Preliminary results on low molecular weight organic substances dissolved in the waters of the Gulf of Gdańsk

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Abstract

The content of low molecular weight LMW (< 1000 Da) dissolved organic substances was determined by ultrafiltration (concentration factor of 2) in different water samples collected in the Gulf of Gdańsk. The proportion of this fraction (based on organic carbon concentration) ranged from 24 to 57%. The lowest percentage was detected in riverine samples. The DOC concentrations in ultrafiltrates was similar in all the samples analysed. The absorbance proportion (at 250 nm) due to the low molecular fraction in the overall absorbance ranged from 14 to 45% and in all but one sample was lower than the DOC percentage. There was no relationship between DOC and absorbance in the LMW fractions ($r^2 = 0.08$), in contrast to the characteristics of the 'bulk' samples ($r^2 = 0.88$).

1. Introduction

The molecular size of the operationally defined dissolved organic matter (DOM) in seawater is used for separating it into the 'truly' dissolved

The complete text of the paper is available at http://www.iopan.gda.pl/oceanologia/

(low molecular weight – LMW) and colloidal fractions (Guo et al. 1994). Clearly, various proportions of these fractions influence the biogeochemical behaviour of DOM in the aquatic environment. For example, the LMW fraction, in contrast to colloids, is not subject to flocculation and subsequent sedimentation. Recent investigations into the bioavailability of DOM have demonstrated significant differences between LMW and the high molecular weight fractions (Amon & Benner 1994, Amon & Benner 1996, McCarthy et al. 1996, Skoog & Benner 1997). Thus, a knowledge of the molecular weight distribution of organic matter may be of some use in predicting (or modelling) its fate in the environment.

The aim of this research was to assess the relative and absolute quantities of the low molecular weight (LMW) fraction of organic matter in coastal and river water. An ultrafiltration technique with a nominal 1000 Dalton cut-off filter (~1 nm) was used to separate it from the bulk dissolved organic mater (DOM). The 'dissolved' matter is defined here as the size fraction passing through a 0.2 μm pore diameter filter. Since the Gulf of Gdańsk is dominated by riverine input, the sampling sites were selected with the intention of obtaining samples variously affected by organic matter originating from river water. In this paper we present preliminary data on the concentration and percentage of the LMW fraction in DOM from Gulf of Gdańsk water (expressed in dissolved organic carbon units). Additionally, the absorbance of both the bulk samples and their ultrafiltrates (comprising low molecular weight substances) was measured in order to evaluate the relationship between absorbance and DOC.

2. Methods

The water samples were collected from on board the r/v 'Oceanograf II' of Gdańsk University during cruises in May 2000. The sampler used was a Close–Open–Close type bottle (volume 3 dm^3, HydroBios Gmbh, Germany), which opens automatically under pressure at 10 m depth and is closed at the sampling depth by a 'messenger' sent down the wire. River water samples were taken with a polyethylene pail from the Świbno-Mikoszewo ferry across the Vistula (Wisła), in midstream, approximately 3 km from the river mouth. Fig. 1 shows the location of the sampling points.

The samples were immediately passed through pre-combusted (450°C, 12 h) Whatman GF/F glass fibre filters, the filtered water then being stored in a pre-combusted 250 ml glass bottle. Before being filled, each bottle was rinsed with the filtered sample water it was to contain. The samples were stored in a refrigerator (4°C), after which they were vacuum-filtered through 0.2 μm Millipore filters. Prior to use, these filters had been rinsed

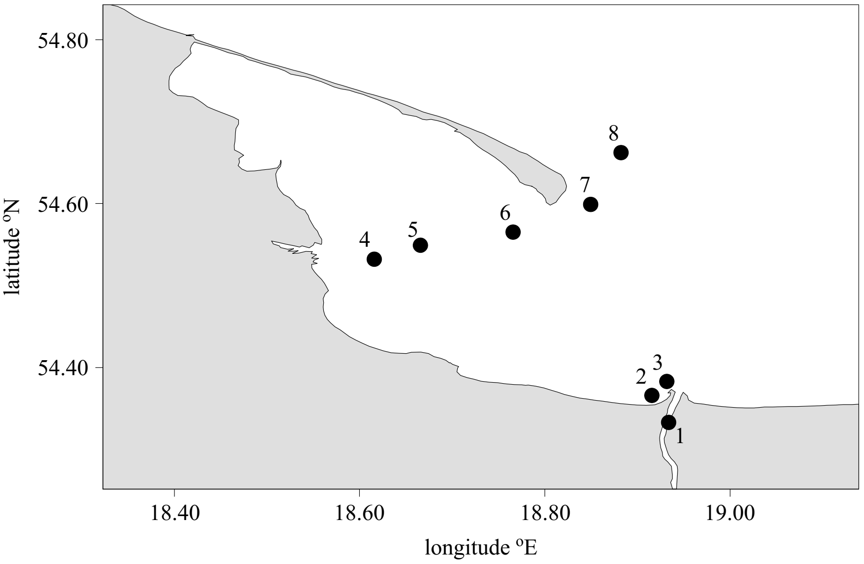

Fig. 1. Map of the Gulf of Gdańsk showing the sampling locations

with 1–2 dm^3 of Milli-Q water. Rinsing quality was checked by comparing the absorbance of Milli-Q water before and after this was passed through the filtration set.

Ultrafiltration was carried out with the high-pressure Amicon 402 ultrafiltration system (400 ml volume), which has a flat surface filter equipped with a magnetic stirrer. Brand new filters (polycellulose AE – 76 mm diameter) were cleaned according to the manufacturer's instructions and then extensively flushed with Milli-Q water. The samples were 'pushed' through the filter with nitrogen at a pressure of ~2 atm. The first 50 ml of a permeate was discarded. One new filter was used for 3–5 samples of seawater. The ultrafiltration cell and the filter itself were rinsed with Milli-Q water prior to the ultrafiltration of each sample. Ultrafiltration was carried out at ambient temperature and its duration did not exceed 8 h.

Absorbance measurements were done on a Perkin-Elmer Lambda 3B dual-beam spectrophotometer with 10 cm optical path length silica cells and an absorbance readout of four decimal places. Scanning was performed at a rate of 120 nm min^{-1}, with 1 nm resolution. The spectrophotometer lamps were heated for 2 h before scanning took place. Milli-Q water filtered through the previously rinsed 0.2 μm Millipore filter served as reference. Measurements were made in matched 100 mm quartz cells. During the measurements the spectrophotometer was calibrated every 2 h.

Before the dissolved organic carbon was analysed, inorganic carbon was removed from the samples by acidification to pH = 2–3 (HCl, Merck, Suprapure) and purging with high-purity nitrogen for 5 min. The DOC analyser used in this study was the commercially available Shimadzu Total Organic Carbon Analyser 5000. The analytical procedure given in the instrument manual was followed. Sample transfer was performed using the motorised syringe of the autoinjector. The injected volume was 0.100–0.250 ml, 3 to 4 injections being made for each sample.

The measurement standards were prepared in Milli-Q water using reagent grade potassium hydrogen phthalate. The calibration curve was determined before each series of measurements from a four point calibration. The results were calculated from the peak areas.

3. Results and discussion

3.1. Blanks and precision of DOC measurement

The intra-sample precision of the analytical procedure (replicates from one bottle), defined as the relative standard deviation, was $< 3\%$, whereas the inter-sample precision (separate bottles containing water collected from one location) was 6%. The variability did not depend on the DOC content of the samples. It should be noted that the precision achieved here was better than that reported for samples collected in the same area by Grzybowski (1996); one probable reason for this was the smaller pore size of the filter used (0.2 μm as against $\sim$0.7 μm), which improved sample homogeneity.

The magnitude of the systematic error introduced during filtration was checked by comparing the UV absorbance spectra of Milli-Q water before and after this was passed through the filtration set equipped with brand new filters. This method proved to be more sensitive than DOC analysis in blanks. In the case of the preliminary 0.2 μm Millipore filtration, approx. 1 dm^3 of Milli-Q water was sufficient to achieve comparable 'before' and 'after' filtration spectra of Milli-Q water.

However, the contamination introduced by the ultrafiltration set with the 1000 Da filter was definitely more persistent. Carlson et al. (1985) encountered similar problems, the greatest contamination being caused by filters with low nominal cut-offs. In the present study it was necessary to filter up to 3 dm^3 of Milli-Q water before stable absorbance readings were obtained. It should be noted, however, that an absorbance check of this kind may be insufficient if filters bleed UV-transparent organic substances. What is more, the applicability of Milli-Q blanks for correcting the seawater blanks is limited: large pH and ionic strength differences may affect bleeding from ultrafiltration membranes (Buesseler et al. 1996). Additionally, where natural waters are concerned, it is impossible to predict whether the error introduced by ultrafiltration is positive (contamination introduced by the filter) or negative (irreversible sorption on the filter). We therefore decided to report the DOC concentration values of the low-molecular weight fractions (LMW) without subtracting the blank values.

Blanks and precision of absorbance measurements

The attainable detection limit of the absorbance measurements (defined as the triple SD of the blank) within the 250–500 nm spectral range ranged from 0.0008 to 0.0022 (Milli-Q water, 100 mm optical pathlength). This value, however, could not be used as a measure of precision in the analysis of natural waters: comparison of absorbance of supposedly the same water (obtained from repeated sampling at the same location) showed absorbance differences at the third and even the second decimal place.

The systematic error introduced by preliminary filtration with the 0.2 m Millipore filter was reduced to 0.003 (at 300 nm) absorbance units after this had been flushed with ~1 dm^3 Milli-Q water. By the time rinsing had been completed, the readings for the blanks passed through the ultrafiltration set were comparable. The final blank values represented $< 0.5\%$ of sample absorbances. Since the differences in seawater absorbance resulting from water ‘patchiness’ were 1–2 orders of magnitude higher, the Milli-Q absorbance blanks were not subtracted from the sample absorbance.

The influence of the concentration factor (CF – ratio of the initial sample volume against the retentate volume) was checked by monitoring the spectra of subsequent portions of the ultrafiltrate. Preliminary experiments showed that in most cases absorbance values started to increase at CF > 4. Since the object of interest was the LMW fraction (the ultrafiltrate), it was decided to stop the ultrafiltration when the volume of the sample had decreased from 400 ml to 200 ml (CF $= 2$).

Evaluation of ultrafiltration efficiency

To check for systematic errors introduced by the ultrafiltration, total DOC (and absorbances at 250 nm in a 100 mm optical cell – A_{250}) in the ultrafiltrate (< 1000 Da) and the concentrate were compared with DOC and A_{250} determined in the bulk samples. The efficiency of the ultrafiltration procedure was defined as:

$$E_x = \frac{V_{\mathrm{ultrf}}\ X_{\mathrm{ultrf}} + V_{\mathrm{conc}}\ X_{\mathrm{conc}}}{V_{\mathrm{bulk}}\ X_{\mathrm{bulk}}}\ 100\%,$$

where

V_{ultrf} – volume of ultrafiltrate,

X_{ultrf} – DOC or A_{250} in the ultrafiltrate,

V_{conc} – volume of concentrate,

X_{conc} – DOC or A_{250} value in the concentrate,
V_{bulk} – volume of the bulk sample,
X_{bulk} – DOC or A_{250} value in the bulk sample.

$\mathrm{E}_{\mathrm{DOC/A}}$ values > 100% indicate sample contamination introduced by the filter and/or the ultrafiltration set, while $\mathrm{E}_{\mathrm{DOC/A}} < 100\%$ suggests irreversible sorption on filters and/or reservoir walls. Table 1 presents the 'mass' balance of DOC and absorbance values measured in the samples.

Table 1. Efficiency of ultrafiltration of natural water samples from the Gulf of Gdańsk based on DOC and absorbance values

Sampling location number and depth	1 0 m	1 0 m	2 0 m	2 18 m	3 0 m	3 22 m	4 0 m	4 34 m
$\mathrm{E}_{\mathrm{DOC}}$ [%]	101	105	97	104	102	104	106	111
$\mathrm{E}_{\mathrm{A250}}$ [%]	93	98	98	90	91	98	106	102
Sampling location number and depth	5 0 m	5 30 m	6 0 m	6 57 m	7 0 m	7 70 m	8 0 m	8 66 m
$\mathrm{E}_{\mathrm{DOC}}$ [%]	109	111	105	106	99	108	112	114
$\mathrm{E}_{\mathrm{A250}}$ [%]	97	93	101	97	92	103	105	96

The efficiency of our ultrafiltration system with respect to DOC values lay within the ranges reported in the literature (Guo et al. 1994, Guo & Santschi 1996, Powell et al. 1996). However, in contrast to these, all but one result was in excess of 100%, which may have been due to the lack of an ultrafiltrate blank correction. The efficiency based on absorbance values was closer to 100%. The balance did not depend on the DOC concentration or the absorbance of the ultrafiltered samples, that is to say, the efficiency of the ultrafiltration of riverine water with an absorbance up to 3-fold higher than that of saline water (see Table 2) was comparable.

Ultrafiltration results

Table 2 gives the results obtained from the ultrafiltration of 14 samples of Gulf of Gdańsk water and 2 river water samples.

The DOC concentrations in the bulk samples ranged from 0.50 to 0.84 mM dm^{-3}. These results are lower than those previously measured in the same area (Pęcherzewski & Ławacz 1976, Pempkowiak et al. 1984, Grzybowski 1996). The reason was the ~0.7 μm pore preliminary filter

Table 2. Dissolved organic carbon concentrations and absorbances (at 250 nm in a 100 mm optical cell) in bulk samples and ultrafiltrates (< 1000 Da) of coastal and riverine water in the Gulf of Gdańsk

Sampling location number – depth of sampling	Salinity [PSU]	DOC concentration in the sample [mM dm^{-3}]	DOC concentration in the ultrafiltrate [mM dm^{-3}]	Absorbance of the sample (at 250 nm in 100 mm optical cell)	Absorbance of the ultrafiltrate (at 250 nm in 100 mm optical cell)
1 – 0 m	0.5	0.76	0.18	1.05	0.14
1 – 0 m	0.3	0.84	0.24	1.12	0.20
2 – 0 m	3.8	0.80	0.25	0.82	0.17
2 – 18 m	7.6	0.54	0.25	0.43	0.18
3 – 0 m	6.8	0.62	0.26	0.53	0.15
3 – 22 m	7.6	0.60	0.29	0.46	0.17
4 – 0 m	6.8	0.64	0.30	0.50	0.20
4 – 34 m	7.9	0.52	0.28	0.40	0.15
5 – 0 m	6.4	0.58	0.29	0.47	0.21
5 – 30 m	7.7	0.50	0.22	0.41	0.16
6 – 0 m	7.2	0.58	0.29	0.41	0.17
6 – 57 m	7.9	0.50	0.29	0.37	0.14
7 – 0 m	7.3	0.52	0.29	0.40	0.15
7 – 70 m	8	0.50	0.29	0.38	0.17
8 – 0 m	7.3	0.53	0.25	0.40	0.16
8 – 66 m	8	0.52	0.26	0.40	0.17

used in the earlier studies. The 0.2 μm filter applied in the present work is rarely used in routine DOC determinations owing to the necessity for time-consuming rinsing with 'organic-free' water. Such a nominal cut-off is, however, indispensable both in optical measurements and in research into molecular size distribution. Similar values were reported for Vistula River and Gulf of Gdańsk water in the paper by Ferrari et al. (1996), who used the same type of filter.

Our data show that DOC concentration is inversely proportional to salinity, suggesting that river water is the source of organic matter in the Gulf of Gdańsk. The relationship, however, is weak in the bulk samples ($r^2 = 0.6$, n = 16) and even weaker in the ultrafiltrates ($r^2 = 0.4$). Non-conservative behaviour of DOC along the salinity gradient has been reported by Miller et al. (1993) and Sempéré & Cauwet (1995). Another explanation for this phenomenon is the variability of water masses in the study area: besides estuarine water (samples 1, 2 and 3), open-sea water is also present (7 and 8).

It should be noted that the choice of the concentration factor (CF) may have affected the ultrafiltration results to some extent. It has been observed that DOC concentration measured in discrete samples of permeate increase with CF (Buesseler et al. 1996, Dai et al. 1998). This observation was explained by the retention of LMW substances by the nominally permeable filters (Guo & Santschi 1996) and/or the 'breakthrough' of high molecular weight substances (Dai et al. 1998). The latter assumption was challenged by Guo et al. (2000) in experiments with chemically defined macromolecules. Nevertheless, the permeation behaviour of natural, ill-defined organic substances of complex structures remains an open question. The consequence of the impact of CF on the ultrafiltration efficiency in our case is that by choosing a low CF we may to some extent have underestimated the LMW fraction. None the less, the same CF was consistently used for all samples, so the bias will have affected them all to a comparable extent. Furthermore, since the real membrane pore size ranged from ∼700 Da to ∼1300 Da (Cheryan 1998), we may assume that the measured LMW fraction consists mainly of substances with molecular weights less than the membrane's nominal cut-off (1000 Da).

The variability of the DOC values in the ultrafiltrate was narrower than that in the bulk samples (coefficient of variance 12% and 20% respectively). Assuming river water to be a significant source of organic matter, one may conclude that the LMW fraction is less affected by the mixing of fresh and salt water.

Fig. 2 presents the percentage of DOC and absorbance in the LMW fraction versus salinity. Though the scatter is considerable, it can be seen that there is a general relative increase in the LMW fraction with rising salinity.

The low-molecular weight fraction comprised 38–57% of DOC in the samples of coastal water, and 24% and 28% in the Vistula river water samples. The percentage of the LMW fraction in the DOC content of Gulf of Gdańsk water is lower than that in oceanic water, where reported values range from 65% (Carlson et al. 1985, Guo et al. 1995, McCarthy et al. 1996) to 82% (Amon & Benner 1994) and 85% (Dai & Benitez-Nelson 2001). As Fig. 1 shows, there were no evident differences in the LMW fraction distribution between surface and bottom samples. In the study by Guo et al. (1995) on the depth distribution of LMW DOC in Gulf of Mexico water, the differences between surface and bottom water ranged from 2 to 5%. Those observations suggest the very limited impact

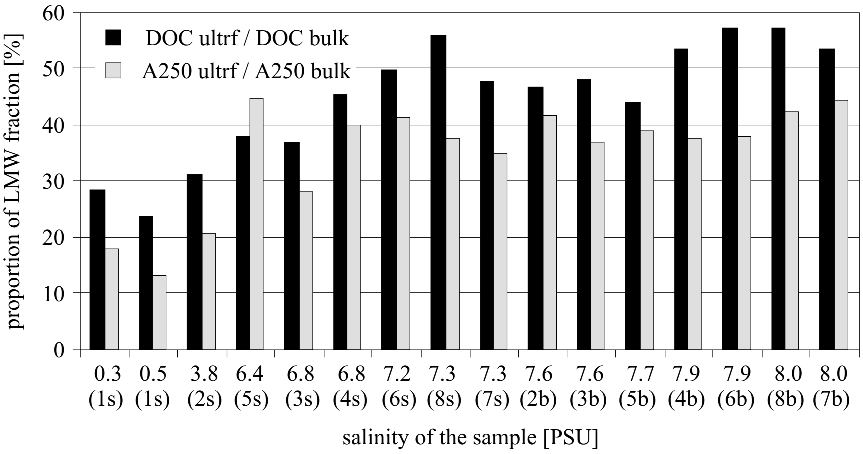

Fig. 2. The proportion of the low-molecular-weight fraction in DOC content and absorbance of water samples collected in the Gulf of Gdańsk, arranged according to increasing salinity; the x-axis codes in parentheses indicate the sampling point number and the sample's origin – surface (s) or bottom (b) water

of primary production on DOC molecular size distribution. The probable reason, however, may be the small number of samples analysed, and, in our case, the well mixed water masses in the study area.

A lower percentage of ultrafiltrate in riverine water was also detected by Amon & Benner (1996) and Dai et al. (1995). This is in agreement with the findings of Whitehouse et al. (1989), who reported that colloidal (> 1000 Da) organic carbon makes up a large proportion of the total DOC in river water. In the study by Powell et al. (1996), the proportion of the LMW fraction was correlated positively with salinity, ranging from 19–25% in freshwater samples to 50–56% in samples with a salinity of ∼10. The seaward increase of LMW organic substances was also reported by Takayanagi & Wong (1984): LMW at salinities 0, 20 and 31 were about 20%, 40% and 60% respectively. On the other hand, Wen et al. (1999) observed practically constant proportions ($55 \pm 3\%$) of the colloidal (> 1000 Da) fraction along a 0–30 salinity gradient.

The fact that the relative content of the LMW fraction was generally higher in samples of higher salinity may be due to the selective removal of the high-molecular-weight matter. This was observed by Sholkovitz (1976) and Whitehouse et al. (1989) in the mixing area: on contact with water of higher ionic strength, the macromolecules dissolved in riverine water first undergo flocculation, then precipitation. As a result, they are not regarded as 'dissolved'. This assumption, however, is not confirmed by the absorbance values obtained in the present study, which to some extent characterise DOC qualitatively. The relationship between absorbance and DOC in the bulk samples, described by the correlation coefficient, reached a value of 0.9, whereas for the ultrafiltrates (permeates) it did not exceed 0.1. This may suggest that the 'open bay' LMW fraction did not originate from river water. The fact that LMW fractions of comparable DOC content have different absorbances may be due to a number of factors. There may be a compositional difference between the organic compounds in river and coastal water, the former containing terrigenous humic substances, whereas the latter originates partly from autochthonous biological activity. Differences in the optical properties of natural waters may also result from the photo-degradation of organic compounds, leading to their conversion into substances with different optical characteristics, while still retaining their DOC content. Vodacek et al. (1997) suggested that it was this phenomenon that could explain the relationship between absorbance and DOC observed in surface coastal water.

4. Summary

Ultrafiltration as applied to the study of low-molecular-weight organics has been proved reliable on the basis of DOC and absorbance balances. The analysis of samples of supposedly different origin showed that while the absolute concentrations of LMW fraction were comparable, this was not the case when relative contents were compared. The samples containing terrigenous material and those affected by it (as indicated by the lower salinity) contained a lower percentage of this fraction. There was a significantly closer relationship between the DOC concentration and absorbance in the bulk samples than in the ultrafiltrates. This fact may suggest that the LMW fraction in coastal water is not merely the outcome of the mixing of freshwater organics with seawater, but that other processes are involved too.

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