Oil Shale, 2001, Vol. 18, No. 3
pp. 239-257

ISSN 0208-189X

CHARACTERIZATION OF BALTIC SEA DISSOLVED ORGANIC MATTER AS OIL SHALE PRECURSOR BY SEPARATION AND FRACTIONATION BY ADSORPTION CHROMATOGRAPHIC XAD METHOD, AND SIZE EXCLUSION CHROMATOGRAPHY

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Dissolved organic matter (DOM) from Baltic Sea water has been isolated and fractionated by adsorption chromatographic XAD method. The XAD-4 and XAD-2 resins were connected in sequence for increasing the adsorption efficiency. The obtained hydrophilic and hydrophobic fractions were characterized by size exclusion chromatography (SEC) with fluorescence detection at 350 nm excitation and 450 nm emission. The percentages of each fraction were estimated using indirect method via size exclusion peak areas. The increased separation efficiency obtained in the present study allowed to separate exclusion peak into several molecular weight fractions. The average molecular weights (weight-average and number-average) were calculated for different size fractions using polystyrene sulfonates as standard substances. The weight-average molecular weight for the fraction of humic substances was found to be characteristic to aquatic fulvic acids.

Introduction

Separation and fractionation of natural organic matter (NOM) are important topics in environmental and analytical sciences because of NOM role in the carbon cycle and of its influence on heavy metal mobility in water systems. The NOM fraction dissolved organic matter (DOM) constitutes the major part, nearly 90 %, of organic matter in seawater. One part of DOM consists of humic substances (HS). This fraction is present in all natural waters where it constitutes between 10–20 % and more than 80 % of dissolved organic carbon (DOC) for open ocean, and river and lake waters, respectively [1, 2]. HS influence groundwater properties and the process of formation of fossil fuels.

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HS form an intermediate phase in the transformation process of living matter (organic carbon), which continue in the organic carbon cycle, or they are deposited as fossil materials. In the environment the transformation processes of HS are influenced by several factors, including plants, fungi, bacteria, plankton, algae, mosses, etc. The organic part in oil shale, kerogen, is known to participate in humus formation. Kerogen is typically enriched in aliphatic carbon, and hence HS originating from kerogen have a greater carbon content and aromaticity than those found in surface waters [3].

The classification of DOM into humic and non-humic substances is operational and based on the isolation method. The Amberlite XAD copolymers (XAD-2, -4, -7, -8, -16 and -2010) have been used for the isolation of humic substances [4–9]. According to the XAD method, the DOM is isolated and fractionated in acidic solutions basing on hydrophobic and hydrophilic interactions between DOM and the surface of the resin. The organic substances that do not interact with resin are referred to as hydrophilic (Hi) and non-humic compounds. The part of DOM that retained onto resin is called hydrophobics. The hydrophobic organic compounds can be further fractionated depending on their elution properties. The exact mechanism of the XAD technique is still unknown, but related to the molecular size of the solutes and to the exclusion properties of the resin.

Historically, XAD-8 resin has been mostly used for isolation and fractionation of DOM [6, 9]. In the case of high-salinity waters, XAD-2 resin had increased adsorption efficiency among other polystyrene resins [10]. The adsorption efficiency of DOM has been improved by connecting different XAD columns in sequences [11, 12], e.g. the recovery obtained by the XAD-7-2-4 column system was up to 65 % of the DOC from a seawater sample.

Although the XAD resin-based fractionation procedure has been used widely in humus chemistry, not much data are available concerning seawater DOM. The studies on Baltic Sea water DOM have focussed on isolation of hydrophobic acid fraction – humic substances – with those resins [13].

Size exclusion chromatography (SEC) is the most convenient and widely used technique for determination of molecular weight distributions of DOM. Molecular weights are estimated with polymer standards, since any proper standard of DOM or HS is not available, with the assumption that the relationship between standards and molecular weight is the same for the standards and samples being analysed. This work has been performed with Biosep SEC column, not widely used for SEC of HS. The only study so far has compared the TSK (Japan) and Biosep columns for characterization of a soil HS [14]. The Biosep column has been applied for aquatic HS characterization by the present author in another study [5].

The objectives of this study were:

i) to employ a modified chromatographic fractionation procedure by applying the XAD-4 and XAD-2 resins connected in sequence for the fractionation of the DOM from different samples of the Baltic Sea

ii) to get information about the molecular size distribution of the eluted various organic fractions by means of the size exclusion chromatography

iii) to estimate the percentages of the various hydrophobic and hydrophilic fractions separated from the DOM of the Baltic Sea

Experimental

Chemicals

XAD-2 and XAD-4 resins were obtained from Sigma-Aldrich (USA). Those polystyrene-based resins had almost the same density (1.07 g/mL) but different pore sizes (330 and 725 m^2/g, respectively) and pore diameters (90 and 50 Å, respectively) according to the manufacturer. The XAD resins were cleaned by washing in batch with dichloromethane, methanol, 0.2M NaOH and 0.01M HCl. The XAD-4 and XAD-2 resins were then packed as water-slurry to the 23-mL glass columns and connected in sequence, and further cleaned by 100 mL of dichloromethane, methanol, 0.2M NaOH and 0.01M HCl. Methanol was removed by rinsing with 2 L of MilliQ water. The cleaning with 0.2M NaOH and 0.01M HCl was repeated until the UV absorbance at 254 nm of the column system effluent coincided with the MilliQ blank. The XAD column system was left in acid state (pH 2) before separation procedures.

Equipment

Glass chromatographic columns of 300 mm with the volume 23 mL and with teflon valves, fittings and tubing were used for separation experiments. SEC was carried out using the Jasco HPLC system, consisting of Jasco 880-PU solvent pump, Jasco 851-AS autosampler and Jasco 821-FP fluorescence detector coupled with the biocompatible BIOSEP-SEC-S2000 column (7.5 × 300 mm, Phenomenex, USA) with a precolumn (7.5 × 75 mm). The column packing consisted of hydrophilic bonded silica with particle size 5 μ and pore size 145 Å. Column efficiency using a protein mixture with 0.1M phosphate buffer, pH 6.8, was 75,900 plates/m. 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 as determined with blue dextran.

Samples

Samples were collected in March 1995 from the Baltic Sea during the expedition with research vessel (RV) Argos. The Baltic Sea is a brackish water

with the salinity of bottom water between 3-15 mgL^{-1}. The Baltic Sea is influenced by freshwater input from surrounding rivers and by seawater input from Atlantic ocean. From each sampling site the water samples were taken from two different depths (the first from *ca* 38 m and the second from near the bottom). Samples were stored in 1-L polyethylene bottles, refrigerated in the dark at 4 °C and prefiltrated through 0.45-mm Millipore filters. Some physical-and-chemical characteristics of seawater samples are shown in Table 1.

Table 1. **Physical-and-Chemical Characteristics of Fractionated Seawater Samples**

Sampling point	Latitude	Longitude	Sampling depth, m	Salinity, mg/L	pH	Oxygen, mg/L	TOC, μM	SEC peak area (AU)
BY1.I	55°00′	13°18′	46	17.9		6.42	151	505,878
BY1.II			31	8.8	8.2	8.75	148	498,909
BY2.I	55°00′	14°05′	44	14.9	8.0	7.73	120	436,679
BY2.II			21	8.2	8.1	8.85	106	404,582
BY4.I	55°23′	15°20′	84	17.1	7.3	0.9	160	527,087
BY4.II			38	7.9	8.1	8.83	124	445,364
BY5.I	55°15′	15°59′	84	16.7	7.3	1.04	192	598,775
BY5.II			40	7.8	8.1	8.77	234	694,210
BCSIII/10.I	55°33.3′	18°24′	80	8.3	7.5	4.87	92	372,948
BCSIII/10.II			52	7.3	8.1	8.86	174	516,974
BY15.I	57°20′	20°03′	208	12.0	7.3	1.72	169	546,923
BY15.II			48	7.2	8.1	8.96	207	633,685

The salinity and oxygen were measured by SMHI (Swedish Hydrological and Meteorological Institute) according to their standard methods. TOC data were supplied by University of Göteborg (Analytical and Marine Chemistry Department) and recorded on a Shimadzu TOC-5000 analyser.

Separation Procedure

Separation and fractionation of DOM was performed as follows. Water samples were acidified to pH 2 with HCl and passed through the column system filled with XAD-4 and XAD-2 resins in sequence (flow rate of 0.35 bed volumes/min). The resins were eluted with different solvents to fractionate adsorbed DOM. The effluent of the XAD column system was named hydrophilic (Hi). DOM adsorbed onto the XAD resins was further fractionated by elution with different solution.

- Step 1: elution with 100 mL 0.01M HCl. This fraction was named as hydrophobic basic (HoB)
- Step 2: 100 mL of 0.2M NaOH was passed through the columns and the hydrophobic acid (HoA) fraction was collected

- Step 3: 100 mL of MilliQ water was passed through the columns and water fraction (HoW) was obtained
- Step 4: elution with 100 mL of methanol. This fraction was named hydrophobic neutral (HoN)

The pH of all the eluted aquatic fractions was immediately adjusted to neutral with NaOH or HCl. The methanol fraction (HoN) was first freeze-dried for evaporation the solvent and then dissolved back in MilliQ water for SEC experiments.

Analysis by SEC

All the DOM fractions collected were analysed by SEC to get information about the molecular size of eluted compounds. The following procedure was used: a 100-μL sample was injected into the size exclusion column, the flow rate of 0.020M phosphate buffer with pH 6.8 was 0.5 mL/min.

The fluorescence at 350/450 nm (excitation/emission) was used for detection. Chromatograms were recorded and processed by using HPLC software Borwin (JMBS Developpements, France).

The SEC column was calibrated using the polystyrene sulfonate sodium salts (NaPSS) (American Polymer Standards Corp.). The PSS standards have narrow relative molecular weight distribution and are characterized by weight – ($\overline{M}_w$) and number-averaged ($\overline{M}_n$) molecular weights. For the calibration the semi-logarithmic relationship between the logarithm of the PSS molecular weight and the retention time was used. The $\overline{M}_w$ and $\overline{M}_n$ for PSS were 1430/1200; 4800/4400; 6500/5900 and 16000/14500. The average molecular weights were calculated using a method where the relative fluorescence intensity after equal retention times was recorded.

The average $\overline{M}_w$ and $\overline{M}_n$ were calculated using the formulas

$$\overline{M}_n = \sum(h_i * M_i) / \sum h_i$$

and

$$\overline{M}_w = \sum(h_i * M_i^2) / \sum h_i * M_i)$$

where h_i is the detector output;

M_i is the molecular weight both on i-th retention time [15].

The results obtained by the used method are to be considered PSS-related average molecular weights because the standards used (PSS) did not have exactly the same conformation and structure as the real seawater DOM.

The percentages of hydrophobic and hydrophilic fractions were calculated using the following formulas:

$$\text{Hi} = (A_{\text{Hi}}/A_{SW}) \times 100\ (\%) \qquad (1)$$

where A_{Hi} and A_{SW} are the total SEC peak areas of the Hi fraction (effluent after the XAD columns) and natural seawater, respectively.

$$Ho = [(A_{Ho}/10)/A_{SW}] \times 100\ (\%) \quad (2)$$

where A_{Ho} is the total SEC peak area of the hydrophobic fraction (either HoB, HoA, HoW or HoN);
10 is the concentration factor (from 1,000 mL seawater to 100 mL fraction volume).

Results and Discussion

XAD Separation

In the present study the XAD-4 and XAD-2 resins have been connected in sequence. This experimental set-up increased the adsorption efficiency of humic substances from seawater up to 65 ± 1.1 % compared to the application of the individual resins, 55.2 ± 2.1 % for XAD-2 and 54.4 ± 1.7 % for XAD-4 [5]. XAD-4 and XAD-2 resins have been reported to be effective for isolation of small molecules with molecular weights below 500 [16]. For isolation of humic substances (HoA) the sorptive power of the XAD-2 resin has been found to be greater than that of XAD-4 [9].

The pore size of the resin in question is the important parameter. Both XAD-2 and XAD-4 have relatively small pore sizes, and for that reason they can exclude some larger-size humic-substance aggregates occurring especially in the fresh water environments. A more detailed discussion about factors influencing the XAD resin separation can be found in the literature [5, 7]. The overall isolation procedure, including the careful cleaning of the resins for obtaining accurate results, is very time-consuming. The acidification of the water samples to pH 2 prior to separation can change some properties of the aquatic DOM. However, it has been reported [17] that this obligatory acidification procedure does not change the fluorescence properties of the marine DOM.

Characterization of DOM by SEC

Fluorescence detection is quite frequent for obtaining more detailed information about humic substances at their natural concentrations in the ocean samples [17]. Two types of fluorescence signals have been observed in seawater: 1) a humic-like which occurs at 420–450 nm at excitation of 230–260 and 320–350 nm, and 2) a protein- or amino acid-like fluorescence [17].

Since humic substances contain a mixture of fluorophores such as aromatic carboxyl and phenolic groups, the fluorescence detection is used in humus chemistry. The number of fluorophores and their individual characteristics are, however, not well-known. The differences between the fluorescence properties of various humic substances originating from different sources may depend on the organic constituents and functional groups forming the humic aggregates. The detection is also sensitive, and a detection

limit of 15 ng has been reported in a fluorescence study of humic substances [18].

It is essential to note that humic substances in seawater consist mainly of fulvic acid. Fulvic acid-type humic aggregates are more aliphatic and their average molecular sizes are significantly smaller than those of their humic acid homologues. Fulvic acids are generally more intensely fluorescent than humic acids. This has been attributed to the highly substituted aromatic structure, higher degree of conjugation, inter- and/or intramolecular bonding or self-quenching of humic acid solutions [19]. It has been reported that the SEC retention times and elution profiles for river-water fulvic acids are similar both for fluorescence and UV detection. The analysis of humic acids revealed the existence of extra peaks at low elution volumes compared to the UV detection. This phenomenon indicates that the fluorescence intensities of humic acids with high molecular weights are lower than those of lower molecular weight humic constituents [20].

Humic substances have anionic groups in their chains, and the stationary phases of SEC columns also have anionic groups on the surface. Therefore the elution of humic substances is affected by ionic exclusion effects. The ionic strengths of the SEC samples in the present study were fairly high but the application of ionic strengths as large as 1.35M has been reported for anionic natural polymers separation with phosphate buffer solutions (pH 8.0).

The low ionic strength, for example 0.003M, is reported to cause elution near the exclusion limit of the column [15]. The early exclusion is due to the ionic exclusion effect between dissociated silanol groups and anionic polymers. With increasing ionic strength, the ionic exclusion effect is suppressed but adsorption effects become remarkable. This effect was observed when the concentration of neutral salt was over 0.1M [21]. Ionic strengths of 0.02 to 0.05M and pH 6.5 to 7.0 have been chosen for SEC elution of humic substances in order to minimize both ionic exclusion and hydrophobic interaction effects [14, 21].

The acidity of the mobile phase and that of the sample solution must be in range of 2–8 because above this pH range the silica-based packing materials will degrade. The acidities of the isolated DOM fractions in the present study were adjusted to neutral (pH 6.8), similar with the buffer solution, being close to acidities characteristic for natural waters. The pH change from 7 to 3.5 has been reported to modify substantially the elution profiles reducing considerably absorbance values and causing variations in elution volumes. The low pH induces the formation of intra- and intermolecular hydrogen bonding between protonated acidic groups and complementary oxygen-containing groups present in humic molecules [22]. This means that under acidic conditions the conformational rearrangements of humic molecules occur, i.e. the original individual constituents will be attached to each other forming larger aggregates causing the decrease of elution volumes.

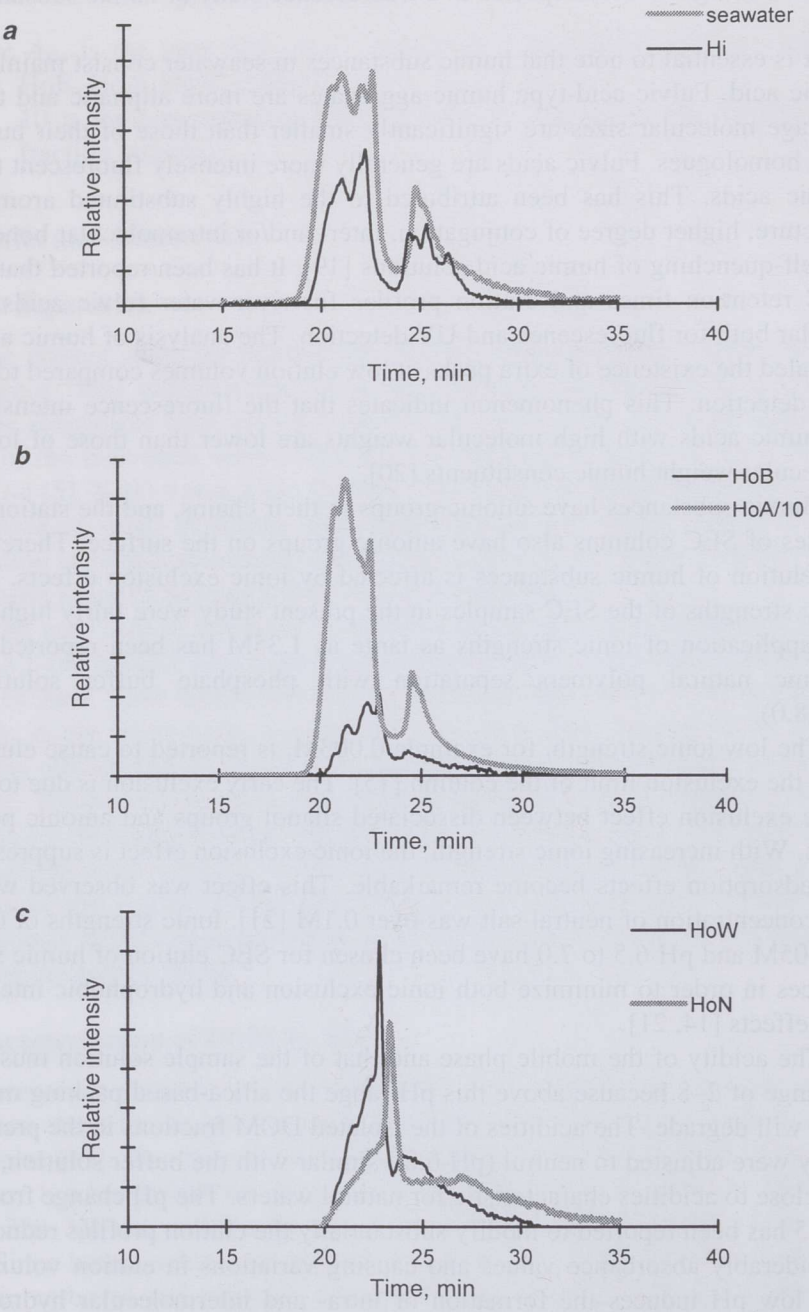

Fig. 1. Size exclusion chromatograms of sea water DOM fractions of BY5.I after XAD-4 + XAD-2 separation; fluorescence detection (λ_{ex} = 350 nm; λ_{em} = 450 nm); *a* – original sample and its hydrophilic (Hi) fraction; *b* – hydrophobic basic (HoB) and hydrophobic acid (HoA) fractions; *c* – water (HoW) and hydrophobic neutral (HoN) fractions

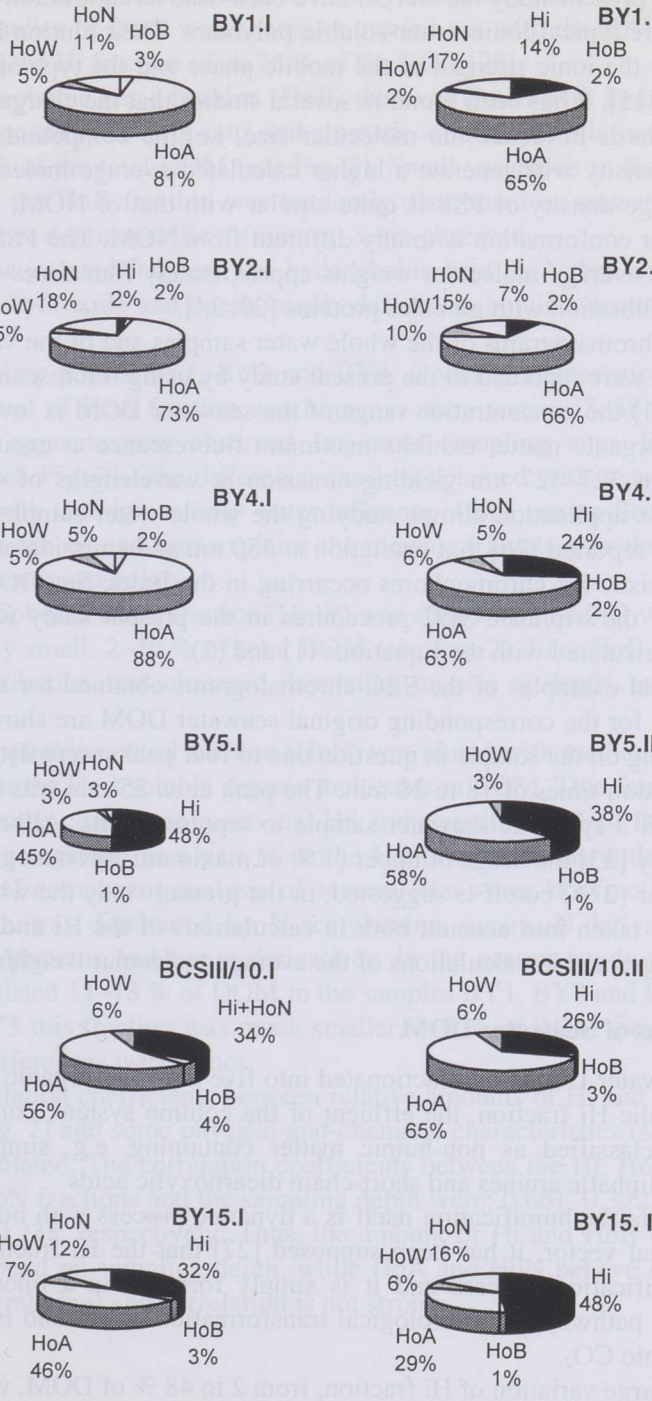

Fig. 2. Percentages of hydrophobic and hydrophilic DOM fractions of the Baltic Sea samples. Indirect estimation *via* size exclusion chromatogram peak areas; for the abbreviations see the text

In the present study the NaPSS have been used as calibration compounds. NaPSS are typical ionic water-soluble polymers. Their elution behavior depends on the ionic strength of the mobile phase and the type of the column packing [15]. It has been found in several studies that the charge densities of the standards influence the molecular size, i.e. the compounds with small charge density will generate a higher calculated average molecular weight. The charge density of PSS is quite similar with that of NOM, however, its molecular conformation is totally different from NOM. The PSS calibration gives the average molecular weights approximately four times smaller than with a calibration with globular proteins [23, 24].

The chromatograms of the whole water samples and of the various DOM fractions were recorded in the present study by using fluorescence detection because 1) the concentration range of the seawater DOM is low, and 2) the aquatic organic matter exhibits maximum fluorescence at excitation wavelengths of 327–427 nm yielding emission at wavelengths of 450–520 nm [25]. This application allows studying the whole water samples directly. It has been reported [26] that excitation at 350 nm and emission at 450 nm are characteristic for chromophores occurring in the Baltic Sea DOM. The precision of the triplicate SEC procedures in the present study was less than 5 % as calculated with the Equations (1) and (2).

Typical examples of the SEC chromatograms obtained for different isolates and for the corresponding original seawater DOM are shown in Fig. 1. Depending on the sample in question one to four peaks were detected within the retention times of 18 to 28 min. The peak at *ca* 25 min was identified by the aid of a synthetic seawater sample to represent salts. Although in a recent study [23] the usage of upper (1 % of maximum chromatograms height) and lower (2 %) cutoff is suggested, in the present study the whole peak areas were taken into account both in calculations of the Hi and Ho fraction percentages and in calculations of the average molecular weights.

Fractions of Seawater DOM

The seawater DOM was fractionated into five different organic classes. The hydrophilic Hi fraction, the effluent of the column system, contained compounds classified as non-humic matter containing, e.g. simple carbohydrates, aliphatic amines and short-chain dicarboxylic acids.

Despite the humification itself is a dynamic process with no unique unidirectional vector, it has been supposed [27] that the Hi fraction indicates the humification process and it is simply formed via a linear, one-way, stepwise pathway of the biological transformation of HS and is at least degraded into CO_2.

The large variation of Hi fraction, from 2 to 48 % of DOM, was observed after data analysis (Fig. 2). Hi fraction was higher at sampling sites BY4, BY5, BCSIII/10 and BY15, which are situated in the central part of the Baltic Sea. Despite the Hi fraction was not further divided into distinct basic

and acid fractions, the results obtained in the present study are in accordance with other studies of fresh water samples carried out with the XAD-8 resin and resulting the Hi fraction of ca 26 % of the DOC [28].

The hydrophobic basic fraction (HoB) that was eluted with hydrochloric acid and supposed to contain salts and aromatic amines was relatively small, about 1–4 % of the total DOM (see Fig. 2). Small variations in the content indicate that the HoB fraction was randomly distributed in seawater, since neither site- nor depth-specific differences were observed.

The major isolated fraction was hydrophobic acid HoA that was eluted with sodium hydroxide and operationally defined as humic substances (Table 2).

For sampling points BY1, BY2 and BY4, it was characteristic that the relative amount of HoA fraction was bigger in deep water, 73–88 % of the DOM. The opposite was observed for other sampling sites BY5 and BCSIII/10 (see Fig. 2). The difference can be explained with seasonal differences or with the high-salinity water influence which causes flocculation of humic substances and thus explains the increased levels near the bottom at the first-mentioned points.

The hydrophobic water fraction (HoW) was eluted with MilliQ water and was relatively small: 2–10 % of total DOM (see Fig. 2). Like HoB fraction, uniform distribution was observed depending on site or depth, except for site BY2.

The hydrophobic neutral fraction (HoN) was eluted with methanol, and it may represent the least soluble organic molecules in DOM. This fraction has been found to be close to conventional HS and constitute 4 % of DOC, and thus has been regarded as a part of acidic HS [8]. The same author states after principal component analysis of several lake-water HS fractions that HoN fraction was dominated by N-acetylamino sugars, N-derivatives of polypeptides/proteins and lignin derivatives. In the present study the HoN fraction consisted 11–18 % of DOM in the samples BY1, BY2 and BY15. In BY4 and BY5 this fraction was much smaller, 3–5 % of DOM (see Fig. 2). The depth differences were minor.

The correlation coefficients between relative amounts of Hi and Ho fractions (see Fig. 2) and some physical-and-chemical characteristics (see Table 1) were calculated. The correlation coefficients between the Hi, HoB, HoA, HoW and HoN fractions and the sampling depth were: 0.661, 0.322, –0.282, 0.121 and –0.238, respectively. Thus, the amount of Hi and HoB fractions seems to depend on sampling depth, while HoA and HoN behave similarly (negative correlation) and correlation is not strong.

Table 2. **Molecular Size Distributions of Different Fractions with Respective Percentages of the Original DOM and Their Averaged Molecular Weights at Different Sampling Sites**

Subpeaks*[1] and averaged molecular weights*[2]	Fraction									
	Hi		HoB		HoA		HoW		HoN	
	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM
BY1.I										
1					1194	23.0				
2			574	1.3	678	43.4	756	1.2		
3			383	0.9			449	1.9		
4			249	0.8	355	14.7	277	1.9	787	11.0
$\overline{M}_w$			**430**		**766**		**457**		**787**	
$\overline{M}_n$			**383**		**650**		**394**		**787**	
BY1.II										
1										
2	Not determined	Σ14 %	662	0.7	859	51.4				
3			412	0.7			388	1.5		
4			256	0.6	336	13.7	190	0.5	655	17.0
$\overline{M}_w$			**453**		**749**		**339**		**655**	
$\overline{M}_n$			**392**		**647**		**308**		**315**	
BY2.I										
1					932	24.5				
2	Not determined	Σ2 %	668	0.6	481	35.6	646	1.3		
3			402	0.8						
4			254	0.7	240	12.8	335	3.7	543	18.0
$\overline{M}_w$			**429**		**590**		**416**		**543**	
$\overline{M}_n$			**372**		**475**		**383**		**543**	

*[1] and *[2]	Hi		HoB		HoA		HoW		HoN	
	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM
BY2.II										
1	Not determined	Σ7 %			962	25.4				
2			663	0.7	450	29.9	789	4.0		
3			368	0.8			383	2.9		
4			228	0.6	156	10.7	244	3.1	346	15.0
$\overline{M}_w$			**426**		**599**		**502**		**346**	
$\overline{M}_n$			**358**		**409**		**395**		**346**	
BY4.I										
1					1020	24.8				
2			669	0.7	551	47				
3			347	0.7						
4			218	0.6	275	16.2	505	5.0	177	5.0
$\overline{M}_w$			**421**		**632**		**505**		**177**	
$\overline{M}_n$			**344**		**522**		**505**		**177**	
BY4.II										
1	Not determined	Σ24 %			1012	23.6				
2			525	0.7	521	29.7				
3			381	0.7						
4			237	0.5	264	9.6	364	6.0	182	5.0
$\overline{M}_w$			**396**		**666**		**364**		**182**	
$\overline{M}_n$			**360**		**539**		**364**		**182**	

Notes: *[1] Numbers 1–4 denote subpeaks of the SEC chromatogram.

*[2] $\overline{M}_w$ and $\overline{M}_n$ are weight- and number-averaged molecular weights calculated for different fractions.

*[3] M is weight-averaged molecular weight proportional to the PSS calibration.

Table 2. Molecular Size Distributions of Different Fractions with Respective Percentages of the Original DOM and Their Averaged Molecular Weights at Different Sampling Sites (end)

Subpeaks*[1] and averaged molecular weights*[2]	Fraction									
	Hi		HoB		HoA		HoW		HoN	
	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM
BY5. I										
1					932	13.2				
2	779	24.4	421	0.3	505	23.3				
3	364	23.6	329	0.4						
4			211	0.3	256	8.6	431	3.0	336	3.0
$\overline{M}_w$	**575**		**321**		**582**		**431**		**336**	
$\overline{M}_n$	**499**		**298**		**480**		**431**		**336**	
BY5.II										
1					1002	22.9				
2	712	18.4	598	0.4	501	25.6				
3	368	10.4	351	0.4						
4	248	9.2	218	0.2	255	9.5	434	3.0		
$\overline{M}_w$	**506**		**423**		**659**		**434**			
$\overline{M}_n$	**417**		**367**		**522**		**434**			
BCSIII/10.I										
1	Not determined	Not determined			1285	18.6			Not determined	
2			460	3.2	685	26.5				
3										
4			175	0.8	346	10.9	306	6.0		
$\overline{M}_w$			**403**		**818**		**306**			
$\overline{M}_n$			**347**		**661**		**306**			

*[1] and *[2]	Hi		HoB		HoA		HoW		HoN	
	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM	M*[3]	% of DOM
BCSIII/10.II										
1					1056	52.3				
2	849	13.7	591	1.5						
3	343	12.3	294	0.8						
4			182	0.6	359	12.7	344	6.0		
$\overline{M}_w$	**610**		**424**		**920**		**344**			
$\overline{M}_n$	**500**		**339**		**766**		**344**			
BY15.I										
1	Not determined	Σ32 %	1043	0.7						
2			723	0.8	773	39.9	939	3.7		
3			459	0.9			467	1.4		
4			294	0.6	301	10.8	299	1.9	479	12.0
$\overline{M}_w$			**633**		**672**		**671**		**479**	
$\overline{M}_n$			**519**		**579**		**527**		**479**	
BY15.II										
1	Not determined	Σ48 %								
2			769	0.3	837	21.4	1040	3.2	665	10.2
3			477	0.4			494	1.0		
4			296	0.3	332	7.6	301	1.9	195	5.8
$\overline{M}_w$			**510**		**705**		**720**		**495**	
$\overline{M}_n$			**446**		**598**		**534**		**355**	

Notes: *[1] Numbers 1–4 denote subpeaks of the SEC chromatogram.

*[2] $\overline{M}_w$ and $\overline{M}_n$ are weight- and number-averaged molecular weights calculated for different fractions.

*[3] M is weight-averaged molecular weight proportional to the PSS calibration.

Fairly good but opposite correlation was obtained with the salinity and the amount of HoA and HoN fractions: 0.452 and –0.442, respectively. The salinity is known to be valuable for water mass analysis and defined as all dissolved ions that contribute to the mass of seawater. High salinity has been reported to cause flocculation of humic acids and the removal of largest molecules [13]. Oxygen content, which is considered to be an indicator for biological and chemical processes, showed good correlation with the amount of HoN fraction (0.662) and fairly good opposite correlation with the Hi fraction (–0.424).

Molecular size distributions were similar for HoB, HoW, and HoN fractions, where average $\overline{M}_w$ and $\overline{M}_n$ values statistically coincided (see Table 2). The HoA fraction differed from other fractions, having $\overline{M}_w$ 700 ± 55 (average for all analysed samples ± confidence, n = 12, α = 0.05) and $\overline{M}_n$ 576 ± 55.

The average molecular weights ($\overline{M}_w$) for HoB and HoW fractions, reported in Table 2, showed fairly good correlation with sampling depth: r = 0.63 for HoB fraction, and r = 0.45 for HoW fraction. No correlation with depth was observed for HoA and HoN fractions. Molecular size characteristics ($\overline{M}_w$ and $\overline{M}_n$) of the HoB and HoW fractions from BY15 sample were clearly different from the characteristics of all other sampling sites. Possibly the physical-and-chemical characteristics of seawater in BY15 influenced the DOM molecular sizes.

The HoA molecular size characteristics were similar for sampling sites BY2, BY4, BY5 and BY15. The fraction from BY1 and BSCIII/10 sites had higher $\overline{M}_w$ and $\overline{M}_n$ values, possibly indicating river water input.

Large variation in $\overline{M}_w$ (177 to 787) was indicated for HoN fraction in general. The samples from the sites BY1, BY2 and BY15 had quite similar sizes but the fraction from BY4 had extremely low $\overline{M}_w$ values (*ca* 180) and showed no dependence on the sampling depth.

Conclusions

1. Increased adsorption efficiency was obtained by the used separation procedure, associated XAD-4 and XAD-2 resin sequence.
2. Improvement in size fractionation of XAD eluted DOM fractions was achieved by the used experimental conditions, thus permitting to resolve the broad SEC peak into four molecular weight fractions.
3. Due to the enhanced detection sensitivity and separation efficiency, new data about Baltic Sea water DOM operational fractions were obtained. Since the knowledge of relative amounts of hydrophilic and hydrophobic fractions is important for understanding the humification process (decay

of organic matter), the indirect estimation method presented in this study is useful for environmental applications.

4. The average molecular weights of HoA/HS fraction were close to those of aquatic fulvic acid.

Acknowledgements

The author is grateful to Prof. M. Wedborg and the members of Analytical and Marine Chemistry Department, University of Göteborg, Sweden, the crew on RV Argos and the Swedish Meteorological and Hydrological Institute for their support, help and some data (TOC).

The scholarships from the Knut and Alice Wallenberg Foundation and the Nordic Council of Ministers and The Swedish Institute and the Council of Science Competence, Estonian Ministry of Education are gratefully acknowledged.

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Presented by J. Kann
Received June 20, 2001