

Variation on the adsorption efficiency of humic substances from estuarine waters using XAD resins

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Abstract

The XAD8 and XAD4 resins, used in series, show different behaviours in relation to the retention of dissolved organic matter (DOM) from estuarine water samples according to different salinities. For the XAD8 resin, the removal of DOM measured in terms of light absorbance at 250 nm varies from $69.4\% \pm 0.5$ to $51.4\% \pm 3.5$ and for the XAD4 resin it varies from $20.0\% \pm 1.0$ to $25.2\% \pm 3.0$ for salinities ranging from 0.6 to 33.4. The isolation, with XAD8, of DOM from samples increasing in salinity, measured in terms of emission fluorescence intensity at selected wavelengths (410, 440 and 460 nm) for an excitation of 340 nm, decreases. In the same experimental conditions, the increase of fluorescent organic matter (FOM) removed by the addition of XAD4 resin is always greater than 20%. However, addition of NaCl to the sample with the lowest salinity, shows that the retention in both resins is related more to the nature of DOM than to the ionic strength of the sample.

1. Introduction

The International Humic Substances Society (IHSS) has used XAD8 resin for the isolation of reference and standard humic substances from fresh-water (Thurman and Malcolm, 1981). XAD resins adsorb dissolved organic matter (DOM) according to the hydrophobic nature of its organic components that interact with the resins. Since it is not possible to predict the exact mechanism of adsorption, laboratory studies must evaluate the efficiency of DOM retention on different matrices.

Most of the previous work on adsorption efficiency of humics has been carried out using samples prepared with humic substances previously isolated according to the IHSS procedure (Stuermer and Harvey, 1977; Sorouradin et al., 1993; Town and Pow-

ell, 1993). These results cannot be extrapolated to obtain the adsorption efficiency of humic substances on XAD resins when using natural water samples. Studies on the adsorption of DOM on XAD resins from estuarine environments under different salinity gradients have not been reported. The present work aims at comparing (using light absorbance and fluorescence) the efficiency of DOM isolation from different estuarine waters using a system of two resins connected in series, XAD8 followed by XAD4. Such data are important to evaluate the matrix effect on the adsorption efficiency from factors such as salinity and, consequently, to discuss the representativeness of the humic substances obtained.

The procedure for extracting humic substances involves a preliminary stage of acidification to increase the protonation and hydrophobicity of the

humic substances and facilitate the adsorption on XAD resins. The degree of protonation θ_i (fraction of sites occupied by protons) and the charge Q is related by $\theta_i = 1 - Q/Q_{\max}$, where Q_{\max} is the maximum (negative) charge of the fulvic acid, i.e. when the sample is fully dissociated (Nederlof et al., 1993). Therefore, at a certain pH the fulvic acids are more negatively charged for high ionic strength. The dissociation is, thus, enhanced with an increasing salt level. Even when all the samples are acidified to the same pH value of 2 before the isolation procedure, the degree of protonation of the humic substances still decreases with increasing salinity (also decreasing the hydrophobicity). The efficiency of removal on the resins therefore seems to depend on the salinity, even for the same pH.

2. Materials and methods

2.1. Sample collection

Samples were collected (in 5 l glass containers) from locations with different salinities in the Aveiro lagoon (Portugal):

- (1) Frei-Gil (freshwater, $S = 0.6$),
- (2) Areão (low salinity, $S = 1.8$),
- (3) Vagueira (medium salinity, $S = 21.4$), and
- (4) Barra (seawater, $S = 33.4$).

After filtration through $0.45 \mu\text{m}$ (Gelman-Supor) filters the samples were stored in a dark room at 4°C .

2.2. Instrumentation

Absorbance readings were performed in a UV-Visible spectrophotometer Shimadzu UV-2101 PC, using a 100 mm quartz cell. The fluorescence measurements, at room temperature, were carried out in a Jasco FP-770 spectrofluorimeter at 410, 440 and 460 nm emission wavelengths and 340 nm excitation ($\lambda_{\text{exc}} = 340 \text{ nm}$), using 5 nm bandwidths and 1 cm quartz cuvettes. All spectra were obtained in the same conditions, although no calibration of the spectrofluorimeter response and correction for scattering were performed. Salinity was measured in the field with a salinometer WTW Microprocessor Conductiv-

ity Meter LF 196, and the pH with a pH meter Orion 250A.

2.3. XAD Resins

The fines, impurities and unpolymerized monomers were removed with a daily washing of the resins (XAD8 and XAD4) with 0.1 M NaOH and distilled water during 10 days, followed by hot soxhlet extraction (two runs) with methanol, acetonitrile and diethylether (5 days in each solvent) finishing the last run with methanol (3 days) (Santos et al., 1994).

Before the isolation of humic substances, the methanol was removed with distilled water until no absorbance was detected at 220 nm, and the resins were then washed with 0.1 M NaOH followed by 0.1 M HCl and 0.01 M HCl.

2.4. Isolation of aquatic humic substances

Four 13 ml glass columns for each resin (XAD8 and XAD4) were packed with 12 cm^3 of resins. All columns were lined with teflon except for the 20 cm tygon portion in the peristaltic pump.

The procedure for the isolation of aquatic humic substances includes delivering 400 ml of the acidified water sample (with HCl to $\text{pH} = 2$), through the XAD8 resin column, collecting the effluent and passing it through the XAD4 resin column, both at a flow rate of 1.7 ml/min. The efficiency of the XAD8 and XAD4 resins was evaluated for a set of four samples for each salinity, measuring A_{250} and fluorescence intensities at 410, 440 and 460 nm for $\lambda_{\text{exc}} = 340 \text{ nm}$, of the influent and effluent of each column.

The desorption was performed by back-elution with 20 ml (\sim three column void volumes; Malcolm et al., 1989) of 0.1 M NaOH.

3. Results and discussion

In the first isolation, the amount of retained DOM was always higher than in subsequent isolations which means that a fraction of the adsorbed DOM was not eluted with the 0.1 M NaOH solution, and that it is strongly retained in the XAD8 and XAD4 resins. Malcolm (1991) referred to the poor recovery of humic solutes from XAD2 and XAD4 ranging

from 75 to 85%. Sorouradin et al. (1993) proposed the use of an alkaline solution of sodium dodecyl sulphate, instead of the traditional 0.1 M NaOH, to enhance desorption efficiency to values greater than 98%. It is well established that XAD4 resins with a pore size of 50 Å are inadequate to isolate DOM with a high molecular weight (Aiken et al., 1979) and, therefore, the fraction of adsorbed DOM on the XAD4 resin must be of low molecular weight.

Table 1 shows that the retention of humic substances on XAD8, measured as A_{250} , decreases linearly with increasing salinity (Pearson correlation coefficient, $r = -0.990$). The same pattern is observed for the XAD4 resin ($r = -0.990$), when adsorption is measured between the influent and effluent of the XAD4 column. In our samples, the increase in the amount of the DOM removed by the introduction of a XAD4 resin depended on the salinity ($r = 0.922$) and ranged from $20.0\% \pm 1.0$ to $25.2\% \pm 3.0$ for freshwater and seawater, respectively. However, the retention of DOM on the system "XAD8 + XAD4" decreased linearly ($r = -0.999$) with increasing salinity, from $89.4\% \pm 1.1$ to $76.7\% \pm 0.5$. The addition of XAD4 to the extraction system only partially compensates the decrease in efficiency of XAD8 removal when the salinity increases.

Table 2 shows a decreasing trend for all emission wavelengths in the amount of FOM, retained on XAD8 resin, with salinity ($r_{\lambda 410} = -0.836$; $r_{\lambda 440} = -0.867$; $r_{\lambda 460} = -0.870$). The DOM that fluoresces at longer wavelengths ($\lambda_{em} = 460$ nm) is more retained in this resin.

The results of Table 3 show that the retention of DOM on XAD4 resin decreases linearly with increasing salinity ($r_{\lambda 410} = -0.927$; $r_{\lambda 440} = -0.913$; $r_{\lambda 460} = -0.904$) when the retention efficiency is cal-

Table 2

FOM from natural water samples, retained on XAD8 resin. The percent (95% confidence intervals) of DOM removal was calculated at the $\lambda_{em} = 410$, 440, and 460 nm, corresponding to the three maxima of the emission spectrum, for $\lambda_{exc} = 340$ nm

Salinity	XAD8 retention (%)		
	$\lambda_{em} = 410$ nm	$\lambda_{em} = 440$ nm	$\lambda_{em} = 460$ nm
0.6	64.6 ± 0.6	66.8 ± 0.6	69.0 ± 0.8
1.8	65.6 ± 0.5	67.5 ± 0.5	69.6 ± 0.6
21.4	63.6 ± 0.6	65.1 ± 0.6	67.3 ± 0.3
33.4	43.2 ± 4.6	51.5 ± 0.5	54.3 ± 0.3

culated on the basis of fluorescence of outlet and inlet of the XAD4 column. The results calculated from the fluorescence intensities of the XAD4 effluent and original sample, do not show a clear linear trend ($r_{\lambda 410} = 0.732$; $r_{\lambda 440} = -0.303$; $r_{\lambda 460} = -0.369$) with salinity, except for the organic matter fluorescent at 410 nm. However, for each sample the removal of DOM on XAD4 is always higher for the organic matter that fluoresces at shorter wavelengths, which is opposite to what we observed on XAD8.

The efficiency of the resin system (XAD8 + XAD4) is increased by slightly over 20% (Table 3) by the addition of XAD4. As also observed for the data in the Table 4, the resin system shows a linear decrease ($r_{\lambda 410} = -0.871$; $r_{\lambda 440} = -0.883$; $r_{\lambda 460} = -0.857$) on the removal of FOM with increasing salinity. As already discussed for the results in Tables 2 and 3 it is interesting to observe that, while XAD8 retains a higher percentage of organic matter fluorescent at longer wavelengths, the XAD4 retains a higher percentage of organic matter fluorescent at shorter wavelengths. However, the data shown in Table 4 demonstrate that the total retention system (XAD8 + XAD4) does not seem to show clear evi-

Table 1

Retention (95% confidence intervals) of humic substances on XAD8 and XAD4 resins, measured as decrease of absorbance intensity at 250 nm (natural water samples)

Salinity	XAD8 retention (%)	XAD4 retention related to the influent of XAD4 (%)	XAD4 retention related to the influent of XAD8 (%)	Total retention (XAD8 + XAD4) (%)
0.6	69.4 ± 0.5	65.2 ± 2.7	20.0 ± 1.0	89.4 ± 1.1
1.8	67.8 ± 0.5	64.6 ± 1.4	20.9 ± 0.5	88.7 ± 0.6
21.4	60.2 ± 2.1	54.7 ± 4.5	21.7 ± 0.8	81.9 ± 2.7
33.4	51.5 ± 3.5	51.8 ± 2.4	25.2 ± 3.0	76.7 ± 0.5

Table 3

Percent of FOM, retained on XAD4 resin after the passage of natural water samples through the XAD8 resin. The retention percent (95% confidence intervals) was calculated with the fluorescence intensity, at $\lambda_{em} = 410, 440$ and 460 nm; and for $\lambda_{exc} = 340$ nm, of outlet of XAD4 column and the inlet of XAD4 or XAD8

Salinity	Retention on XAD4 resin (outlet and inlet of XAD4) (%)			Retention on XAD4 resin (outlet of XAD4 and inlet of XAD8) (%)		
	(a)	(b)	(c)	(a)	(b)	(c)
0.6	73.7 \pm 1.1	72.7 \pm 0.5	74.4 \pm 1.4	26.2 \pm 0.3	24.2 \pm 0.6	23.1 \pm 0.5
1.8	71.4 \pm 1.1	71.2 \pm 0.6	72.0 \pm 0.8	24.6 \pm 0.3	23.2 \pm 0.3	21.9 \pm 0.5
21.4	68.2 \pm 1.0	66.8 \pm 2.2	67.9 \pm 2.9	24.8 \pm 0.3	23.3 \pm 0.5	22.8 \pm 0.8
33.4	56.1 \pm 0.3	48.5 \pm 6.5	47.2 \pm 3.0	31.8 \pm 1.0	23.6 \pm 3.2	21.8 \pm 1.0

(a) $\lambda_{em} = 410$ nm; (b) $\lambda_{em} = 440$ nm; (c) $\lambda_{em} = 460$ nm.

dence of fractionation of FOM since there are no significant differences for the retention of FOM at different wavelengths for the same salinity level.

In order to find out whether the obtained results are influenced by salinity or DOM composition, freshwater from Frei-Gil was treated with different amounts of NaCl to obtain salinities of 1.3, 4.8, 23.6, and 32.3. The influence of other seawater ions was not tested.

The results presented in Table 5 apparently show the same trend as observed for the data of Table 1:

Table 4

Total FOM of natural water samples, retained on the system of the resins, XAD8 followed by XAD4. The retention (95% confidence intervals) was calculated for the $\lambda_{em} = 410, 440$ and 460 nm for an $\lambda_{exc} = 340$ nm excitation

Salinity	Retention on (XAD8 + XAD4) resins, (%)		
	$\lambda_{em} = 410$ nm	$\lambda_{em} = 440$ nm	$\lambda_{em} = 460$ nm
0.6	90.7 \pm 0.8	91.0 \pm 0.2	92.1 \pm 0.8
1.8	90.2 \pm 0.6	90.7 \pm 0.6	91.4 \pm 0.8
21.4	88.4 \pm 0.8	88.4 \pm 1.1	90.1 \pm 1.0
33.4	75.0 \pm 3.8	75.0 \pm 2.9	76.1 \pm 1.4

Table 5

Retention percent on XAD8, XAD4 and (XAD8 + XAD4) resins of the sample with different salinities (amounts of NaCl) and the same organic matter content. The retention percent (95% confidence intervals) was calculated on the base of absorbances at 250 nm wavelength

Salinity	XAD8 retention (%)	XAD4 retention (%)	XAD4 retention related to the original sample (%)	Total retention (XAD4 + XAD8) (%)
1.3	70.7 \pm 3.5	63.9 \pm 2.1	18.8 \pm 1.3	89.6 \pm 2.2
4.8	69.1 \pm 3.3	63.7 \pm 1.1	19.8 \pm 2.1	88.9 \pm 2.5
23.6	66.5 \pm 4.5	60.8 \pm 3.2	20.3 \pm 1.0	86.8 \pm 4.1
32.3	66.1 \pm 4.6	60.4 \pm 3.2	20.5 \pm 0.8	86.6 \pm 4.3

the removal of DOM decreasing with salinity in the XAD8 ($r = -0.965$) and in the system XAD8 + XAD4 ($r = -0.980$), and increasing with XAD4 ($r = 0.886$). However, these results for each resin and for the resins system are not significantly different with an increase in salinity. It is the nature of DOM, associated to different salinities, that plays a major role in the isolation efficiency by XAD resins.

FOM adsorption on XAD8, XAD4 and XAD8 + XAD4 in series was also evaluated using the samples described above, with the salinity increased using NaCl.

The results of Table 6 show a total retention percent of fluorescent ($\lambda_{em} = 410, 440, 460$ nm; $\lambda_{exc} = 340$ nm) organic matter greater than 87.7% with the system of XAD8 and XAD4 resins in series. The XAD4 resin contributes more than 20% of this total. Salinity does not affect the adsorption of FOM on the resins, since no correlation was found between total FOM adsorbed and salinity ($r_{\lambda 410} = -0.437$; $r_{\lambda 440} = -0.190$; $r_{\lambda 460} = -0.538$). Comparison of the results from Table 6 with the freshwater data from Tables 2–4 shows no significant differences. The ionic strength (NaCl) is not an interferent

Table 6
FOM percent (95% confidence intervals) retained on XAD8, XAD4 and XAD8 + XAD4 resins from a sample with different ionic strength (NaCl). The measurements were made at $\lambda_{em} = 410, 440$ and 460nm for an $\lambda_{exc} = 340\text{ nm}$

Salinity	Retention on XAD8 (%)			Retention on XAD4 (%)			Retention on XAD4 related to the original sample (%)			Total retention (XAD8 + XAD4) (%)		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
1.3	68.5 ± 4.3	69.1 ± 1.4	71.5 ± 1.9	72.4 ± 0.6	72.3 ± 1.6	73.4 ± 0.5	22.4 ± 2.5	22.4 ± 1.6	20.9 ± 1.3	90.9 ± 3.3	91.5 ± 3.0	92.5 ± 3.0
4.8	67.6 ± 4.3	68.2 ± 1.9	70.6 ± 2.1	70.7 ± 2.4	70.8 ± 1.6	72.6 ± 1.1	22.4 ± 2.7	22.6 ± 0.8	21.4 ± 0.5	90.0 ± 3.3	90.8 ± 2.5	92.0 ± 1.4
23.6	67.3 ± 4.5	67.8 ± 2.2	69.6 ± 2.7	69.0 ± 0.6	70.0 ± 1.6	70.8 ± 0.6	20.4 ± 3.8	21.9 ± 0.8	21.5 ± 0.8	87.7 ± 1.3	89.7 ± 2.7	91.1 ± 3.2
32.3	68.9 ± 5.3	69.4 ± 3.0	71.2 ± 3.3	70.6 ± 0.8	72.1 ± 1.0	72.1 ± 1.1	21.4 ± 1.3	22.1 ± 1.3	20.8 ± 1.0	90.3 ± 4.6	91.5 ± 4.1	92.0 ± 4.0

(a) $\lambda_{em} = 410\text{ nm}$; (b) $\lambda_{em} = 440\text{ nm}$; (c) $\lambda_{em} = 460\text{ nm}$.

on adsorption of natural FOM on XAD8 and XAD4 resins.

4. Conclusions

For the salinity range $S = 0\text{--}33$, the isolation of DOM, characterised by UV absorbance and fluorescence intensity, is increased by more than 20% using a system of XAD8 and XAD4 resins in series, instead of a XAD8 resin only. These results agree with the range of 17–30% obtained by Santos et al. (1994) for freshwater.

In first instance, salinity is thought to be a ruling parameter in the efficiency of humics isolation with the system XAD4 + XAD8. The data obtained with natural water samples show a decreasing trend of adsorption of humics, measured as UV and fluorescence radiation, with increasing salinity. However, the XAD8 and XAD4 show different behaviour patterns concerning the effects of salinity on the adsorption. While adsorption on XAD8 decreases with salinity, the opposite is found for the XAD4 resin, partially compensating the decrease observed in the XAD8 resin. The DOM that fluoresces at longer wavelengths ($\lambda_{\text{em}} = 460\text{ nm}$) is more retained on XAD8 than the one that fluoresces at lower wavelengths. An opposite trend is observed on XAD4, resulting in a bulk of extracted organic matter more representative of humic substances in terms of fluorescence.

An experiment with a freshwater sample to which NaCl was added has shown that salinity may not be the master parameter to explain the decrease in DOM extracted when salinity increases. No clear trend was observed for adsorption of DOM on the resins by adding different amounts of NaCl to the freshwater sample. The change of nature of the DOM appears to be the main reason for different behaviour of the resins adsorption of organic matter from estuarine waters with different salinities.

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