Comparison of DAX-8 and DEAE for Isolation of Humic Substances from Surface Water

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Abstract: Aquatic humic solutes were isolated in parallel by the non-ionie macroporous DAX-8 and diethylaminoethyl cellulose (DEAE-cellulose) resins from Nile river. Non-humic substances and humic substances which were separated from the water samples were determined. In addition, humic and fulvic acids were also determined. Quantitative measurement of aquatic humic substances was carried out by Total Organic Carbon Analyzer (TOC). Generally, concentrations of the Non-humic substances were greater than the humic substances in water samples. The fulvic acid concentration of the water samples examined ranged between 1.36 and 4.67 mg C L⁻¹, while the level of humic acid ranged between 0.72 and 3.04 mg C L⁻¹. DEAE-cellulose gives a useful conventional sorbing solid in the isolation of the bulk of aquatic humic substances over DAX-8.

Key words: Nile river, humic acid, fulvic acid, resins, adsorption

INTRODUCTION

Virtually all water at the surface of the Earth contains organic matter (White, 1998). Organic matter in the environment can be divided into two classes of compounds that are Non-Humic Substances (NHS) such as hydrocarbons, carbohydrates, amino acids, etc. and Humic Substances (HS) (Zouboulis et al., 2003; McDonald et al., 2004). These two groups are not completely, neither physically or chemically, distinguishable from each other, because some natural NHS such as carbohydrates can be an integral part in the structural composition constructing HS (Puravuori et al., 2005). Humic substances described to be composed of chemically complex, non-biochemical organic components, which are largely hydrophilic, amorphous, dark colored and resistant to chemical and biological degradation (McDonald et al., 2004). Humic substances are ubiquitous natural organic materials occurring in huge amounts in soils, sediments and waters as a product of the chemical and biological transformation of animal and plant residues (Péña-Méndez et al., 2005). Humic substances may constitute 95% of the total dissolved organic matter in aquatic system (Koopal et al., 2001; Galambos et al., 2004). Humic substances play an important role in aquatic chemistry and therefore have attracted the attention of the researcher. The presence of humic substances in natural waters can cause various environmental and health problems. For example, humic substances cause water to have undesirable color and taste and they bind with heavy metal ions and other organic substances, facilitating their transportation in the water systems (Liu and Gonzalez, 2000) or removing them from water (Davies and Ghahbour, 1999). Humic substances can be divided into three categories, according to solubility as humic acid (HA), fulvic acid (FA) and humin (MacCarthy, 2001; Puravuori et al., 2005). HA is the fraction of HS that are not soluble in water under acid conditions (below pH 2) but become soluble at greater pH. They are often referred to as being the high molecular weight fraction, with weights being estimated to range from 1500 to 5000 Daltons in streams and from 50,000 to

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500,000 Daltons in soils. FA is the fraction of HS that are soluble under all pH conditions and are referred to as moderate molecular weight substances ranging from 600 to 1000 Daltons in streams and 1000 to 5000 Daltons in soils. The third fraction, Humin, is defined as the fraction that is not soluble in water at any pH value. Determination of Aquatic Humic Substances (AHS) begins by separation of the sample into dissolved (containing AHS) and particulate organic carbon fractions. Although there is no distinct size that separates these two groups, 0.45 μm is used as the compromise between acceptable flow rate and rejection of small colloidal materials (McDonald et al., 2004). The second step of the measurement of AHS is the concentration, isolation or extraction of HS which can be achieved by various methods including vacuum evaporation, precipitation, Liquid-Liquid Extraction (LLE), ultra-filtration, freeze concentration, Solid-Phase Extraction (SPE) (e.g., adsorption XAD resin and adsorption diethylaminoethyl (DEAE) cellulose) and reverse osmosis (Peuravuori and Pihlaja, 2000; Peuravuori et al., 2005). The most frequently applied procedures for isolating HS are the adsorption chromatographic methods by non-ionic sorbing solids (such as XAD resins or analogous solids) and by weakly basic anion exchangers (such as DEAE-cellulose) (Peuravuori et al., 2005). The final step is the quantification of HS by measuring dissolved organic carbon (Standard Methods, 1998). The concentrations of HS in surface and ground waters depend on the concentration of total organic carbon (Aiken et al., 2002; Gadmar et al., 2005). In the present study, two different sorbing solids (namely DAX-8 and DEAE) were applied for isolation, fractionation of humic-type organic solutes from surface water samples.

MATERIALS AND METHODS

A fresh water samples were collected from Nile river in different locations. The water samples were collected between October 2006 and August 2007. The water samples were collected 1 m below the surface in amber organic free glass containers. The original samples were filtered immediately after collection through 0.45 μm membrane filter and thereafter, stored in dark at 4°C during the analysis and isolation procedures. Each sample was divided into three portions: The first portion was acidified to pH 2 and the inorganic carbon content (CO₂) was removed by purging with stream of nitrogen for about 10 min and then the Dissolved Organic Carbon (DOC) of this portion was measured and referred as DOM. The second portion was adjusted to pH 2 and the AHS were isolated using the DAX-8 procedure. The third portion was adjusted to pH 6 and the AHS were isolated using the DEAE procedure.

Isolation and Analysis of AHSs

The adsorption and recovery of AHS from the water samples were compared using column chromatography isolation procedure with DAX-8 resin and DEAE cellulose (Supelco and Sigma-Aldrich, USA). The cleaning and preparation of DAX-8 and DEAE columns have been thoroughly reported previously (Standard Methods, 1998; Tatar et al., 2002; Peuravuori et al., 2005).

Isolation and Fractionation of AHS by DAX-8 Resin

A suitable volume of the pre-filtered portion of the water sample being adjusted to pH 2 was passed through the column at a flow rate of about 2 mL min⁻¹. The column effluent was collected and acidified to pH 2 and labeled as non-humic substances portion (NHS). The CO₂ of this effluent was removed by purging with nitrogen for 10 min. and then the DOC was measured. The adsorbed humic substances were eluted with 30 mL, 0.1 N NaOH at a flow rate of about 1 mL min⁻¹. The elute was acidified to pH 2 and labeled as Humic Substances (HS). Similarly the CO₂ of this elute was removed and the DOC was measured. Another portion of the humic substances elute sample was acidified to pH 1 with HCl and left in dark for 24 h to precipitate humic acid. The precipitated humic acids and the
supernatant fulvic acid solution were separated by filtration through 0.45 μm membrane filter. Similarly the CO₂ of the supernatant was removed and the DOC was measured and referred as FA. The precipitated humic acid was re-dissolved with 0.1 N NaOH, neutralized with HCl and the DOC was measured as the supernatant solution but referred as HA.

Isolation and Fractionation of AHSs by DEAE Cellulose

A suitable volume of the pre-filtered portion of the water sample being adjusted to pH 6 was passed through the column at a flow rate of about 2 mL min⁻¹. The column effluent was collected and acidified to pH 2 and labeled as non-humic substances portion (NHS). The CO₂ of this effluent was removed by purging with nitrogen for 10 min. then the DOC was measured. The column was rinsed with 5 mL bidistilled water (pH 6) and the adsorbed humic substances were eluted with 30 mL 0.1 N NaOH at a flow rate of about 1 mL min⁻¹. The elute was acidified to pH 2 and labeled as Humic Substances (HS). Similarly the CO₂ of this elute was removed and the DOC was measured. Another portion of the humic substances eluate sample was acidified to pH 1 with HCl and left in dark for 24 h to precipitate humic acid. The precipitated humic acid and the supernatant fulvic acid solution were separated by filtration through 0.45 μm membrane filter. Similarly the CO₂ of the supernatant was removed and the DOC was measured and referred as FA. The precipitated humic acid was re-dissolved with 0.1 N NaOH, neutralized with HCl and the DOC was but referred as HA.

Dissolved Organic Carbon (DOC) of all samples was measured using Phoenix 8000 TCC analyzer (Tekmar Dohrmann Company) utilizing persulfate-ultraviolet oxidation technique. Dissolved organic carbon is oxidized to CO₂ by persulfate in the presence of ultraviolet light. The CO₂ produced is measured by a nondispersive infrared (NDIR) analyzer (Standard Methods, 1998).

For quality assurance and quality control, each sample has been analyzed in triplicate. In addition, procedural blanks and standard reference materials were analyzed routinely with each batch of samples. The International Humic Substances Society (IHSS) reference materials of Leonardite humic acid standard (1S104H) and Suwannee river fulvic acid standard (1S101F) were used as control samples. These reference materials used also for recovery of resin and cellulose columns. Organic carbon standard solution (potassium biphthalate C₁₂H₁₀O₆) was used to determine instrument response. Blank corrections were applied by subtracting the values of DOC of bidistilled water blank from that the standard and sample. Mean concentrations and standard deviations (±SD) of DOC values were used as statistical measurements to verify the accuracy of the results. Analysis of variance test (ANOVA) was used to determine the significance variations between both isolation techniques.

RESULTS AND DISCUSSION

The recovery experiments using mixture of IHSS references samples (humic and fulvic acids) were conducted in parallel utilizing DAX-8 resin and DEAE cellulose with conventional chromatographic technique. However, the desorption recoveries of the humic substances mixture were 83 and 96% for the DAX-8 and DEAE columns, respectively.

Dissolved Organic Matters (DOM) in natural waters can be broadly classified into two fractions of hydrophobic (humic) and hydrophilic (non-humic) substances (Owen et al., 1995; Burdon, 2001; Baker and Khalili, 2005; Zazouli et al., 2007). The structure and concentrations of these two fractions in natural waters are contrasted due to natural ecosystem differences and the human activities. In the present study, the ability of sorbing solids, DAX-8 resin and DEAE cellulose, to isolate and divide the original DOM into humic (HS) and non-humic (NHS) substances is similar. In general, the concentrations of the NHS isolated by DAX-8 and DEAE were higher than HS. The results in Table 1 show that the average concentration of NHS isolated by DAX-8 and DEAE accounted for 59 and 50% of the original DOM, respectively. While the average concentration of HS constituted
Table 1: Concentrations of natural organic matter (mg L\(^{-1}\) DOC) and percentage distribution in water samples form Nile river

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Original sample</th>
<th>Non-humic substances (NHS)</th>
<th>Humic substances (HS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Range</td>
</tr>
<tr>
<td>DXA-8 resin</td>
<td>8.38±16.92</td>
<td>11.86±3.50</td>
<td>4.46±9.90</td>
</tr>
<tr>
<td>DEAE cellulose</td>
<td>8.38±16.92</td>
<td>11.86±3.50</td>
<td>4.20±8.87</td>
</tr>
</tbody>
</table>

Percentage distribution of NHS and HS of original water samples (DOM)

Table 2: Concentrations of humic and fulvic acids (mg L\(^{-1}\) DOC) and percentage distribution in water samples from Nile river

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Humic acid (HA)</th>
<th>Fulvic acid (FA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>DXA-8 resin</td>
<td>0.73±2.82</td>
<td>1.45±0.82</td>
</tr>
<tr>
<td>DEAE Celulose</td>
<td>0.72±3.04</td>
<td>1.88±0.73</td>
</tr>
</tbody>
</table>

Percentage distribution of HA and FA of original water sample (DOM)

32 and 48% of the original DOM for DAX-8 and DEAE, respectively. Although, the efficiency of DAX-8 to isolate higher proportion of NHS than DEAE, but without significant difference (p>0.05). It can be noted that water from large rivers like Mississippi river (Semmens and Staples, 1986) the Colorado river (Collins et al., 1986), the Seine river (Legube et al., 1990) Mayenne and Loire rivers (Croué et al., 1993) was found to contain less hydrophobics (humic) than hydrophilics (non-humic) such as the case of Nile river in the current study.

Table 1 shows the high retaining capacity of the DEAE cellulose for HS was 16% more than with DAX-8 resin. This is of the same magnitude as reported previously (Peuravuori et al., 2002, 2005). It seems that the concentration of HS isolated by DEAE was relatively higher that those isolated by DAX-8 but without significant difference (p>0.05). It has been verified (Peuravuori et al., 1997, 2001) by fine-structural analyses, that the integrated whole of macromolecular organic acids isolated by the DEAE technique resembles both qualitatively and quantitatively very closely to an average combination of the four different acidic fractions obtained by the multi-stage DAX-XAD procedure. This is an essential advantage from the structural chemistry point of view and shows that the DEAE isolate represents a clear-cut average HS for fresh water.

The statement of high binding capacity of the DEAE cellulose for HS over the DAX-8 resin has been verified in the present study. The DAX-8 column was able to isolate the NHS fraction significantly more than HS fraction (p<0.05). In contrast, HS fraction was isolated not significantly but slightly 2% less than NHS fraction by DEAE column (p>0.05).

Table 2 shows the concentrations of humic and fulvic acid (HA and FA) isolated by DAX-8 resin and DEAE cellulose chromatographic columns. The concentrations of FA were about 1.5-2 times higher than those of HA in both sorbing solids. HA fraction obtained by DAX-8 and DEAE accounted for 12 and 16%, respectively of the original DOM. Whereas, the FA fraction predominated in all water sample, accounting for 18 and 29% of the original DOM with DAX-8 and DEAE, respectively. It has been verified (Yamada et al., 2000; Huizhong et al., 2001) that the predominance of FA fraction over HA one in the natural water isolated by different sorbing solids. Again, the higher levels of HA and FA fraction obtained by DEAE cellulose than those obtained by DAX-8 resin proves the high retaining capacity of the DEAE.

Surprisingly, a significant correlation between the original DOM and HA levels with both sorbing solids was noted (p<0.05). In contrast, the correlation between the original DOM and FA levels was not significant (p>0.05) as shown in Fig. 1. Since this study is performed on the same water samples and the FA exhibited higher levels than HA, the only possible explanation might be due to the results are caused by some methodical bias related to the concentration of both fractions.
Fig. 1: Correlation between DOM and HS (A: HA and FA with DXA-8 resin; B: HA and FA with DEAE cellulose)

Generally, the problems of analytical determination of concentration of aquatic humic substances in natural water are closely related to their properties and the lack of strict boundaries between humic and non-humic substances. Also distinction between humic and fulvic acids is causing problem due to the complex chemical properties, analytical problems, sensitivity and interferences. It is evident that no ideal system is available for isolating pure hypothetical humic substances from water sample, as shown in this study and also verified previously (Klavins et al., 1999; Huizhong et al., 2001; Peuravuori et al., 2005).

Unless substantial efforts to develop analytical methods for isolation and determination of humic substances in aquatic environments, the problem still is open and versatile, efficient and unified methods are needed.

CONCLUSION

It is evident that no ideal system is available for isolating pure hypothetical humic substances from a water sample. According to this study the DEAE cellulose serves as a practical choice for isolating humic substances from fresh water better than DAX-8 resin. The advantage of DEAE cellulose is that adjustment of the original acidity of the water sample is not needed.

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REFERENCES


