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Isolation of Marine Dissolved Organic Matter: Evaluation of Sequential Combinations of XAD Resins 2, 4, and 7

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The sorptive behavior of resins XAD2, -4, and -7 for dissolved organic carbon (DOC) derived from marine phytoplankton was compared. The resins were arranged in varying sequences, which proved to be critical for determining the efficiency of DOC isolation. The sequence XAD7/2/4 allowed the isolation of a very high percentage of DOC (65%) from the seawater matrix. A mixed bed of the same resins showed a significantly lower recovery (39%). Varying amounts of the material adsorbed by the individual resins could not be recovered by NaOH/methanol elution. The percentage of DOC firmly retained by each resin was 32, 21, and 2% in XAD2, XAD4, and XAD7, respectively.

Characterizing the nature of dissolved organic matter (DOM) in seawater by direct analysis typically accounts for less than 15% of the total.¹ Alternatively, DOM can be isolated from seawater, thereby providing improved analytical conditions in regard to ionic strength and concentration levels. Various methods have been employed to isolate DOM: the adsorption of DOM from acidified seawater onto XAD resins has proved to be useful, even though these are selective for mainly hydrophobic compounds. Some workers use a combination of XAD and ion-exchange resins to recover the hydrophilic fraction of DOM and, hence, achieved almost 100% recovery of dissolved organic carbon (DOC).² Unfortunately this methodology is not appropriate for high-salinity matrices such as seawater. Tangential-flow ultrafiltration through 1000-Da cutoff filters only recovers approximately 30% of DOM in seawater.¹ Currently there is much speculation about the amount and form of DOM in the world's oceans. However, our understanding of the generation of this pool, its transformation by the trophic network, and the humification of organic matter in the water column is hindered by these technical difficulties.

Lara and Thomas³ presented results from a study following the humification of DOC recently produced by laboratory diatom cultures. It was found that ca. 30% of the material adsorbed by the XAD2 resin used for DOC fractionation could not be recovered by standard NaOH/methanol elution. In an attempt to separate and operationally delimit the nature of this tightly bound DOM fraction, we subsequently compared the sorptive behavior of three different XAD resins arranged in varying sequences. Our main research is orientated toward nitrogenous compounds and organic acids in the humification

of marine DOM, and so the combination of XAD2, XAD7, and XAD4 is most appropriate. According to the manufacturers product information (Serva, Heidelberg, Germany) XAD7 should have good adsorptive properties for polypeptides and enzymes, and XAD4 for small molecules, including low-molecular-weight acids.⁴ XAD7 and XAD8 have been shown to have the best performances for soil fulvic acid.⁴ XAD8 and XAD2 have similar performances for marine humics, XAD2 being preferred to XAD8 because of lower bleed.^{5,6}

EXPERIMENTAL SECTION

¹⁴C radiolabeled DOM was produced by cells of the Antarctic diatom *Thalassiosira antarctica* grown in Antarctic seawater as described in Lara and Thomas.³ Thus, the terms "DOM" or "DOC" used to discuss results from the present study refer to algal-derived material. The seawater containing 7 month old DO¹⁴C was filtered through cellulose nitrate filters (0.2 μ m, Sartorius) and acidified to pH 2 with HCl. Subsequently, 100-mL samples were passed through 3 series each comprising column combinations containing respectively 5 mL of the XAD resins 2, 4, and 7 (particle size: 0.3–1.0 mm) as described in Figure 1. Thereafter, the resin was washed with 50 mL of 0.01 N HCl. The retained DOM was fractionated by eluting with 150 mL of 0.2 N NaOH (hydrophobic acid fraction, HbA), 75 mL of distilled water (H₂O fraction), and 100 mL of methanol (hydrophobic neutral fraction, HbN). The fraction not retained by the resin was termed hydrophilic (HI). The radioactivity of subsamples of each fraction was measured in triplicate subsamples by liquid scintillation counting. The results are expressed as percentages of the total activity applied to the column series.

RESULTS AND DISCUSSION

The analytical approach used here permitted a direct measurement of the carbon retained by the resins. In a comparison of those resins at the beginning of the fractionation scheme (Figure 1), XAD4 retained 34% less carbon than XAD2. Strong sorption was ca. 10–15 times lower on XAD7 (2% of the DO¹⁴C) compared to XAD4 and -2, respectively. Considering only XAD2 and -4, it is evident that, despite the different amount of DO¹⁴C tightly retained (32% and 21%, respectively), HbA was almost the same for both, but HbN

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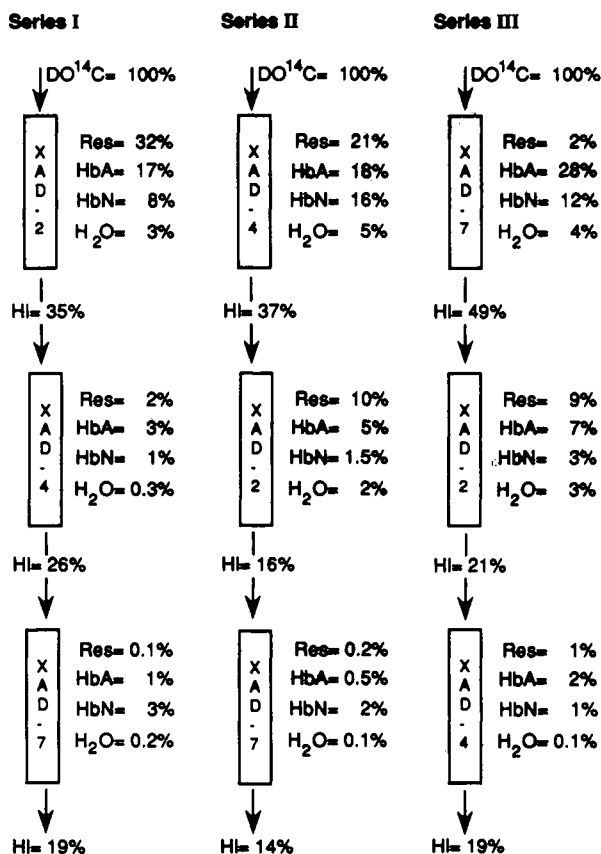


Figure 1. Resin sequences of three fractionation series. Key: Res, fraction tightly retained by the resin; HbA, hydrophobic acid fraction; HbN, hydrophobic neutral fraction; H₂O, fraction eluted with distilled water; HI, hydrophilic fraction. Results from each series are listed as a percentage of total DO¹⁴C in the unfractionated sample.

was significantly higher for XAD4. It would seem therefore that XAD-2 firmly binds a part of HbN. However, if XAD2 and -7 are compared, the difference in the firmly bound fraction is mainly reflected in the much higher HbA fraction from XAD7. From this, one would conclude that XAD2 firmly retains a part of HbA, which seems contradictory to the comparison between XAD2 and -4. The differences in the firmly bound fraction are partially reflected in the different HbA and HbN fractions but also in the HI counts which increase in the sequence XAD2 < XAD4 < XAD7. From this comparison it seems that XAD2 may also be retaining a part of the hydrophilic compounds. This clearly indicates that the operational delimitation of the sorptive characteristics of the fraction strongly bound by XAD2 is not straightforward. Thurman⁷ indicated that, because of interaction between aromatic rings on the resin and in humics, these elute poorly from styrene-divinylbenzene resins such as XAD2. However, in the present study this is not a likely explanation. It does not seem realistic that 30% of the carbon present in phytoplankton exudates or in its degradation products is part of aromatic structures.

The difference between XAD7 and -2 or -4 was not just a higher carbon content in the HbA fraction but also (due to the extremely low amount tightly retained by XAD7) higher DO¹⁴C in the hydrophilic fraction. In series III the hydrophilic

fraction from XAD7 was passed through XAD2. This showed that, although XAD7 yielded by far the highest HbA, its hydrophilic fraction still contained a significant amount of HbA substances that could be adsorbed by XAD2 and be subsequently recovered in the NaOH eluate. Assuming that the sum of HbA in both resins in series III represents 100% of the humic and fulvic acids in the sample, then XAD2 in series I recovered only 49% and XAD7 in series III about 80%.

A column rinse after extraction of humics from seawater is necessary to avoid precipitation of insoluble salts during alkaline elution. This rinse is made using distilled water acidified at the same pH of the sample (pH 2), thereby avoiding desorption of a DOM portion. However, the radioactivity in the acid wash was on average about 5% of the total carbon, similar to that recovered by H₂O elution. This amount of carbon greatly exceeds (ca. 10 times) that expected to come from cross contamination from the preceding sample. The existence of such a hydrophobic basic fraction in XAD extracts has been mentioned by others,^{8,9} although no explanation has been proffered. The recoveries from the acid rinses are not included in Figure 1, since the radioactivity in these was not measured in all instances.

Considering the fraction of organic acids associated with humics on XAD4 in series II as compared to XAD2 in series I, the carbon content in the alkaline eluates does not seem to reflect a more efficient adsorption/desorption by XAD4. On the other hand, the H₂O fraction from XAD4 contains ca. 100% more counts than the one from XAD2, which might be a reflection of the increased capacity of XAD4 for small organic acids.

Not only the type but also the sequence of the resins proved to be important. In series I, the HbN fraction from XAD4 and -2 together recovered 9% of the total DO¹⁴C, whereas in series II these resins recovered 17%. Whether or not the substances contained in HbN should be considered humic or not is controversial. Nevertheless, this fraction represents a substantial portion of the total DOC. The different percentages of DOC isolated from seawater by each series are strongly determined by the resin sequence as seen in the summary given in Table 1. The high efficiency of series III and the degree of tight sorption of DOM of each resin suggested that the "ideal" sequence would be XAD7/4/2, i.e. in order of decreasing elution efficiency. This was tested in an additional experiment, although without further improvement, the overall DOC recovery being ca. 65%, including the acid rinse.

Mixed beds of XAD resins have been successfully used by Van Rossum and Webb¹⁰ for the extraction of organics from water. The significant difference in performance among the three series raised the question about the efficiency of a mixed bed of the three resins. This was tested using a mixed bed containing 5 mL of each XAD2, -4, and -7, compared with a column containing 15 mL of XAD2. The percentage of "humic" DOC (HbA) was practically the same in both cases, 18 and 17% for the mixed bed and XAD2, respectively (Table 1). The percentage of DO¹⁴C firmly retained by the columns

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Table 1. Summary of Results from Each Series as a Percentage of Total DO¹⁴C in the Unfractionated Sample

	series				
	I (XAD2/4/7)	II (XAD4/2/7)	III (XAD7/2/4)	mixed bed (XAD2 + 4 + 7)	XAD2 (15 mL)
% DOC in HbA	21	23	37	18	17
% DOC in HbN	12	19	16	13	11
% DOC in H ₂ O	3	7	7	3	2
% DOC in acid rinse	n.m.	n.m.	5	5	4
% DOC recovered	36 ^a	49 ^a	65	39	34
% DOC on resin	34	31	12	33	41
% DOC in final H1	19	14	19	22	22
sum	89	94	96	94	97
% unaccounted DOC	11 ^a	6 ^a	4	6	3

^a This refers to the effect of losses in the acid rinse, which was not measured (n.m.) for series I and II. Thus, the values cited are probably slightly underestimated for "% DOC recovered" and overestimated for "% unaccounted DOC".

was lower for the mixed bed (33%) compared with 41% for XAD2 alone. However, the latter was not much greater than the 32% for only 5 mL of XAD2 in series I, especially considering that the amount of resin was three times higher. This points again to the importance of resin sequence compared to mixed beds. It appears that the influence of XAD2 in the amount of carbon firmly bound is fundamental for the overall efficiency of the mixed bed.

Including the acid rinse, the combination XAD7/2/4 in series III recovered 65% of algal-derived DOC. This is substantially more than the 5–15% considered to be a usual recovery of DOM from seawater using XAD resins.¹ However, it must be taken into account that the behavior of the algal DOM used in this study might differ from truly marine DOM, especially from the deep ocean. The use of radiolabeled DOC in this study permitted an accurate investigation of the performance of XAD7, -4, and -2 at low, oceanic DOC concentrations. Despite the potential contamination due to bleed of XAD7,⁴ if the released compounds do not interfere with the substances of interest being specifically investigated, this resin can be a useful alternative for extraction and characterization of marine DOM, especially in productive surface waters.

CONCLUSIONS

The percentage of DO¹⁴C firmly retained by each resin was 32, 21, and 2% in XAD2, XAD4, and XAD7, respectively. A result of this is that the sequence of the individual resins in a series is critical for the overall efficiency in isolating algal

DOM from seawater: the percentage of DO¹⁴C recovered by each series was inversely related to the percentage of carbon tightly bound by it. The main difference among the series is that the unrecoverable DO¹⁴C is much lower for series III. XAD7 adsorbs reversibly components of the HbA fraction which are irreversibly adsorbed by XAD2 and, to a lesser extent, by XAD4. According to the properties of XAD7, these components might be polypeptides or enzymes. The combination XAD7/2/4 allowed the isolation of a very high percentage of DO¹⁴C (65%) from the saltwater matrix, and we would recommend the use of this combination, whose effectivity might be further enhanced by the use of reverse flow elution. The combination of this procedure (XAD) and ion-pair extraction of H1 onto solid phases¹¹ may considerably increase the amount of isolated DOM. The series XAD7/4/2 showed a similar performance and might be more useful for other chromatographic applications. A mixed bed of all three resins had no substantial improvement over that of XAD2 alone, and the overall efficiency of the mixed bed was evidently strongly influenced by the amount of carbon firmly bound by XAD2. The precise operational characterization of the fraction strongly bound by XAD2 remains elusive.

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