–	46.3	–	49.2	–	–	–
		O09a	25.9		33.4		35.0		–	
		O09b	31.5		35.5		43.6		–	

Note: Standard deviations are indicated between brackets.

as well as their DOM content, are different and the % results differ among them. On other hand, it must be noticed that the blanks from DAX-8 are higher than those from C18 and special concern must be taken with DAX-8 isolation procedure for obtaining low blanks that may affect more the samples with lower DOM content.

Comparing the isolation procedures used in this work, results in Table 3 emphasize what has been already seen in Figs. 2 and 3: the C-18 isolation procedure allows for a higher concentration of a DOM that is more representative of the global rainwater matrix, recovering a higher percentage of protein-like compounds and a similar percentage of humic-like compounds than the DAX-8 isolation procedure, which preferentially separates humic-like compounds. The possible reasons behind the preferential concentration of protein-like compounds by the C-18 procedure and the similar concentration of humic-like compounds by DAX-8 and C-18 procedures may be associated with the interactions between the compounds and the sorbents, being stronger between the protein-like compounds and the DAX-8 sorbent, as evidenced by the higher retention and lower recovery (elution) of this fraction. The results emphasize that the humic-like and protein-like compounds may have different chemical properties such as the molecular size or functional groups conducting to the different results obtained on the isolation of the compounds

4. Conclusions

In the present work, rainwater DOM isolated by two different procedures, the DAX-8 and the C-18 ones, was compared using UV–visible and molecular fluorescence spectroscopies, evidencing that:

(1) With respect to molecular weight (inferred by *S*), both isolation procedures extracted preferentially the larger molecular weight DOM.
(2) The C-18 based isolation procedure applied allowed for the isolation of a higher percentage of DOM from rainwater than the DAX-8 based one. Also, a higher percentage of protein-like compounds were extracted by the C-18 procedure compared with the DAX-8 one, while the percentages of humic-like compounds extracted by both procedures were in the same range.
(3) Furthermore, protein-like and humic-like compounds were equally extracted from rainwater by the C-18 based isolation procedure, which thus allows to concentrate DOM that is more representative of the global matrix. On the contrary, the DAX-8 procedure extracts the humic-like compounds preferentially than the protein-like ones.

UV–visible and molecular fluorescence have been very useful to highlight that caution must be taken when comparing DOM fractions isolated from rainwater by the DAX-8 or by the C-18 procedures. However, further work, using other analytic techniques, such as high-resolution mass spectrometry, or nuclear magnetic resonance spectroscopy, should be carried out to further compare DAX-8 and C-18 solid phase extraction of rainwater DOM.

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