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# Direct Gel Chromatographic Characterization and Quantification of Marine Dissolved Organic Carbon Using High-Sensitivity DOC Detection

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## *Introduction*

A special issue of *Marine Chemistry* on the analysis of dissolved organically bound carbon (DOC) and dissolved organically bound nitrogen (DON) in seawater (*1*) has motivated us to apply a high-sensitivity gel filtration liquid chromatographic system with continuous DOC detection for the characterization and quantification of DOC in seawater.

The advantages of on-line chromatographic DOC detection are obvious: analysis of organic matter is virtually carried out matrix-free because the gel filtration mechanism separates the inorganic salts from the organic matter to a large extent. Interferences occur only for that small organic fraction that coelutes with the inorganic salts. The different organic fractions reflect groups of compounds with distinct chemical properties. This can be used for the characterization of organic matter.

The system allows the direct chromatographic analysis of organic matter in 1 mL of seawater without pretreatment.

In 1983, an older design of the DOC detector—but without chromatographic fractionation—was used for the direct analysis of seawater DOC (*2*).

## *Materials and Methods*

The objective of this research was to investigate the applicability of a chromatographic system with DOC detection to marine samples. Our intention was neither to study marine systems nor to give details that could be used for comparison of our results with existing data obtained by other methods. Therefore, we can supply only the following information: sampling took place in July 1993 in the Mediterranean Sea. We received three seawater samples that came from the same collection batch from a depth of about 20 m. The samples had been glass-filtered on the vessel and had been treated in the following way: one was left unaltered, one was acidified to a pH of 2 with phosphoric acid, and one was stabilized with mercury chloride to suppress biological activity. The mercury concentration was later determined by hydride atomic absorption spectrometry to be 82 mg/L. The samples arrived in the laboratory in 100-mL brown glass flasks with hard plastic caps. They had been shipped by surface mail and thus had been exposed to normal temperatures for probably 1 week. Thereafter, the samples were kept at 4 °C for 2–3 weeks until analysis. Aging processes are likely to have taken place. This was not necessarily a disadvantage, because method development requires sample "stability" rather than sample "authenticity".

## *Analytical System*

The DOC detector and the chromatographic system were described before (*3*, *4*). The separation of organic and inorganic carbon is carried out on-line in a rotating thin-film reactor with a central low-pressure mercury lamp. Both species are quantified simultaneously with two infrared detectors in two separate reactor outlets. No external oxidizing agents are used for the oxidation process: active oxygen is produced in an anoxic nitrogen atmosphere by UV radiolysis of water.

An outline of the system with a total of five detection units is given in Figure 1. In our study, detectors 1, 2, and 4 were used.

**Chromatographic Conditions.** Column: TSK HW 40 (S), 21-cm length, 1.6-cm diameter, temperature 24 °C ± 1%. Elution: isocratic (1 mL/min) with phosphate-buffered mobile phase (pH 6.58, 28 mmol/L) of UV-irradiated double-distilled water. Sample: injection volume of 1.5 mL (loop injection with reduced pressure). Detection: simultaneous detection of UV (210/254/350 nm, Linear Instruments, USA, detector no. 1); fluorescence (ex 258 nm/em 430 nm, Linear Instruments, USA, detector no. 2), DIC ("dissolved inorganic carbon", Siemens Ultramat 3, Germany, detector no. 3), DOC ("dissolved organic carbon", Siemens Ultramat 3, Germany, detector no. 4), and UV active oxidation products after irradiation in the thin-film reactor (210/254 nm, Linear Instruments, USA, detector no. 5).

## *Results and Discussion*

In Figure 2, the results for the untreated seawater sample with the detection of DOC (detector no. 2), UV (detector no. 1), and inorganic carbon (DIC) (detector no. 3) are shown. At the top of the figure, a chromatogram for molecular mass calibration (polyethylene glycols, oligosaccharides, and methanol) is included (*5*). The exclusion limit is around 4000 g/mol, and the permeation limit (methanol) is around 30 g/mol according to this calibration. For DOC detection, the integration area for total quantification of chromatographic DOC is graphically enhanced. A value of 1170 μg/L DOC was found. The dominant fraction of DOC eluted in the high molecular mass range close to the exclusion limit.

For UV detection, only one peak was found with extreme "fronting" elution behavior. By comparison, this peak was found to be derived mainly from sodium chloride, but nitrate may have contributed as well. In the DOC chromatogram, a signal depression, or "trough", can be seen at the corresponding retention time (arrow). It is assumed that sodium chloride acted as a "scavenger" of radicals and prevented the complete oxidation of DOC during this stage of analysis (*6*), resulting in a negative response in the infrared detector. We have described this phenomenon before (*4*).

The high molecular mass material is essentially aliphatic. A UV/DOC ratio was found which was about 20 times lower than that of a standard fulvic acid (IHSS Standard Stream Fulvic Acid). A moderately UV-absorbing double-peak appeared between 28 and 30 min (see inlet in Figure

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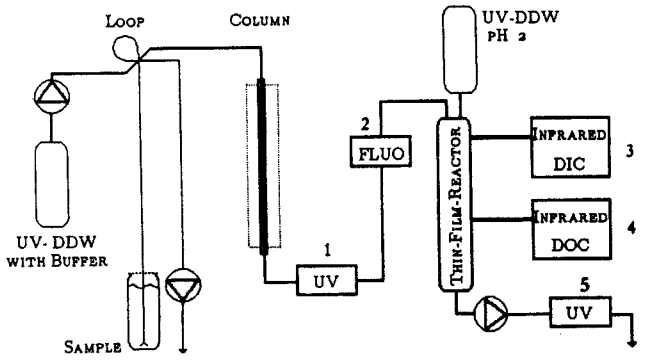


**Figure 1.** Schematic diagram of the liquid chromatographic system with a total of five detectors (UV-DDW: UV-irradiated double-distilled water).

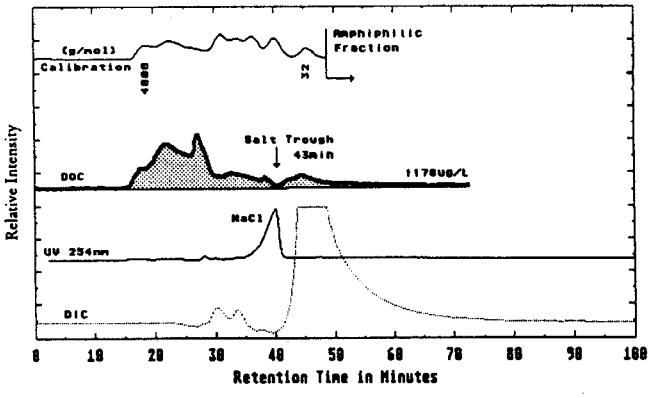


**Figure 2.** Chromatogram of the untreated seawater with the detection of DOC, UV, and DIC.

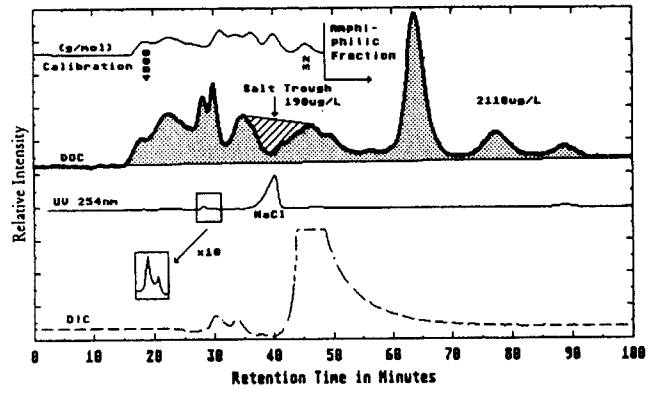


**Figure 3.** Chromatogram of the seawater stabilized with mercury chloride. Detection of DOC, UV, and DIC.

3). This double-peak corresponded well with a double-peak in the DOC chromatogram, with the second peak appearing as a shoulder due to signal broadening in the DOC detector.

Fluorescence did not show pronounced signal responses and, therefore, was not included in Figure 2. At the retention time of the chloride/nitrate peak, a negative response was found, presumably due to quenching effects. For DIC detection, several signals were found which could not be interpreted at the present. Hydrogen carbonate elutes around 45 min and corresponds well with the large (capped) peak.

In Figure 3, the results for the same sample stabilized with mercury chloride are shown. DOC detection showed a more structured chromatogram with longer retention times for some substances. Total DOC was much higher than in the untreated sample and was determined to be

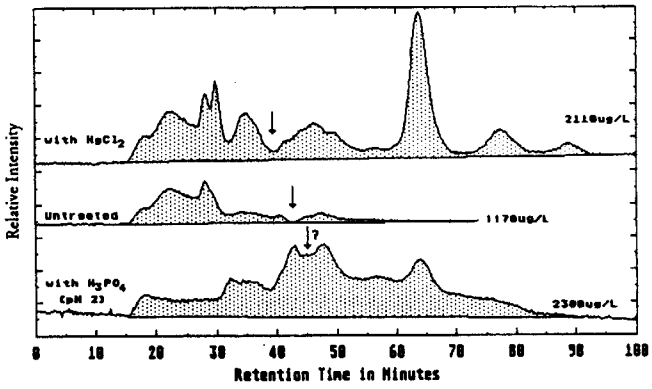


**Figure 4.** Impact of sample pretreatment on gel chromatographic elution behavior of DOC (DOC detection).

around 2110 μg/L. According to the calibration, a large portion of the DOC eluted beyond the permeation limit of the column at around 50 min. This fraction can be characterized as slightly hydrophobic or amphiphilic, because this DOC must have interacted with the stationary phase. The true molecular masses are presumably in the low molecular mass range because peak shapes are good and show no significant peak broadening. The salt trough is more pronounced this time and was shifted by about 3 min. The area described by the trough corresponds to a concentration of 190 μg/L DOC. Total DOC rises to 2300 μg/L if this area is included. For the other detectors, responses similar to those for the untreated water sample were found.

In comparing the stabilized sample with the untreated sample (Figure 2), one is tempted to assume that a significant portion of DOC was not stable and had disappeared in the original sample. This unstable DOC was of low molecular, amphiphilic origin.

The chromatograms obtained for the acidified seawater sample differ strongly from those of the first two samples. In Figure 4, the results for DOC detection are shown, together with the chromatograms obtained for the untreated water and the mercury-stabilized water. Total DOC was found to be around 2330 μg/L and, thus, is similar to the mercury-stabilized sample.

A shift of the high molecular mass fraction toward lower masses was found. Several explanations seem to be viable: (a) The initial high molecular mass fraction was composed of compounds with weak acidic functional groups which were protonated by acidification and hence exerted a "hydrophobic interaction" force with the stationary phase. (b) Acid hydrolysis resulted in a partial breakdown of this fraction. (c) Contraction of humic-like substances as a result of the decrease in negatively charged functional groups caused a decrease in average sizes.

The salt trough is obliterated, which could be the reason for the slightly higher DOC value. For the mercury-stabilized sample, a very similar DOC value (2300 μg/L) was obtained when the area beneath the salt trough (190 μg/L) was included in the total area integration (see Figure 3).

An experiment was carried out in order to obtain information on the photooxidizability of seawater DOC. A total of 50 mL of the untreated seawater was placed in a small UV batch reactor made of high-purity quartz glass and was exposed to low-pressure Hg UV irradiation for 50 and 100 h.

The results are shown in Figure 5. Irradiation led to a

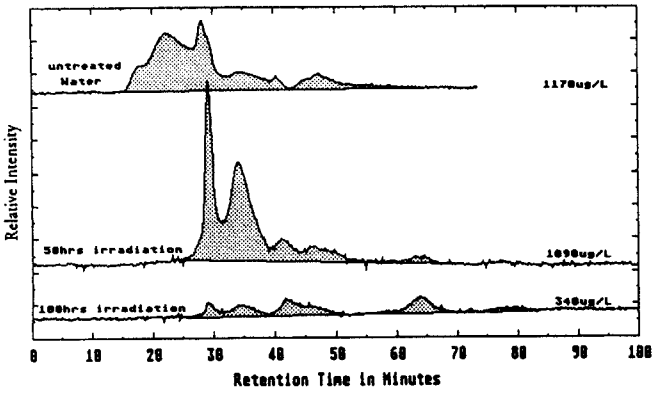


**Figure 5.** Oxidation of the original sample in a small UV batch reactor for 50 and 100 h.

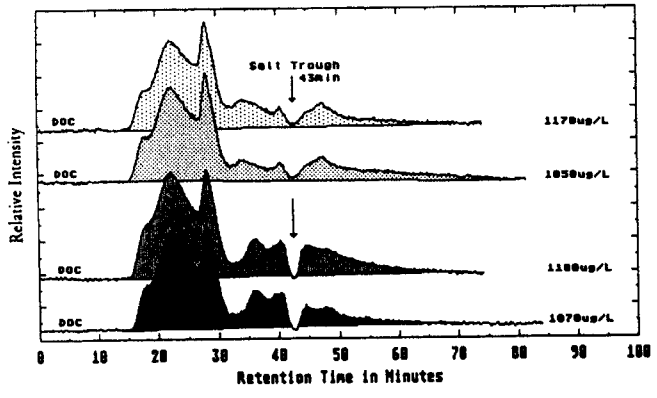


**Figure 6.** Four measurements of the untreated seawater sample (DOC detection). The lower two chromatograms were obtained after the addition of persulfate to the acidification flow of the DOC detector.

complete reorganization and a partial destruction of high molecular mass organic material. After 50 h, all high molecular mass material was broken down into smaller molecules, but the total DOC did not decrease significantly. After 100 h, a large part of this restructured material was completely oxidized.

### *Reproducibility*

In Figure 6, the results for four repetitive analyses of the untreated seawater sample are shown. The calculated DOC values are roughly within an error of about 10%. The lower two chromatograms are distinctively different, especially in the low molecular mass region. The salt troughs are deeper than in the upper two chromatograms. The reason for this is that an oxidizing agent (persulfate, 1 mg/L) has been added to the acidification flow of the DOC reactor (*4*). This had obviously no positive effect on the recovery of DOC, but has effectuated a rise in the baseline due to persulfate contamination with organics (about 100 μg/L DOC). The elevated baseline led to a more pronounced "erosion" of the trough. Comparing the two sets of chromatograms, one can see that the signals before and after the troughs are distinctively different. Thus, a primarily operational quality should be assigned to these areas.

For the mercury-stabilized samples (Figure 7), the reproducibility was similar but significantly lower for the lower two chromatograms, which again were obtained by adding the oxidizing agent. It is likely that the deeper salt troughs here are the cause for the lower DOC recoveries.

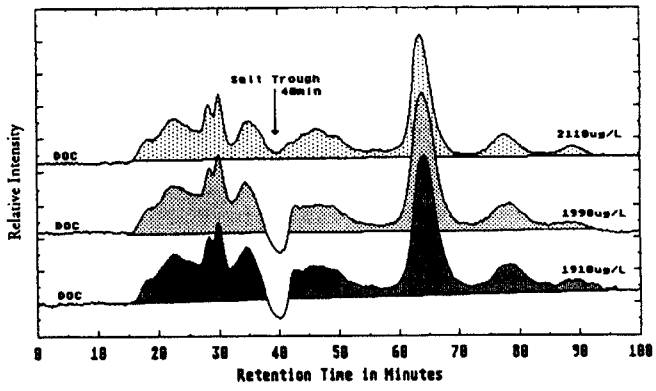


**Figure 7.** Three chromatograms of the mercury-stabilized seawater sample (DOC detection). The lower two chromatograms were obtained after the addition of persulfate to the acidification flow of the DOC detector.

### *Research Needs*

We have presented some results from a 1-week measuring session. Some effects have been encountered which need further clarification before routine analysis can be envisaged.

Reproducibility was not good at the beginning. We assumed that this was due to memory effects, and we assumed that reproducibility should improve with time. This was in fact the case, but with the beginning of the analyses of the mercury-stabilized sample, reproducibility deteriorated again. The baseline sometimes showed poorly reproducible undulations for the DOC and UV detectors (210 nm), but without clear correlation. The baseline stabilized only after extended measuring times (3–10 h). We came to the conclusion that severe memory effects, originating from complexation reactions of mercury with chloride, and/or slow ion-exchange processes of mercury with cations fixed on the stationary phase affected the results. The interactions are documented by a shift of the salt trough (see Figure 4). The precise processes have to be studied in more detail.

Further research needs include the evaluation of potential complexation reactions of mercury with the organic matter itself and the assessment of potential organic impurities brought into the system by the addition of the mercury salt and the phosphoric acid. Another source of contamination may be salt-induced "column bleeding". However, we expect that column bleeding may not by a major contributor to DOC. This can be derived from Figure 5, in which the organic matter in the 100-h oxidized seawater sample was composed of several distinct and attributable fractions with an overall DOC value of only 340 μg/L.

### *Conclusions*

The results show that gel filtration chromatography with high-sensitivity DOC and UV detection can be used for the characterization and quantification of seawater dissolved organic matter. Depending on the kind of sample pretreatment, marked differences in the quantity and the quality of organic matter were found.

Total DOC in the unpreserved sample was around 1 mg/L. The acidified sample and the sample stabilized with mercury chloride showed DOC values around 2.3 mg/L.

The major part of DOC is primarily aliphatic and of high molecular mass origin. This is evident from the elution behavior, from the UV detection responses, and from the oxidation experiments.

The mercury-stabilized sample showed high amounts of new, amphiphilic, low molecular mass organic matter. This material could have been material which was lost in the original water during storage. However at the present, it is not clear whether this new material is authentic material, whether it has been hydrophobicized by mercury, or whether it could have originated from the mercury salt itself.

The acidified sample showed a very different elution behavior with a general shift toward lower molecular masses. Acidic hydrolytic breakdown of high molecular mass compounds, contraction, and/or protonation of acidic functional groups are assumed to have influenced the results. DOC recoveries were comparable to those obtained for the mercury-stabilized sample (2330 $\mu$g/L versus 2300 $\mu$g/L).

The spectrometric detection systems (UV, fluorescence) showed generally weak responses for organic matter. The only exception was a pronounced double-peak in the apparent middle molecular mass range that showed moderate absorption in the UV range. From these results, we got the general impression that spectrometric detection systems are not very useful for the characterization of the bulk of marine organic matter. DOC detection seems to be a promising approach.

Chromatographic reproducibility was reasonable, but it should be possible to improve this by replacing isocratic elution with pH- or salt-controlled gradient elution. Moreover, this would probably solve problems with the definition of the uncharacteristic upper integration limit.

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