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Comparison of XAD resins for the isolation of humic substances from seawater

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Abstract

The XAD-2010 and XAD-16 polystyrene based resins have for the first time been used for the isolation of humic substances (HS) from seawater in this study. The adsorption efficiencies and recoveries of HS were compared using batch and column isolation procedures with different XAD resins (XAD-2, -4, -16, -2010 and XAD-4+XAD-2, XAD-2010+XAD-16, XAD-16+XAD-2010). The recoveries of adsorbed HS were evaluated by the comparison of the size-exclusion chromatogram (SEC) peak areas of the seawater sample and of the NaOH effluents from different XAD columns. The batch recoveries of adsorbed HS varied between 35.2±2.4% (XAD-4) and 72.5±5.2% (XAD-2010). The XAD-16 resin had the best adsorption properties (column procedure, recovery 89.5±0.5%) and XAD-2010 the best elution properties for seawater HS. The batch isolation procedure for seawater HS has been worked out.

Keywords: XAD resins; Polystyrene resins; Water analysis; Environmental analysis; Humic substances

1. Introduction

Humic substances (HS) are a complex mixture of coloured organic heterogeneous structures with a wide range of molecular sizes. Both aromatic and aliphatic organic compounds are present with a variety of functional groups, for example, carboxylic and phenolic. HS are refractory end products in the degradation of plant and microbial organic material. HS are operationally defined on the basis of their solubility in water. Humic acid is the fraction of HS that is insoluble at pH<2, whereas fulvic acid is soluble over the whole pH and humins are insoluble at any pH [1–3].

The characterization of water and especially seawater HS has proved to be difficult because of their low concentration and the lack of isolated HS standards [4]. Several isolation procedures have been developed and employed, most of them based on styrene–divinylbenzene and acrylic ester Amberlite XAD resins [1,2,5–11]. Of the alternative methods, isolation based on DEAE anion exchangers is frequently used [12,13]. The XAD method has been used by the International Humic Substances Society for the isolation of standard aquatic HS [6]. The XAD-8 resin has been employed for their standard isolation procedure.

All XAD resins adsorb organic matter mainly by hydrophobic bonding. The exact mechanism of adsorption is still unknown [5]. Among different XAD resins, XAD-2 and XAD-8 have been mostly utilized for the isolation of seawater and aquatic HS [1,5,7,14]. XAD-2 has been favoured for seawater

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HS isolation, but the recovery of the organic fractions varied only from 4 to 31% [4]. In the same study using the XAD-2 adsorption technique the HS isolation efficiency was considered to be below 20% of dissolved organic matter. The isolation efficiency has been improved by using resins XAD-8 and XAD-4 in series [5]. The poor recovery of HS from XAD resins has been explained with interactions between some HS components and resin material. Some of the HS can be trapped onto the resin pores [6]. The adsorption efficiency is influenced by the fact that the hydrophilic compounds present in HS do not adsorb to the resin surface [2,6,9,15].

Size-exclusion chromatography (SEC) has been used for the determination of molecular mass distributions of HS in both aqueous and organic mobile phases. The HS from marine sediments have been separated and characterized by SEC [16]. The HS-form waters have been directly separated and characterized by aqueous SEC [17,18]. As detection techniques, refractive index, UV absorbance and fluorescence are employed. UV absorbance detection has been most frequently used [16,19–21]. Fluorescence detection has been used for isolated fulvic and humic acid characterization in connection with SEC [22] and HPLC [23].

The aim of the present study was to work out an isolation method for seawater HS applicable for the isolation from large volumes of samples. Therefore the adsorption efficiencies and recoveries of HS were compared using batch and column isolation procedures with different XAD resins. The recoveries of adsorbed HS were evaluated by the comparison of the SEC peak areas of the seawater sample and of the NaOH effluents from different XAD columns. In the present study, SEC with fluorescence detection at 350/450 nm (excitation/emission) wavelengths has been used.

Isolation experiments were performed with new XAD-2010 and XAD-16 resins and compared with the XAD-2 and XAD-4. The application of XAD-2010 and XAD-16 resins for HS isolation has not been reported before.

2. Experimental

2.1. Chemicals

Four styrene–divinylbenzene copolymer resins have been used in this study, XAD-2, XAD-4, XAD-16 and XAD-2010, obtained from Sigma–Aldrich (USA). All resins had different pore sizes and surface areas (Table 1). XAD-2010 was not specifically processed or cleaned by the manufacturer and XAD-4 was slightly coloured. Without extensive purification resins cannot be used for isolation experiments. Various cleaning procedures have been reported [1,9–12,14,24,25]. In this study XAD resins were cleaned by first washing in batch with dichloromethane, methanol, 0.2 *M* NaOH and 0.01 *M* HCl. Resins were then packed as water–slurry into 23-ml glass columns and further cleaned using 100 ml dichloromethane, methanol, 0.2 *M* NaOH and 0.01 *M* HCl. Methanol was removed by rinsing with 2 l of Milli-Q water. The cleaning with 0.2 *M* NaOH and 0.01 *M* HCl was repeated until the UV absorbance at 254 nm of the column effluent coincided with the Milli-Q blank. The resin was left in the acid state before isolation experiments.

2.2. Equipment

Glass chromatographic columns of 300 mm, with a volume of 23 ml (H. Jürgens and Co., Germany), with PTFE 3-valves (Bohlender, Germany), fittings and tubing were used for isolation experiments. A Hewlett-Packard diode array spectrophotometer

Table 1
Characteristics of XAD resins (polystyrene structure)

Name	Density dry (g/ml)	Surface area (m^2/g)	Pore diameter (Å)	Mesh size
XAD-2010	–	660	280	20–60
XAD-2	1.07	330	90	20–60
XAD-4	1.08	725	50	20–60
XAD-16	1.02	800	100	20–60

8452A was used for checking the purity of column effluents. The Jasco HPLC system, consisting of a Jasco 880-PU solvent pump, a Jasco 851-AS autosampler and a Jasco 821-FP fluorescence detector, coupled with the BIOSEP-SEC-S2000 size-exclusion column (300×7.5 mm) and guard column (75×7.5 mm) (Phenomenex, USA) were used for the SEC experiments. The column packing consisted of hydrophilic bonded silica, with a particle size 5 μm. The M_r exclusion range for native proteins was according to the manufacturer 1000–300 000. Column efficiency using a protein mixture with 0.1 *M* phosphate buffer, pH 6.8, was 75 900 plates/m.

2.3. Samples

Samples were collected from the Baltic Sea in April 1996 during the expedition with UF *Argos*. The samples were stored in 1-l polyethylene bottles, refrigerated in the dark without any treatment.

2.4. Isolation procedures

2.4.1. Column procedure

A 1-l seawater sample, acidified to pH 2 with HCl (Merck), was passed through the two adsorption columns, both filled with 10 ml of XAD resin at a flow-rate of 0.35 bed volumes/min. The column effluent was collected and neutralized with NaOH for resin adsorption evaluation by SEC. After removal of salts with 100 ml of 0.01 *M* HCl the adsorbed humic substances were eluted with 100 ml of 0.2 *M* NaOH solution. All the NaOH effluent from the column was collected and neutralized with HCl for SEC determinations.

2.4.2. Batch procedure

To the 1-l seawater sample, acidified to pH 2 with HCl, 20 ml of XAD resin were added. In the case of different resins first 10 ml was added. The bottle was shaken for 1 h, then equilibrated for 30 min to allow resin particles to settle. The supernatant was poured into a second 1-l bottle and 10 ml of the different second resin were added. After shaking and settling the water was decanted. A subsample for adsorption evaluation was taken and neutralized with NaOH for SEC. Resins were transferred into columns and, after removal of salts with a 100 ml of 0.01 *M* HCl, the humic substances were eluted with 100 ml of 0.2 *M* NaOH. All the NaOH effluent was collected, neutralized with HCl, and HS were quantified with SEC.

2.5. Quantification of HS

The SEC determinations were performed to evaluate the adsorption of HS to the resins and desorption from resins. The adsorption efficiency from SEC experiments was obtained by first taking chromatograms of natural seawater samples and of the effluent after respective XAD columns. Second, the calculated chromatogram, representing the adsorbed fraction was computed by subtraction of the seawater chromatogram from the effluent chromatogram. The adsorption efficiency was computed by dividing the adsorbed chromatogram peak area with the seawater chromatogram peak area.

The desorption efficiency was obtained by taking chromatograms of natural seawater and the NaOH fraction from the respective XAD column. The peak area of the chromatogram of the NaOH fraction was divided by 10 (100 ml of NaOH was used to desorb HS from 1 l of seawater) and divided with the adsorbed chromatogram peak area (recovery of adsorbed HS) and with the peak area of the seawater chromatogram (recovery related to seawater HS).

The SEC chromatograms were obtained using following procedure: a 100-μl sample was injected into the size-exclusion column, the flow-rate of 0.02 *M* phosphate buffer, pH 6.8, was 0.5 ml/min. Fluorescence at 350/450 nm (excitation/emission) was used for HS detection. The SEC column was calibrated with PSS (polystyrene sulfonate) sodium salts of known molecular masses (American Polymer Standards). PSS had the weight/number average molecular masses of 1430/1200, 4800/4400, 6500/5900 and 16 000/14 500. The total permeation volume of the column (V_t, 11.58 ml) was determined with glucose, the void volume V_0 was 6.43 ml.

Chromatograms were recorded and processed by using HPLC software Borwin (JMBS Developpements, France). Over 1000 data points were integrated in each chromatogram. The total peak area was calculated, including peak areas of all subpeaks.

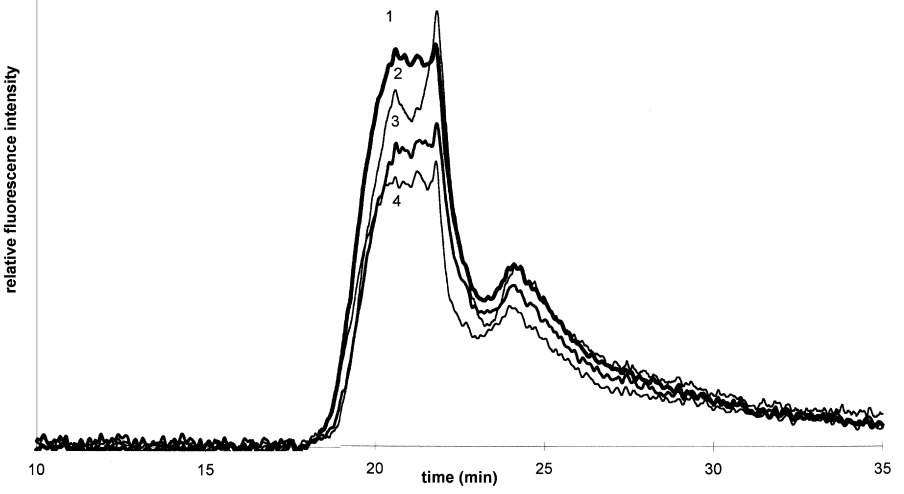

Fig. 1. SEC chromatograms of adsorbed HS. (1) XAD-16; (2) XAD-4+XAD-2; (3) XAD-4; (4) XAD-2. Sample: Baltic Sea, Gotland Deep, salinity 7.17 mg/l, depth 1.5 m. Column, BIOSEP-SEC-S 2000 (300×7.5 mm); eluent, 0.02 *M* phosphate buffer, pH 6.8; flow rate, 0.5 ml/min; fluorescence detection (excitation/emission 350/450 nm).

3. Results

The SEC chromatograms of adsorbed fractions obtained with different XAD resins using batch isolation procedures are presented in Fig. 1. The XAD-16 adsorbed, in the case of batch procedure, up to 70.5±0.2% of HS from water (Table 2). To confirm the significant differences between results obtained, statistical comparison of two experimental means was done using *t*-test. The t_{exp} value was computed using the standard deviations of two means and compared to the t_{theor} value for the 95% confidence level, for certain degrees of freedom. If $t_{exp} > t_{theor}$ the difference was significant.

The adsorption efficiency of XAD resins for batch procedure decreased in the order XAD-16=XAD-2010+XAD-16>XAD-16+XAD-2010=XAD-4+XAD-2>XAD-2010>XAD-4=XAD-2 (equals sign indicates no significant difference for 95% confidence). For the column procedure, the XAD-16 resin had higher adsorption efficiency than XAD-2010, 89.5±0.5 and 82.8±0.6, respectively, with significant difference for 95% confidence.

Examples of SEC chromatograms of HS desorbed with NaOH from different XAD resins are shown in Fig. 2. The highest recovery for adsorbed HS in the case of batch and column procedures was obtained with XAD-2010 resin (Table 2). The recovery of

Table 2
Adsorption efficiencies and recoveries of HS (average±confidence intervals, $\alpha=0.05$, $n=3$), evaluated from SEC chromatogram peak areas, fluorescence detection at the 350/450 nm (excitation/emission)

Resin	Procedure	Adsorbed HS (%)	Recovery (%)	Recovery related to seawater (%)
XAD-2	Batch	55.2±2.1	38.3±3.5	21.1±3.4
XAD-4	Batch	54.4±1.7	35.2±2.4	19.1±2.4
XAD-16 ($n=5$)	Batch	70.5±0.2	53.8±5.2	37.9±5.1
XAD-16	Column	89.5±0.5	47.5±1.4	42.6±1.0
XAD-2010 ($n=5$)	Batch	60.9±3.3	72.5±5.2	44±1.9
XAD-2010	Column	82.8±0.6	72.6±4.3	60±4.3
XAD-4+XAD-2	Batch	63±1.1	45.7±3.0	28.8±2.9
XAD-2010+XAD-16	Batch	68.6±3.2	50±4.9	34±2.2
XAD-16+XAD-2010	Batch	66.1±3.0	55.6±5.2	36±2.7

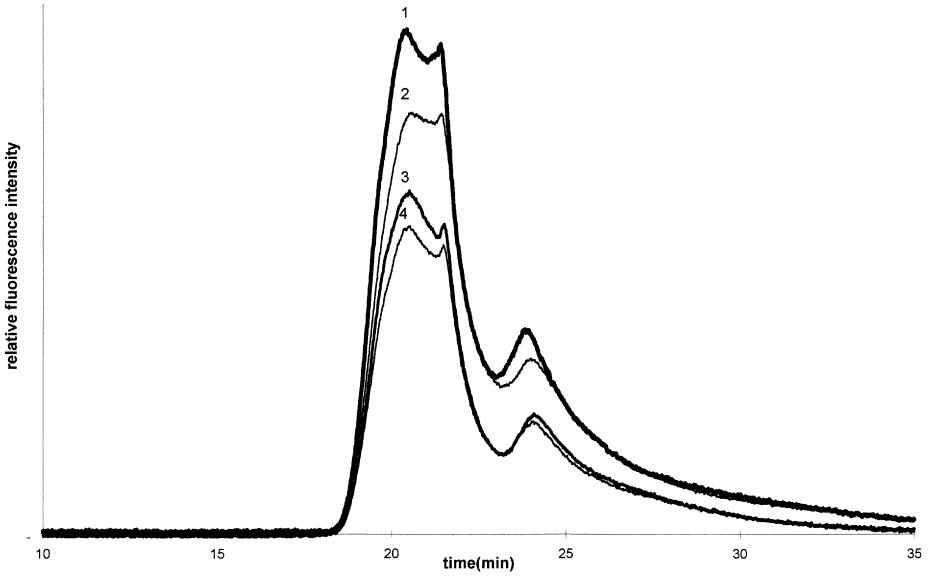

Fig. 2. SEC chromatograms of desorbed HS. (1) XAD-16; (2) XAD-4+XAD-2; (3) XAD-2; (4) XAD-4. Sample: see Fig. 1. Column, BIOSEP-SEC-S 2000 (300×7.5 mm); eluent, 0.02 *M* phosphate buffer, pH 6.8; flow rate, 0.5 ml/min; fluorescence detection (excitation/emission 350/450 nm).

adsorbed HS decreased in the order XAD-2010> XAD-16 + XAD-2010 = XAD-16 = XAD-2010+ XAD-16=XAD-4+XAD-2>XAD-2=XAD-4 (equals sign denotes no statistical difference for 95% confidence). Table 2 presents also the recovery of HS from seawater.

An example of a seawater SEC chromatogram before and after column and batch isolation procedures with XAD-16 resin is presented in Fig. 3. The column procedure is favoured (Table 2). A drawback is that the whole isolation is time consuming and thus not preferred for HS isolation from large volume samples.

A bleed of resin XAD-2010 was noticed at a UV wavelength of 254 nm. To test the possible interference to SEC measurements, the Milli-Q water was treated according to the procedure used for seawater and passed through XAD-2010 and XAD-16 columns. SEC chromatograms recorded at a fluorescence of 350/450 nm indicated baseline. Substances that bleed from XAD-2010 resin did not interfere with SEC determinations with fluorescence detection at 350/450 nm, which is commonly used for HS detection. The XAD-16 absorbance blank at 254 nm was small if compared to XAD-2010, which indicated the absence of resin bleed during the desorption of HS with NaOH.

4. Discussion

All XAD resins used in this study were styrene–divinylbenzene copolymers. They were aromatic, hydrophobic and had no ion-exchange capacity. It has been reported that fulvic acid in ionic state interacts with styrene–divinylbenzene resins by forming $\pi-\pi$ bonds between the aromatic structures of the resin and the HS [2]. The adsorption experiments in the present study were performed at pH 2 when HS were in ionic form. A very efficient adsorption was obtained at lower pH, but there is a possibility of denaturization of fulvic acid [1]. In another paper even pH 2.5 was chosen for soil humic acid isolation and pH 2.0 for isolation from natural waters. The authors state that pH was not lowered under 2.5 to minimize the precipitation and dissolution processes [6]. In the case of XAD-2 it has been found that the risk for denaturization increases with acidity, and the adsorption efficiency only increases by 2% when the pH is lowered from 2.2 to 1.5 [11].

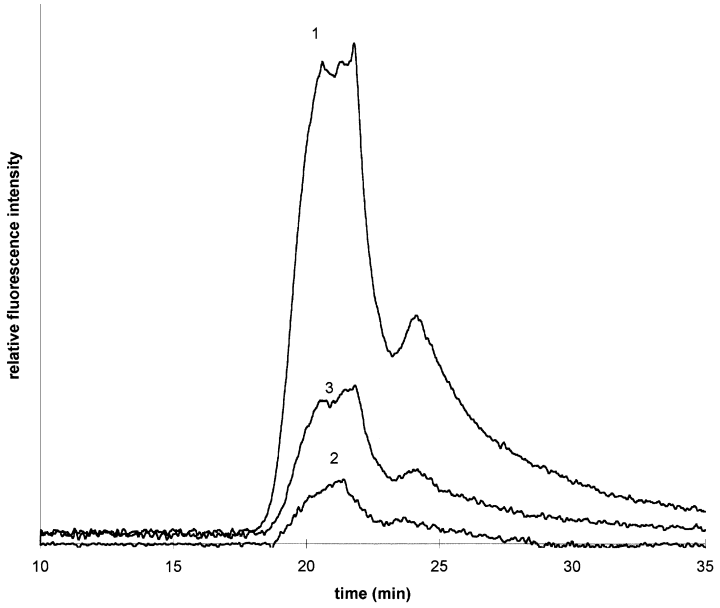

Fig. 3. SEC chromatograms of seawater before and after XAD-16 isolation. (1) Seawater; (2) column procedure; (3) batch procedure. Sample: see Fig. 1. Column, BIOSEP-SEC-S 2000 (300×7.5 mm); eluent, 0.02 *M* phosphate buffer, pH 6.8; flow rate: 0.5 ml/min; fluorescence detection (excitation/emission 350/450 nm).

The effects of other pH values on the adsorption efficiency were not tested in this paper.

The data in Table 2 indicate that complete adsorption and desorption of HS was not achieved with XAD resins used. Part of HS remained irreversibly bound to XAD resins, although 0.2 *M* NaOH was used for elution.

The differences obtained for adsorption efficiency of HS are partly due to the surface area of the resin. XAD-4, XAD-16 and XAD-2010 have all double the surface area of XAD-2 (Table 1). XAD-4 has been reported to have increased adsorption capacity for low-molecular mass acids [1]. The same authors found that XAD-2 had twice the capacity of XAD-4, when fulvic acid adsorption was investigated. The phenomenon has been explained with size-exclusion during the adsorption. The fulvic acid particles have extreme steric restrictions to penetration of pores smaller than 100 Å. In this aspect XAD-4 has the lowest capacity, then XAD-2 and XAD-16 are almost comparable, having pore sizes of 90 and 100 Å, respectively. The XAD-2010 is favoured, with 280 Å, being comparable with XAD-8 acrylic ester resin, not used in this study. The resin pore size explains differences in adsorption and desorption behaviour of all used resins exept XAD-16. This resin had the highest surface area.

The combined columns improved the adsorption and elution efficiency of HS compared to separated columns, in case of XAD-4+XAD-2. The combination of XAD-2010+XAD-16 and XAD-16+XAD-2010 did not improve results compared to XAD-2010 and XAD-16 alone (Table 2).

SEC with fluorescence detection at 350/450 nm (excitation/emission) enabled to detect the molecular mass distributions of natural seawater samples and of isolated HS. It has been stated that isolation with XAD resins irreversibly changes the structure and chemistry of HS [16]. The results of the present study indicated no major differences in molecular mass distributions. The XAD-2010 resin had the best qualities for recovering also high-molecular mass HS fractions. The XAD-2010 bleed problem was not thoroughly investigated or eliminated, the blank subtraction was considered to be sufficient since no interference was observed for fluorescence detection.

5. Conclusions

HS are difficult to isolate from seawater. In comparison with different XAD resins, separately and in combined columns, using batch and column isolation procedures, the XAD-2010 resin is favoured among other resins studied for HS isolation from seawater. This resin had high adsorption efficiency and recovered also the high-molecular mass HS fractions. For obtaining pure HS isolates the blank problem must be solved. The XAD-16 resin had the best adsorption efficiency for seawater HS. Both XAD-2010 and XAD-16 resins had better adsorption/desorption properties than XAD-4 and XAD-2.

The XAD resin isolation did not markedly change the molecular mass distributions of HS as seen from the comparison of SEC chromatograms of natural seawater and of NaOH effluents.

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