

Re-examination of cross-flow ultrafiltration for sampling aquatic colloids: evidence from molecular probes

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Abstract

Application of cross-flow ultrafiltration techniques to marine systems has greatly increased in recent years. However, the retention and permeation behavior of macromolecules and their associated trace elements during ultrafiltration is still controversial. In addition, the optimum concentration factor (CF) and the possibility for a “breakthrough” of high molecular weight (HMW) dissolved organic carbon (DOC) during ultrafiltration are still a matter of contention. The permeation and retention behavior of natural DOC, standard macromolecules and selected metals was examined on a 1 kDa Amicon S10N1 ultrafiltration cartridge, using molecular probes and radioactive metals spiked to natural seawater. Laboratory results from molecular probes show that significant fractions (> 40%) of low molecular weight (LMW) molecules (0.5 kDa rhodamine 6G and 0.6 kDa glutathione) are retained by a 1 kDa ultrafilter membrane, even under a CF of ~ 50. Therefore, the retention of LMW molecules can give rise to an overestimate of the colloidal fraction, especially under lower CFs. The percentage of HMW molecules passing through the 1 kDa membrane decreases rapidly with increasing size or MW. On average, ~ 15% of vitamin B₁₂ (1.3 kDa) and ~ 3% of a 3 kDa dextran pass through the 1 kDa membrane. However, permeation of a 10 kDa dextran through the 1 kDa membrane becomes negligible (< 0.6%). Thus, the permeation of HMW molecules is minimal during ultrafiltration, even under high CFs. The ultrafiltration behavior of natural DOC and LMW metals can be well predicted by a permeation model, and consistently shows an increasing concentration in the permeate with increasing CFs. Standard dextrans, rhodamine 6G and natural DOC are recovered at 92–95%, while losses of glutathione and vitamin B₁₂ to the membrane can be significant. Possible sorptive losses and overall mass balance of molecules are thus dominated by their physicochemical properties but not by their MWs. Most retained LMW DOC can be further removed during diafiltration whereas loss of HMW DOC during diafiltration is minimal. Since retention of LMW molecules is the main problem rather than the permeation of HMW molecules, a high CF (> 40) is recommended for isolating marine colloids by ultrafiltration. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: ultrafiltration; molecular probes; colloids; dissolved organic carbon; metal radiotracer

1. Introduction

Colloids, defined here as the size fraction between 1 nm and 0.2 μm, which includes macromolecules

and microparticles, are thought to play a central role in regulating concentration and speciation, and thus, fate, transport, bioavailability and toxicity of many trace elements in marine environments (Wells and Goldberg, 1991; Honeyman and Santschi, 1992; Gustafsson and Gschwend, 1997). The cross-flow ultrafiltration (CFUF) technique has been increas-

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ingly used to isolate marine colloids (e.g., Buesseler et al., 1996; Guo and Santschi, 1997 and references therein). There is a consensus that CFUF, which is an operational separation technique, requires considerable care in quantifying blanks, assessing the membrane's cut-off and obtaining a good mass balance. However, controlled laboratory studies on the interaction between macromolecules and ultrafiltration membranes are still few. Without a full understanding of the retention and permeation behavior of macromolecules and their associated trace elements in seawater during ultrafiltration, it will be difficult to properly interpret results from studies where colloids were sampled using CFUF.

Recent studies have recommended CFUF for sampling marine colloids (e.g., Benner et al., 1992; Guo et al., 1994; Buesseler et al., 1996; Guo and Santschi, 1996; Gustafsson et al., 1996; Wen et al., 1996; Benner et al., 1997; Dai et al., 1998). However, these studies disagreed on, among other things, the choice of an optimal concentration factor (CF, i.e., the ratio of initial sample volume to final retentate volume). For example, higher CFs (> 40) are recommended for sampling marine colloids based on laboratory studies (Guo and Santschi, 1996) and model calculations (Benner et al., 1997). On the other hand, low CFs (< 5 – 10) have also been suggested (Wen et al., 1996; Dai et al., 1998) and have been used by a number of investigators. Most recently, Dai et al. (1998) reiterated concerns raised in earlier studies and recommended a CF of < 5 . However, systematic laboratory studies on the permeation and retention behaviors of both low molecular weight (LMW) and high molecular weight (HMW) molecules are needed to draw firm conclusions on proper ultrafiltration protocols.

A considerable portion of the dissolved fraction with a molecular weight less than the ultrafiltration membrane's cut-off can be actually retained during ultrafiltration (e.g., Guo and Santschi, 1996; Benner et al., 1997). This retention can be expected to be worse at low CFs, which may lead to significant over-estimates of the colloidal abundance. On the other hand, it has been alleged that HMW colloids can break through and pass the ultrafiltration membranes to a significant extent (Dai et al., 1998). If this were true, choosing a high CF would lead to an underestimate of the colloidal fraction of chemical

species. However, it is also possible that the ultrafiltration membranes used might not have met the specification of the manufacturer, since HMW molecules larger than the average pore size should be mostly retained by the membrane when it functions properly (Amicon, 1995; Cheryan, 1998). In addition, improper experimental protocols and operational procedures during ultrafiltration could have caused biased sampling and thus resulted in varying results. Large variations in reported percentages of colloidal fractions for different chemical species could have been a consequence of different CFs (see review in Guo and Santschi, 1997), as well as the malfunctioning or improper use of the CFUF technique (e.g., Harvey, 1995). Therefore, a re-examination of the ultrafiltration behavior of macromolecules through controlled laboratory experiments is desperately needed.

Here, we report results from a suite of rigorously controlled laboratory experiments using both molecular probes and radioactive metals spiked in natural seawater systems at ambient concentration levels. Molecular probes used here included both LMW and HMW organic molecules. LMW and HMW are defined here relative to the nominal molecular weight cut-off (MWCO) of a 1 kDa ultrafiltration membrane. In addition, the ultrafiltration behavior of natural dissolved organic carbon (DOC) in seawater was also investigated and compared with the results of molecular probes. Last but not least, diafiltration experiments with both nanopure water and pre-ultrafiltered seawater were carried out to examine possible artifacts during the desalting process.

2. Methods

2.1. Ultrafiltration system

In order to compare our laboratory study with results from previous calibration studies (e.g., Guo and Santschi, 1996; Gustafsson et al., 1996; Wen et al., 1996; Dai et al., 1998), a spiral-wound 1 kDa Amicon S10N1 ultrafiltration cartridge (polysulfone) was chosen. The other reason for choosing the Amicon S10N1 cartridge is that most recent biochemical and isotopic characterization studies of marine colloids used S10N1 ultrafiltration cartridges and high

CFs (e.g., Benner et al., 1992; Amon and Benner, 1994; Bianchi et al., 1995; Guo et al., 1995; Santschi et al., 1995; Guo et al., 1996; Aluwihare et al., 1997; Guo et al., 1997; McCarthy et al., 1997, 1998). Therefore, it is crucial to find out if there were possible sampling artifacts in these previous field studies.

Briefly, a Teflon diaphragm pump head (Cole-Parmer) was equipped with Teflon fittings and tubings to decrease sorptive losses and any possible contamination from the apparatus (Wen et al., 1996). Before use, the ultrafiltration cartridge was first calibrated and its integrity checked with vitamin B₁₂ (Guo and Santschi, 1996). After that, the ultrafiltration cartridge was thoroughly cleaned before each experiment with 1–2% Micro detergent, 0.05 M NaOH, and 0.02 M HCl, respectively. Each chemical was recycled for 20–30 min and allowed to soak for another 20–30 min. Between each solution, ~ 40 l of nanopure water (DOC < 2 μM) were flushed through the ultrafiltration system, and after chemical cleaning, another 40–80 l of nanowater were flushed again under normal ultrafiltration operating conditions (i.e., 207–276 kPa or 30–40 psi at 50–60% pump power efficiency).

2.2. Molecular probes

Thanks to available and sensitive analytical techniques, individual macromolecules tagged with fluo-

rescein can be added to experimental systems at trace levels (e.g., Gustafsson et al., 1996), which allows the monitoring of the dynamic changes of specific molecules during permeation and retention. Therefore, possible methodological artifacts during ultrafiltration and diafiltration can be investigated.

Standard macromolecules used include both LMW (e.g., 0.5 kDa rhodamine 6G and 0.6 kDa glutathione) and HMW molecules such as 1.3 kDa vitamin B₁₂, a 3- and a 10-kDa dextran (Table 1). These macromolecules were chosen because most of them had been used during previous studies (e.g., dextran in Gustafsson et al., 1996; Dai et al., 1998; and vitamin B₁₂ in Guo and Santschi, 1996).

2.3. Ultrafiltration

In order to avoid changes in ionic strength and pH between seawater and nanopure water, laboratory experiments were conducted using natural seawater media. Seawater samples were collected and pre-filtered in situ through a 0.2 μm Nuclepore cartridge from Galveston Bay and the Gulf of Mexico, with salinities between ~ 26 and ~ 35 (Table 1). Before ultrafiltration, 1 l of pre-filtered seawater, containing no molecular probes, was used to condition the S10N1 cartridge. Standard macromolecules were added into pre-filtered seawater, and the well mixed spiked seawater was then sampled to quantify the initial concentration of a specific molecule. During

Table 1
Standard macromolecules used and experimental parameters in seawater media

Exp. ID	Macromolecule	Molecular weight (kDa)	Concentration of tracers	Seawater salinity	Seawater bkg DOC (μM)
1	B ₁₂	1.33	0.2 μM	26	245
	Dextran	3	20 nM		
2	B ₁₂	1.33	0.50 μM	35.2	84
	Dextran	10	20 nM		
3	Glutathione	0.612	1 μM	30	125
	Dextran	10	20 nM		
4	Rhodamine 6G	0.495	50 nM	30	125
	Dextran	10	20 nM		
5	Rhodamine 6G	0.495	5 nM	35	76
6	Rhodamine 6G	0.495	5 nM	35	78
	Dextran	10	10 nM		

Glutathione (oxidized form, C₂₀H₃₂N₆O₁₂S₂) and vitamin B₁₂ (or cyanocobalamin) were purchased from Sigma while rhodamine 6G (C₂₇H₂₇ClN₂O₅) and dextran (carbohydrate) are fluorescein-tagged and were purchased from Molecular Probe (Eugene, OR).

ultrafiltration, the ultrafiltration reservoir was under continuous stirring using a magnetic stirring bar. Time series samples from both permeate and retentate lines were collected as discrete samples. Duplicate experiments were carried out without collecting time series samples to evaluate the mass balance of macromolecules during ultrafiltration. In this case, samples were collected from the ultrafiltration reservoir, before and after processing, and the integrated permeate for determining the concentrations of macromolecular tracers in the initial solution, final retentate, and integrated permeate fractions. In a separate ultrafiltration experiment of natural seawater ($< 0.2 \mu\text{m}$) without any tracer added, time series DOC samples from discrete permeate were also collected to examine the permeation behavior of natural DOC for comparison with results from the molecular probe experiments.

In order to further characterize the retention behavior of LMW DOC during ultrafiltration, a pre-filtered ($0.2 \mu\text{m}$) fresh water sample collected from the Mississippi River was pre-ultrafiltered using a 1 kDa ultrafilter. The < 1 kDa dissolved fraction (permeate) was then re-ultrafiltered using the same ultrafiltration system to examine how LMW DOC is being retained during replicate ultrafiltration. DOC concentrations in all ultrafiltration samples were measured to determine the percentage of DOC in permeate and retentate fractions.

2.4. Diafiltration

The behavior and speciation of aquatic macromolecules may change due to differences in ionic strength and pH between nanopure water and seawater (Ghosh and Schnitzer, 1980; Perdue, 1989; Buffle, 1990). In order to evaluate the effect of cations, such as calcium and magnesium, on the retention of macromolecular DOM in seawater during desalting, diafiltration experiments were carried out using both nanopure water and pre-ultrafiltered seawater. "Diafiltration" of molecular probes using pre-ultrafiltered seawater was designed to simulate the diafiltration process and thus, the effect of major cations on the retention and permeation behavior of macromolecules. These experiments were made possible by the fact that pre-ultrafiltered seawater contained

negligible levels of background signals for all molecular probes we used. Time series samples from the permeate line were collected to examine how macromolecular DOM is being retained or washed away during diafiltration.

2.5. Ultrafiltration using complexed radioactive metals

Radioactive metals (^{54}Mn , ^{59}Fe , ^{60}Co , ^{65}Zn , ^{109}Cd , $^{110\text{m}}\text{Ag}$, ^{133}Ba , and ^{234}Th) were added into either pre-ultrafiltered seawater, containing mostly < 1 kDa DOC, or a 1 mM EDTA (di-sodium salt) solution to simulate the ultrafiltration of LMW species. The spiked solution was allowed to equilibrate overnight before further ultrafiltration. Time series samples were collected from both permeate and retentate lines and gamma counted for isotopic activity to study the ultrafiltration behavior of these LMW metals.

2.6. Determination of DOC, molecular probes and radioactive metals

Concentrations of DOC were measured on a high temperature combustion TOC analyzer (MQ Scientific 101). The total DOC blank (including water and instrument) is usually $< 4\text{--}6 \mu\text{M}$. Precision, in terms of the coefficient of variation, is better than 2%, depending on DOC concentrations. Further detailed procedures are described in Guo et al. (1994).

Fluorescein labeled molecular probes (dextran and rhodamine 6G) were quantified on a HPLC equipped with a scanning fluorescence detector (Waters 474), a size exclusion column and a temperature controlled auto-sampler (Waters 717 plus), while concentrations of vitamin B₁₂ were measured on an absorbance detector (Waters 2487 Dual λ). Wavelengths used for dextran analysis were 490 nm for excitation (18 nm band width) and 524 nm for emission (10 nm band width). Rhodamine 6G has slightly different excitation/emission (524/550 nm) wavelengths for maximum intensity but was measured at 490/524 nm. Glutathione was detected via pre-column-derivatization of SBDF and fluorescence detector. For the case of two molecular probes in the same experiment, samples were first separated by a size exclusion column (Bio-Rad G250) before analysis. Further

details on these analytical procedures are described in Tang (1999).

The activities of ^{54}Mn , ^{59}Fe , ^{60}Co , ^{65}Zn , ^{109}Cd , $^{110\text{m}}\text{Ag}$, ^{133}Ba , and ^{234}Th were measured on a high purity Ge well detector with a Canberra Series 100 multichannel analyzer. Counting time was adjusted to yield a propagated error of $\sim 5\text{--}10\%$ or higher, depending on the metals. Decay corrections were applied to correct to the initial day of the experiment, and sample count rates determined for the same geometry.

2.7. Ultrafiltration permeation model

If chemical species (metal ions, organics, and complexes) with dimensions or sizes higher than the MWCO of a specific ultrafiltration membrane (i.e., the HMW fractions) are quantitatively retained and those with MWs lower than the MWCO (i.e., the LMW fractions) pass through the membrane without much retention, concentrations of LMW chemical species in the discrete permeate would be constant. According to the membrane filtration theory, however, any particle or molecule with a gyration radius of R_p less than the membrane's pore size of R_m can pass through the membrane at a rate (permeation fraction) which is proportional to $(R_m - R_p)^2 / (R_m)^2$. Since R_p is always > 0 , the ratio of $(R_m - R_p)^2 / (R_m)^2$, will be always $< 100\%$ for the LMW case (e.g., Johnson and Wangersky, 1985).

According to Logan and Jiang (1990) and Kilduff and Weber (1992), the permeation model can be described as:

$$\log C_p = \log(P_c C_f^0) + (1 - P_c) \log(\text{CF})$$

where C_p is the concentration of a given chemical species in the permeate, CF is the concentration factor, P_c is the permeation coefficient defined as the ratio of C_p to C_f (feed concentration), while C_f^0 is the initial concentration of LMW or permeable species in the upstream feed solution. The general application of this model has improved our understanding of ultrafiltration behavior of macromolecules even though this model cannot strictly be applied to complex molecule mixtures and those compound classes with significant sorptive losses to the membrane.

A linear relationship between $\log C_p$ and $\log \text{CF}$ indicates that the permeation behavior follows the permeation model with a constant P_c , which is always < 1 . Therefore, retention of LMW materials

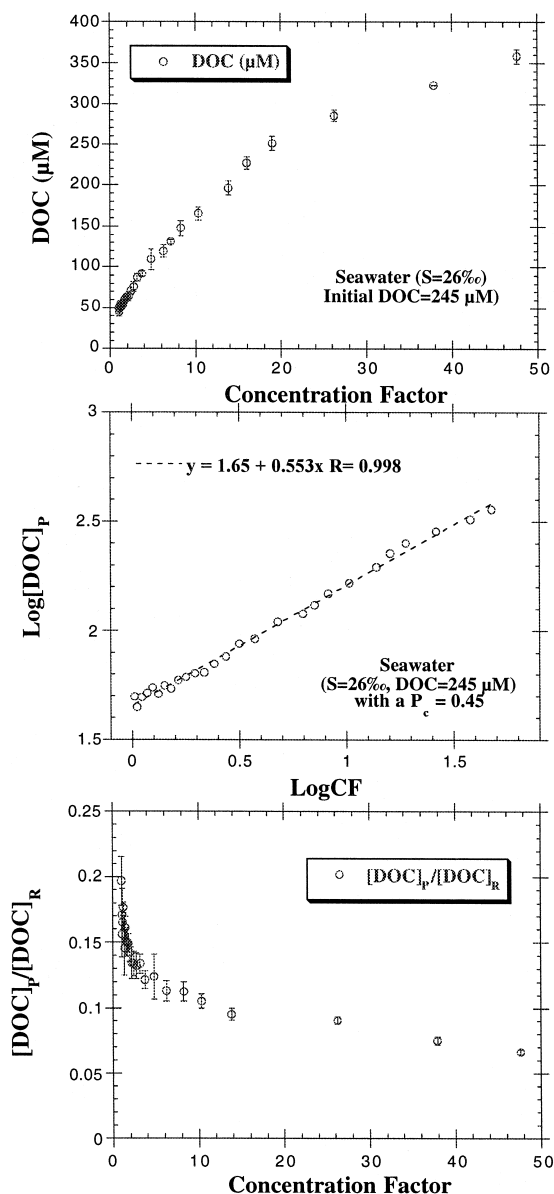


Fig. 1. Variations of DOC concentrations in discrete permeate samples ($[\text{DOC}]_p$) with concentration factor, CF (upper panel), the relationship between $\log \text{CF}$ and $\log [\text{DOC}]_p$ (middle panel), and the concentration ratio of DOC in the permeate ($[\text{DOC}]_p$) to that in the retentate ($[\text{DOC}]_R$) vs. CF (lower panel), resulting from the ultrafiltration of natural seawater DOC.

Table 2
Permeate coefficients (P_c) of natural DOC and standard macromolecules added to seawater during ultrafiltration

Molecule	Slope	P_c	R	Level of significance (p)
DOC in seawater I	0.55	0.45	0.99	0.001
Glutathione	0.84	0.16	0.98	0.001
Rhodamine	0.40	0.60	0.99	0.001
Vitamin B ₁₂	0.85	0.15	0.95	0.001
3 kDa dextran	–	0.03 ^a	–	–
10 kDa dextran	1.09	~ 0	0.99	0.001
DOC in seawater II	0.33	0.67	0.97	0.001

^a Estimated from the measured percentage of 3 kDa dextran in the permeate fraction.

during ultrafiltration is an inevitable consequence of their low (i.e., < 1) P_c values. As a result, concentrations of seawater DOC in the permeate will increase with increasing CF (Fig. 1). However, this does not constitute a ‘‘breakthrough’’ of HMW DOC, suggested by Dai et al. (1998).

The P_c value of a given permeable species can be calculated from the slope ($1 - P_c$), derived from the linear relationship between $\log C_p$ and $\log CF$ (e.g., Fig. 1 and Table 2). On the other hand, a combination of the values of the intercept, $\log(P_c C_f^0)$, and the slope ($1 - P_c$) allow an estimate of the initial feed concentration (C_f^0) of the LMW or permeable fraction, which can be used to estimate the initial colloidal fraction. Therefore, the colloidal (or retainable) fraction (i.e., $1 - C_f^0/C_T^0$) of a specific chemical species can be predicted from the estimated initial LMW concentration (C_f^0) and the measured total dissolved concentration, C_T^0 .

3. Results and discussion

3.1. Ultrafiltration characteristics of natural DOC

The main concerns are how macromolecular DOC in natural waters is being isolated during ultrafiltration and what kinds of artifacts, if any, exist under given operational conditions. Fig. 1 shows the variations of seawater DOC concentrations in the discrete permeate (C_p) with CF, and a linear correlation between $\log C_p$ and $\log CF$ during ultrafiltration of a

coastal seawater sample. As is evident from Fig. 1, the concentration of DOC in the permeate increases with increasing CF. This relationship between C_p and CF has been reported previously (e.g., Buesseler et al., 1996; Guo and Santschi, 1996; Dai et al., 1998). Increase of C_p (or $\log C_p$) of seawater DOC with increasing CF (or $\log CF$) can be explained either as a significant retention of LMW DOC (e.g., Guo and Santschi, 1996) or as a breakthrough of HMW DOC across the filter membrane (e.g., Dai et al., 1998). However, the results of our laboratory experiments do not support this latter interpretation.

The following questions were addressed. (1) Is the increase in C_p during ultrafiltration mainly due to the breakthrough of HMW DOC, or because of the retention of the LMW DOC in seawater? (2) If the HMW DOC can pass a 1 kDa pore size ultrafilter membrane, how does this so-called ‘‘breakthrough’’ vary with CF and MW? (3) What fraction of LMW DOC can be retained during ultrafiltration, and how does this retention of LMW DOC overestimate the colloidal fraction under different CFs? (4) What artifacts, if any, occur during the diafiltration or desalting operation of macromolecular DOC?

3.2. Retention behavior of LMW DOM

The retention characteristics of LMW molecules (i.e., 0.495 kDa rhodamine 6G and 0.612 kDa glutathione) are shown in Fig. 2. Similar to the case of LMW raffinose (0.595 kDa) reported in Guo and Santschi (1996), permeate concentrations of both 0.5 kDa rhodamine 6G and 0.6 kDa glutathione increased with increasing CF. This is clear evidence of retention of LMW organic molecules during ultrafiltration. Indeed, concentrations of LMW rhodamine 6G (0.5 kDa) in the retentate time series samples were also found to increase with increasing CF (Fig. 2). Using the simplified permeate model to fit the permeate time series data, a linear correlation between $\log C_p$ and $\log CF$ results in slope values of 0.83 for glutathione and 0.40 for rhodamine, which correspond to a permeation coefficient (P_c) of 0.17 for the 0.6 kDa glutathione and 0.60 for the 0.5 kDa rhodamine 6G, respectively. These results indicate that, overall, only 17% of the LMW glutathione and 60% of rhodamine pass through the 1 kDa filter membrane during each cycle of ultrafiltration. The

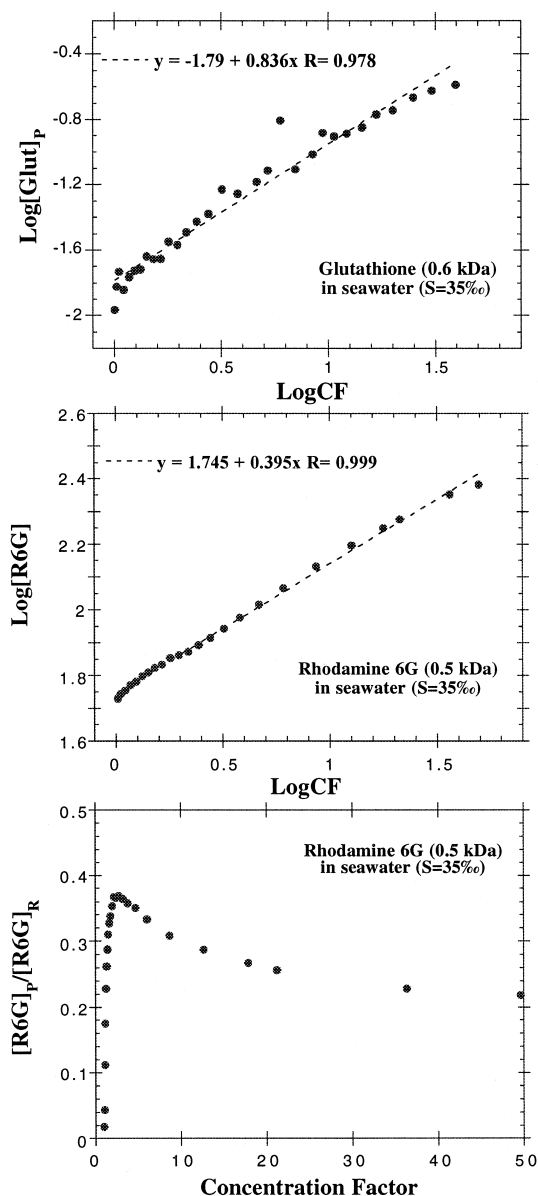


Fig. 2. Permeation behavior of LMW rhodamine 6G (R6G, 0.5 kDa) and glutathione (Glut, 0.6 kDa) spiked into pre-filtered ($< 0.2 \mu\text{m}$) seawater during ultrafiltration. The linear relationship between log CF and log C_p (data of R6G are those of retentate time series) indicates here a constant (but < 1) permeation coefficient and thus, the retention of these LMW molecules.

lower P_c value for glutathione than for the rhodamine 6G could be due to glutathione's lower solubility, higher MW and tendency to polymerize (Tang, 1999).

Using available time series data, the concentration ratio of rhodamine 6G in the permeate to rhodamine 6G in the retentate (i.e., $[\text{R6G}]_p/[\text{R6G}]_R$ or C_p/C_R) is plotted against CF (see Fig. 2). In the low CF region, this concentration ratio of rhodamine 6G is somewhat lower, likely due to sorptive effects at the beginning of the ultrafiltration. However, the overall C_p/C_R ratio is < 0.4 which corresponds to an apparent P_c value of < 0.4 . The C_p/C_R ratio is somewhat lower than the P_c value derived from model fitting for rhodamine 6G (Fig. 2), likely resulting from deviations of model assumptions from the situation during actual ultrafiltration, such as sorption, concentration polarization, and membrane fouling (Buffle et al., 1992; Cheryan, 1998). Similarly, a somewhat lower C_p/C_R ratio (< 0.25) than the model derived P_c value (0.45) was also found for natural DOC (Fig. 1, lower panels). Nevertheless, the overall low C_p/C_R ratio and low P_c value all point to a significant retention of LMW DOC and thus the increase of LMW DOC concentration in the permeate with increasing CF during ultrafiltration, consistent with our previous conclusion derived from a LMW raffinose (0.595 kDa) experiment (Guo and Santschi, 1996). Thus, the retention of LMW DOC can result in an overestimate of the colloidal fraction in seawater, especially under lower CFs. This is just the opposite of the speculations given in Dai et al. (1998).

Fig. 3 shows the retention of operationally defined LMW DOC by a 1 kDa ultrafilter, which was consecutively ultrafiltered using a CF of ~ 20 . When the permeate solution from the first ultrafiltration experiment (UFE #1) was re-ultrafiltered, $\sim 27\%$ of the LMW DOC which had passed through the 1 kDa ultrafilter was retained by the same ultrafilter (Fig. 3). Similarly, about 23% of the LWM DOC in the permeate fraction from the second consecutive ultrafiltration experiment (UFE #2) was again retained during the third ultrafiltration experiment (Fig. 3). These results are consistent with those from our molecular probe experiments (Fig. 2) and confirm that a significant fraction of LWM DOC can indeed be retained during ultrafiltration.

One may still argue that the retention of LMW DOC could rise from the coagulation of LMW DOC while the permeation of LMW DOC could largely result from the "breakthrough" of HMW DOC.

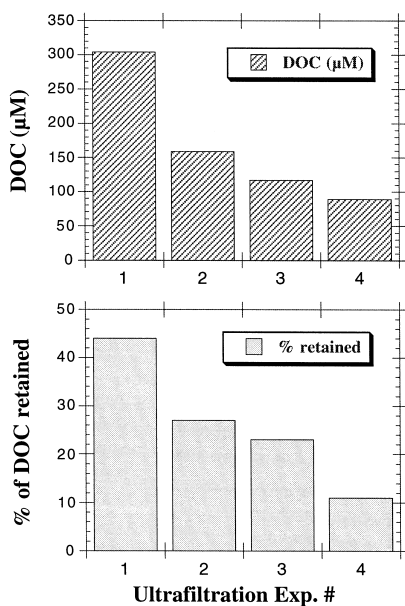


Fig. 3. Results of consecutive ultrafiltration experiments using a pre-ultrafiltered (< 1 kDa) water sample. Initial DOC concentrations are those of previous permeate solutions.

However, there is no evidence for efficient coagulation of LMW DOC (Stumm and Morgan, 1982; Gu et al., 1995). Furthermore, COC/DOC ratios show little change during seawater sample storage for up to 120 h (Guo and Santschi, 1996), indicating that results given in Fig. 3 simply represent the natural retention of LMW DOC but not the coagulation of < 1 kDa LMW DOC. In order to investigate the extent of HMW DOC breakthrough during ultrafiltration, especially under higher CFs, the permeation behavior of HMW DOM is further investigated and discussed in Section 3.3.

3.3. Permeation behavior of HMW DOM

Fig. 4 shows the permeation behavior of HMW molecules added to seawater, including vitamin B₁₂, with a nominal MW of 1.3 kDa, and a fluorescein-tagged 10 kDa dextran. Both vitamin B₁₂ and the 10 kDa dextran show a concentration increase in the permeate with increasing CF (Fig. 4). Note, however, that the levels of 10 kDa dextran concentration in the permeate are very low. When the concentrations of these HMW DOM in the permeate (C_p) are compared with those in the retentate (C_R) fraction,

the ratio of C_p/C_R is only < 0.006 for the 10 kDa dextran, 0.03 for the 3 kDa dextran (without time series data but calculated from integrated permeate and initial concentrations) and ~ 0.14 for vitamin B₁₂ (Fig. 4 and Table 2). Therefore, the so-called “breakthrough” for the HMW molecules is far less than 0.6% for the 10 kDa dextran, even when the CF reaches up to 45. The very small fraction of dextran being detected in the permeate may change with individual membranes. However, this amount of loss to the permeate (< 0.6% in this case) is within the analytical error for most analytical techniques. With a mass balance of at best $\pm 5\%$ in most ultrafiltration experiments, < 0.6% is truly negligible.

Without being able to identify whether HMW or LMW molecules are responsible for the elevated DOC concentration in the permeate, Dai et al. (1998) surmised that HMW molecules breakthrough dominates during ultrafiltration of seawater. Our results here from the HMW molecules contradict their conjecture, and further support our conclusion that the increase of seawater DOC in the permeate with increasing CF is a consequence of the retention of LMW DOC and its constant permeation behavior. In other words, under high CFs, higher concentrations of seawater DOC in the permeate line are predominantly due to the permeation of LMW DOC fractions, and not the breakthrough of the HMW DOC fraction.

For the 3 kDa dextran and vitamin B₁₂, the percentage passing through the 1 kDa pore size is ~ 3% (Table 2) and ~ 15% (Fig. 4), respectively. The fact that the percentage of HMW molecules passing through a rated 1 kDa membrane decreases with increasing sizes or MWs is not unexpected as it is consistent with the nature of the membrane’s pore size distribution (Cheryan, 1998). The membrane’s stated pore size is a nominal MWCO (Amicon, 1995; Cheryan, 1998), which means that a 1 kDa ultrafiltration cartridge actually has pore sizes ranging from, e.g., ~ 0.7 to ~ 1.3 kDa, or even farther, with the majority at ~ 1 kDa. In addition, the nominal ratings are based upon a rejection rate of ~ 90% for specific globular molecules (Cheryan, 1998) and the rejection rate is not necessarily the same for other molecules having the same MW but different molecular configurations. Therefore, it is expected that a portion of HMW molecules, such as vitamin B₁₂,

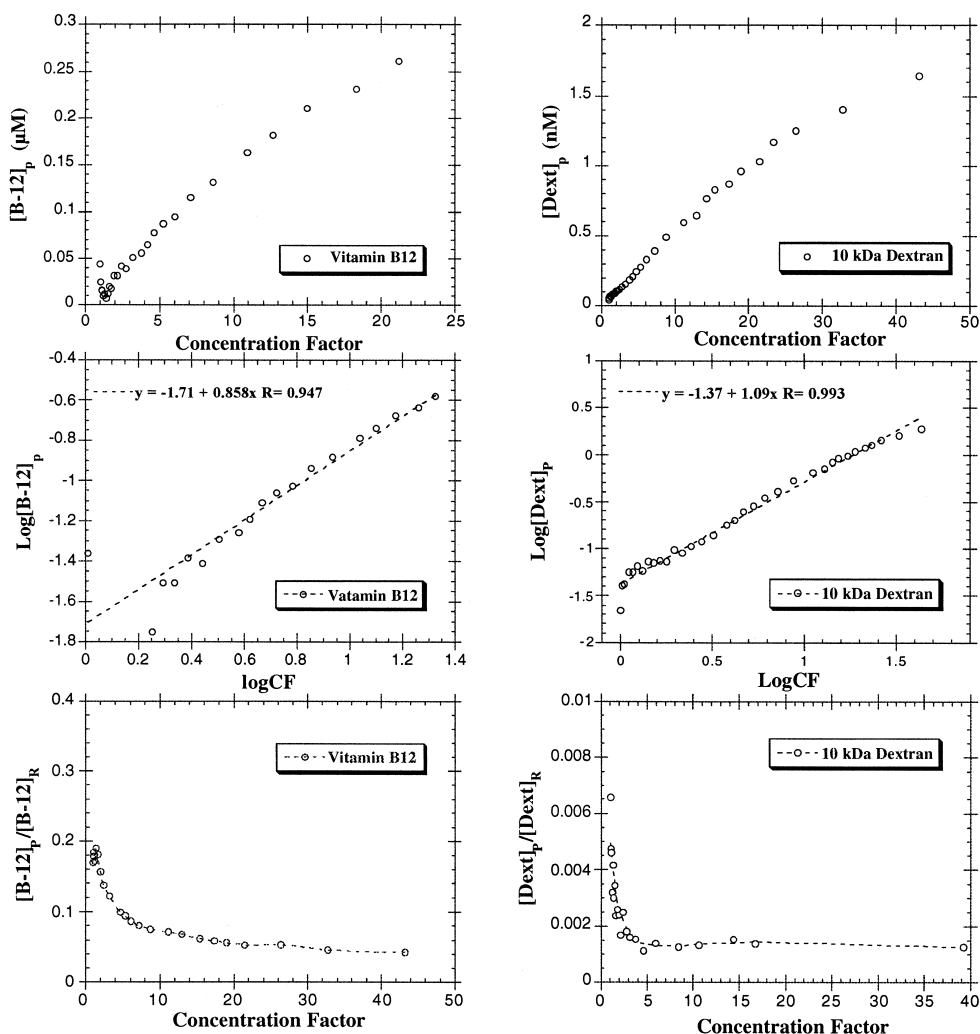


Fig. 4. Permeation behavior of HMW macromolecules (a 1.3 kDa vitamin B₁₂ and a 10 kDa dextran) in seawater. While concentrations of HMW macromolecules in the permeate increase with increasing CF, the concentration ratio of permeate (C_p) to retentate (C_R) decreases with increasing CF and is < 0.002 and < 0.1 for the 10 kDa dextran and vitamin B₁₂, respectively.

will pass through the ultrafiltration membrane since its nominal MW (1.3 kDa) is close to the filter's MWCO. This is also consistent with the manufacturer's specification and our previous calibration results using various standard macromolecules (Amicon, 1995; Guo and Santschi, 1996). As the molecular weight increases to 10 kDa, the amount of HMW DOM permeating through the filter membrane becomes truly negligible (see dextran results in Fig. 4). We thus show that the overall permeation of HMW DOM decreases with increasing sizes or

molecular weights, which is consistent with the stated distribution feature of pore sizes in the ultrafilter membranes (Cheryan, 1998).

Our results also show that the percentage of HMW molecules which permeate through the ultrafiltration membrane actually decreases as the CF increases (Fig. 4), possibly due to the "fouling" of the membrane (Cheryan, 1998). For example, the concentration ratio of the 10 kDa dextran in the permeate to that in the retentate is 0.005 at CF = 2 and decreases to ~ 0.001 at CF > 5 (Fig. 4). Therefore, the small

losses of HMW DOM to the permeate are worst at lower CFs.

3.4. Permeation characteristics of macromolecules during diafiltration

Chemical characterization of many biomolecules and isotopes in marine colloids involves a procedure of desalting or diafiltration (e.g., Benner et al., 1992; Santschi et al., 1995; Guo et al., 1996; Aluwihare et al., 1997; McCarthy et al., 1997). Losses of seawater DOC during diafiltration have been reported in previous studies (Benner, 1991; Guo et al., 1994; Guo

and Santschi, 1996). However, it is still unclear whether this loss is mainly from the LMW or HMW DOC.

Our results from diafiltration experiments show that LMW rhodamine 6G retained during ultrafiltration can be further removed during diafiltration (left panels in Fig. 5). The permeation behavior of rhodamine 6G during ‘diafiltration’ using pre-ultrafiltered seawater or nanopure water is remarkably similar (Fig. 5). In addition, the trends in rhodamine 6G permeation during diafiltration under different initial rhodamine 6G concentrations are basically the

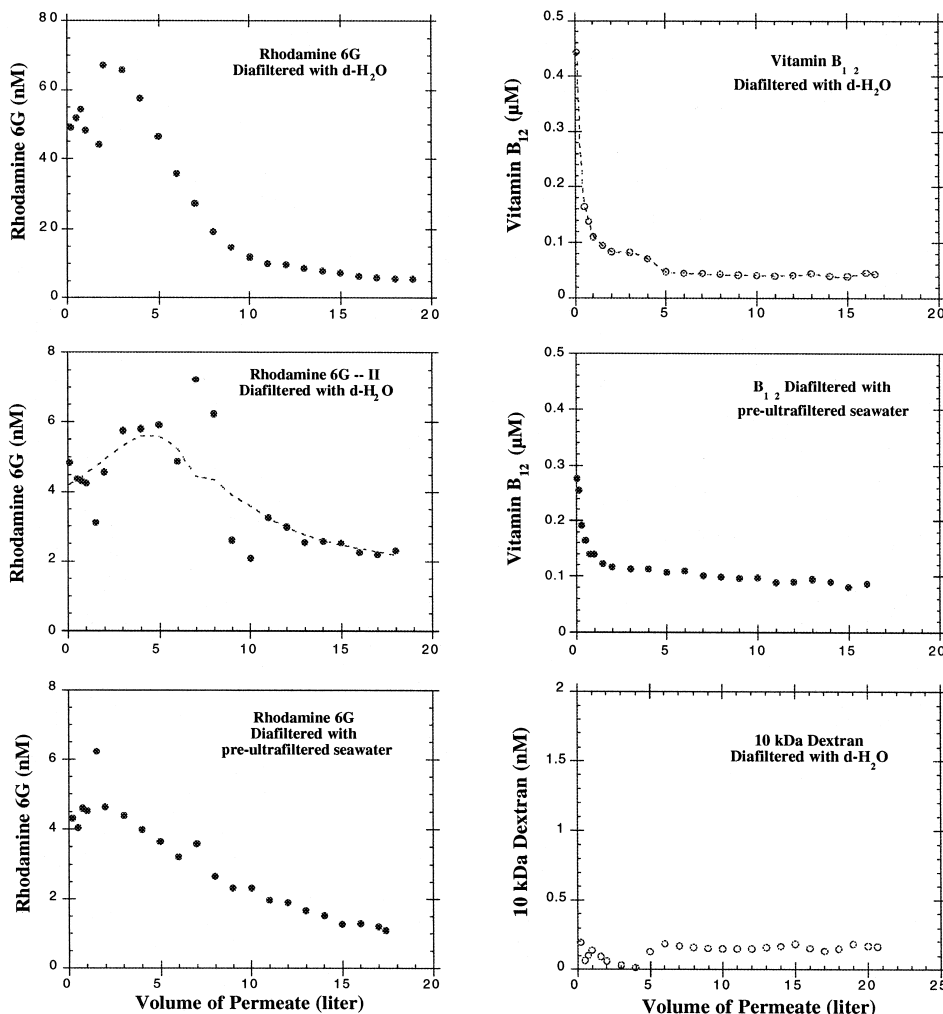


Fig. 5. Permeation behavior of LMW (0.5 kDa rhodamine 6G) and HMW (1.3 kDa vitamin B₁₂ and 10 kDa dextran) macromolecules in seawater during diafiltration (with results diafiltered with nanopure water vs. pre-ultrafiltered seawater). For comparison, results from a natural DOC diafiltration experiment are shown in Fig. 6.

same. This indicates that diafiltration with nanopure water negligibly affects the permeation behavior of LMW DOM, even though changes in ionic strength and pH may affect the speciation, charge and interactions of aquatic macromolecules (Ghosh and Schnitzer, 1980; Perdue, 1989).

As for the HMW counterparts, undetectable or negligible amounts of 10 kDa dextran were found in the diafiltration permeate (Fig. 5 and Table 3). Very low concentrations of HMW molecules in the diafiltration permeate indicate that diafiltration using nanopure water does not significantly affect the retention of HMW molecules. For example, in a diafiltration experiment with pre-ultrafiltered seawater, concentrations of 10 kDa dextran in the permeate fraction were below the detection limit. In other diafiltration experiments with nanopure water, the 10 kDa dextran concentrations in the permeate were either below the detection limit or close to instrumental background levels. Therefore, diafiltration with nanopure water without major seawater cations (e.g., calcium and magnesium) probably does not noticeably affect the retention of macromolecules even though it is known that these cations, especially Ca, could enhance coagulation of gels and microparticles in seawater (e.g., Chin et al., 1998).

Fig. 6 (lower panel) shows the variation of natural seawater DOC concentration in the permeate during diafiltration. The measured apparent COC percentage in the total DOC in this seawater sample is $\sim 68\%$ under a CF of 34. The predicted COC percentage for this sample is actually $\sim 47\%$ from the permeation model fitting with a P_c value of 0.67, an initial total $[\text{DOC}] = 241 \mu\text{M}$, and an intercept of 1.88 in the relationship between $\log C_p$ and $\log \text{CF}$ (Fig. 6). After diafiltration, DOC in the integrated permeate

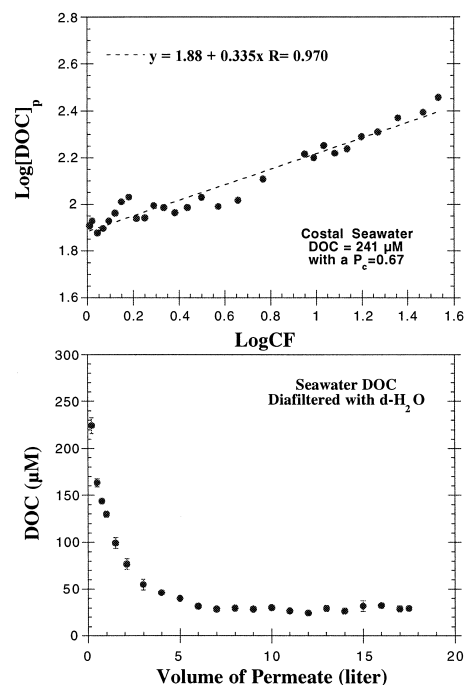


Fig. 6. Permeation behavior of natural DOC during diafiltration. Interestingly, the final retained DOC after diafiltration is similar to the predicted COC percentage using the model derived P_c and the initial LMW DOC concentration, indicating that losses of seawater DOC during diafiltration are dominantly in the LMW fraction.

accounts for $\sim 17\%$ of the initial DOC, which is surprisingly very close to the difference (21%) between the measured % of COC and the model predicted % of COC (i.e., $21\% = 68\% - 47\%$). This demonstrates that the loss of natural DOC during diafiltration is indeed dominated by the LMW DOC, consistent with our conclusions derived from both LMW and HMW molecular probes discussed earlier.

Table 3

Comparison of macromolecule concentrations between permeate and final retentate during diafiltration experiments

Diafiltered with	Molecules	Molecular weight (kDa)	C_p (nM)	Final C_R (nM)	C_R/C_p (average)
Nanopure water	Rhodamine 6G	0.495	2.5	0.21	0.1
Nanopure water	Vitamin B ₁₂	1.33	60	1450	24
Nanopure water	Dextran	10	BD	> 200	Infinite
Pre-ultrafiltered seawater	Rhodamine 6G	0.495	2.4	17	7
Pre-ultrafiltered seawater	Vitamin B ₁₂	1.33	52	1550	30
Pre-ultrafiltered seawater	Dextran	10	BD	225	Infinite

BD = below detection limit.

3.5. Overall mass balance and sorptive loss of macromolecules

Mass balance and overall loss to the membrane of standard macromolecules and seawater DOC are listed in Table 4. The percentage of seawater DOC recovered during ultrafiltration ranged from 94% to 95% under a CF of 40–100. This corresponds to an overall DOC loss of 5–6% to the ultrafiltration membrane (Table 4). Recovery rates of DOC lower than 100% may largely result from the difficulty of complete recovery under high retentate DOC concentrations when using high CFs.

Recovery rates and sorptive losses of standard macromolecules during ultrafiltration differed amongst molecules. For example, LMW rhodamine 6G (0.5 kDa) had an overall recovery of 92% whereas 0.61 kDa glutathione only had a 30% recovery (Table 4). Correspondingly, losses to the membranes were 9% and 70% for rhodamine 6G and glutathione, respectively. The recovery rate for vitamin B₁₂ was only 69%, i.e., about 30% was lost to the membrane, while losses of dextran with different MWs were minimal. Glutathione, a peptide with six amino acids, had the highest sorptive loss among macromolecules we used (Table 4). Higher sorptive losses of protein standards (e.g., lactalbumin) to membranes have been reported previously (Gustafsson et al., 1996; Dai et al., 1998). It seems that sorptive losses to the membrane and thus the overall mass balance of macromolecules are largely determined by their physicochemical properties, such as solubility, hydrophobicity, and isoelectric points

(Gustafsson et al., 1996), and to lesser extent by their sizes or MWs.

For those HMW molecules, 3 kDa dextran had a 93% recovery and 10 kDa dextran had a 95% recovery (Table 4). Dextran has previously been shown to exhibit low sorptive loss by most membranes compared with protein standards (Cheryan, 1998). Indeed, both 3- and 10-kDa dextrans had low percentage loss (5–7%) and high recovery rates in our experiments. Gustafsson et al. (1996) systematically studied the sorption behavior and loss mechanisms of macromolecules to different types of membranes. They also found that losses of the 3 kDa dextran to the Amicon S10N1 membrane were amongst the lowest, with one case without any loss. Thus, our results reported here using the 3 kDa dextran are consistent with those reported in Gustafsson et al. (1996). However, Dai et al. (1998) recently reported up to 40% losses of the 3 kDa dextran to the same Amicon S10N1 membrane. Higher losses of dextran reported in Dai et al. (1998) could have been related to their operational protocols.

3.6. Ultrafiltration behavior of LMW metals

In order to further examine the ultrafiltration behavior of LMW metals, selected radioactive metals (⁵⁴Mn, ⁵⁹Fe, ⁶⁰Co, ⁶⁵Zn, ¹⁰⁹Cd, ^{110m}Ag, ¹³³Ba, and ²³⁴Th) were added to a 1 mM EDTA solution or pre-ultrafiltered seawater containing < 1 kDa DOC to produce predominately LMW complexes. Time series results show that concentrations of radioactive metals in the permeate (C_p) increased with increas-

Table 4

Fraction of macromolecules and seawater DOC in the ultrafiltration permeate (UP), ultrafiltration retentate (UR), diafiltration permeate (DP), and diafiltration retentate (DR), as well as recovery and loss

Molecule	F (UP)	F (UR)	Recovery during UF	F (DP)	F (DR)	Overall recovery ^a	Overall loss
DOC	0.31	0.63	0.94	–	–	–	0.06
Rhodamine 6G	0.34	–	–	0.43	0.15	0.92	0.08
Glutathione	0.29	0.01	0.30	–	–	–	0.70
Vitamin B ₁₂	0.19	0.51	0.69	–	–	–	0.31
3 kDa Dextran	0.03	0.90	0.93	–	–	–	0.07
10 kDa Dextran	0.004	0.95 ^b	0.95	–	–	–	0.05
DOC-2	0.36	0.59	0.95	–	–	–	0.05

^aOverall recovery rate includes all fractions in ultrafiltration and diafiltration (100% recovery = 1).

^bIncludes fraction of cartridge washing with nanopure water.

ing CF (Fig. 7), as is expected from a permeation model with a $P_c < 1$. All metals measured showed a

constant slope value in the relationship between $\log C_p$ and $\log CF$, indicating a constant P_c value.

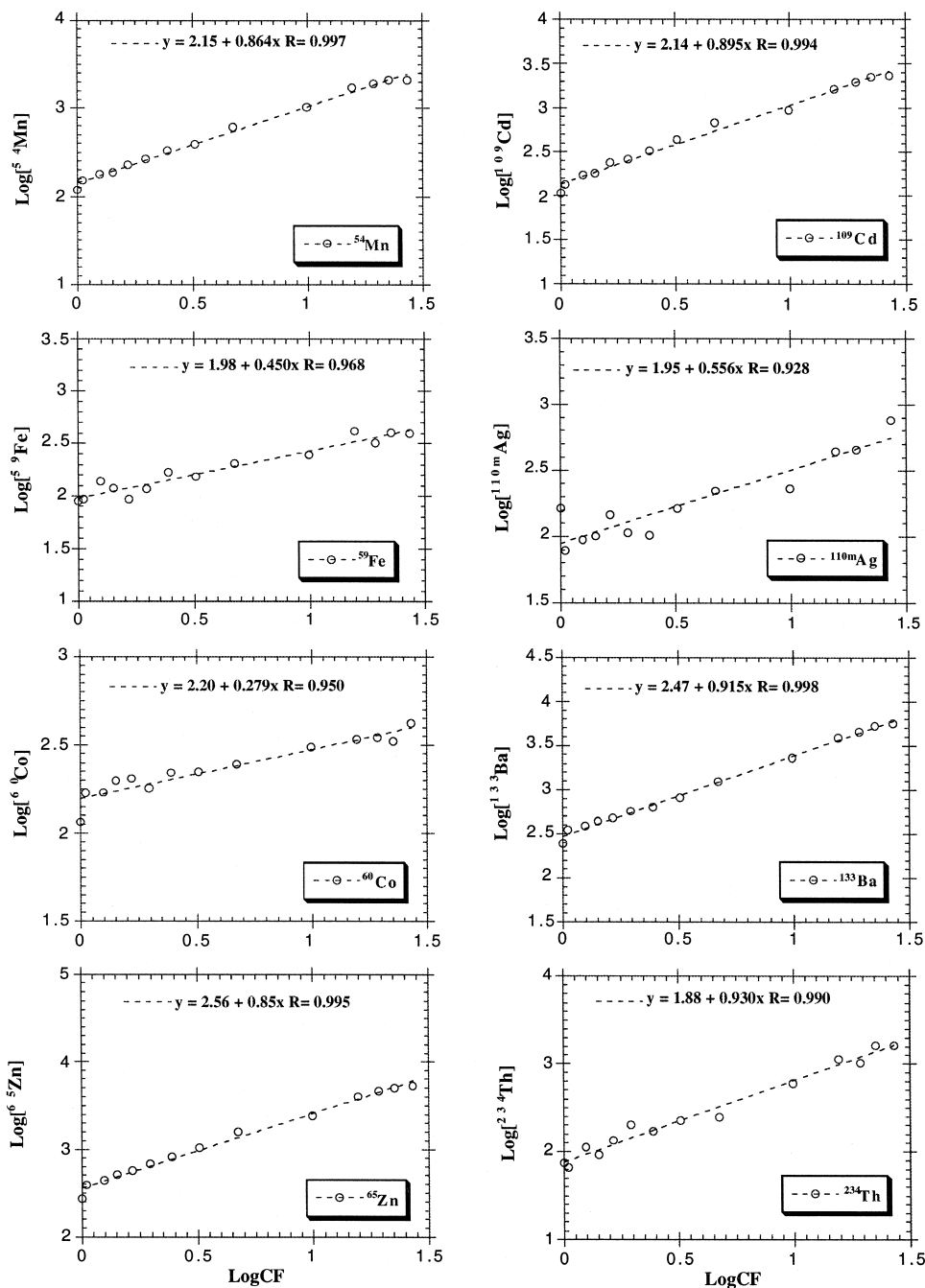


Fig. 7. Log–log plots using radioactive metals (dpm) complexed by EDTA in the discrete permeate vs. CF during ultrafiltration (Ag data are from retentate time series samples). While the slopes (and thus the permeation coefficients) are different for different metals, their permeation behavior can be predicted by the permeation model.

Similar to the situation in macromolecules, if LMW metals pass through the ultrafilter without being concentrated, the concentrations of metals in the permeate should be constant, i.e., $P_c = 1$. However, the opposite is true. From slope values in Fig. 7, P_c values ranged from ~ 0.1 to 0.72, depending on metals (Table 5). Constant P_c values for each metal and P_c values less than 1 imply that varying fractions of LMW trace metals can be retained during ultrafiltration. Therefore, a low CF may significantly overestimate the colloidal abundance of certain metals as it does for the ultrafiltration of DOC.

Our results from spike experiments reported here are also consistent with those measured for selected stable trace metals in natural seawater (Wen et al., 1996). For example, Wen et al. (1996) demonstrated that Cu concentrations in discrete permeate samples increased with increasing permeation volume or CF and the corresponding “colloidal” percentage of Cu decreased from $\sim 42\%$ of the total dissolved Cu at a CF of ~ 11 to $\sim 25\%$ when the CF approached ~ 23 . As the CF increased, the percentage of colloidal Cu gradually decreased. Since the ultrafiltration behavior of Cu could be well predicted by the permeation model with a constant P_c of 0.56 for the LMW Cu fraction (Wen et al., 1996), higher CFs are required for those metals which are strongly bound to LMW DOC.

3.7. Recommended optimum CF

It seems that most seawater ultrafiltration time series data sets can be fitted by the permeation model, which gives rise to a P_c value < 1 for both

DOC and certain metals (e.g., Guo and Santschi, 1996; Dai et al., 1998). If a LMW chemical species in seawater has a P_c value of < 1 , then its retention is a consequence of ultrafiltration. From this point of view, a higher CF is the natural choice for the ultrafiltration of seawater to quantify marine colloids.

One may speculate that higher CFs retain less HMW materials. From our experimental results using vitamin B₁₂ with an MW of 1.3 kDa which is close to the membrane’s MWCO (i.e., 1 kDa), the permeation was actually the highest when the CF was < 5 , with the C_p/C_R concentration ratio decreasing from ~ 0.2 at lower CFs to < 0.05 at higher CFs (Fig. 4). This demonstrates that breakthrough of HMW molecules is less likely under high CFs as was previously concluded by Guo and Santschi (1996) and proven by this new evidence using molecular probes.

Guo and Santschi (1996) previously recommended an optimum CF of > 40 . The results from the present study support this recommendation, and also suggest a high CF. Since the permeation of HMW molecules is minimal even under high CFs, using a higher CF will minimize the retention of LMW materials. However, there are lesser known effects resulting from high CFs, such as concentration polarization, clogging, and losses to the membrane (Buffle et al., 1992; Cheryan, 1998), which, however, were not dominant in our experiments, but would need to be further evaluated. Most importantly, we recommend to keep a similar CF for all processed samples. Ideally, the LMW fraction, and thus, the colloidal percentage of specific chemical species in seawater can be determined from model fitting of the time series permeate data, as shown previously in Guo and Santschi (1996) and in the present study.

Table 5
Permeate coefficients (P_c) of LMW radioactive metals resulting from analysis of relationships between $\log C_p$ and $\log CF$

Metal	Slope	P_c	R	Level of significance (p)
Co	0.28	0.72	0.95	0.001
Zn	0.85	0.15	0.99	0.001
Cd	0.89	0.11	0.99	0.001
Ba	0.91	0.09	0.98	0.001
Fe	0.45	0.55	0.97	0.001
Mn	0.86	0.14	0.99	0.001
Ag	0.56	0.44	0.93	0.001
Th	0.93	0.07	0.99	0.001

4. Conclusions

Through controlled laboratory experiments, the permeation and retention behavior of molecular probes during ultrafiltration were re-examined using an S10N1 Amicon ultrafiltration cartridge (1 kDa MWCO). All tracer ultrafiltration experiments were carried out from low to high CFs in a natural seawater.

ter medium. The permeation and retention of macromolecules during diafiltration were also examined to investigate possible artifacts during the desalting process. Seawater was also ultrafiltered to compare the ultrafiltration behavior of natural DOC with those of standard macromolecules.

Significant amounts of LMW molecules are retained during ultrafiltration, resulting in an increasing permeate concentration with increasing CF. Concentration ratios of a LMW rhodamine 6G (0.5 kDa) in the permeate to that in the retentate are lower than 0.4, indicating that over 60% of the rhodamine 6G is actually being retained, even when CF reaches 50. Only small amounts of HMW molecules, on the other hand, permeate across the ultrafilter membrane. The percentage of HMW molecules passing through the membrane decreases rapidly with increasing MW and becomes truly negligible for a 10 kDa dextran.

It was found that most LMW molecules, which are retained during ultrafiltration, can be further removed during diafiltration, and losses of HMW materials during diafiltration are generally negligible. The permeation behavior of macromolecules is remarkably similar during diafiltration with nanopure water and pre-ultrafiltered seawater, indicating that the influence of diafiltration on the retention and permeation behavior of macromolecules is minimal.

The ultrafiltration behavior of seawater DOC can be well predicted by a permeation model, resulting in a permeation coefficient (P_c) of < 1 . LMW complexed radioactive metals show a similar ultrafiltration behavior to that of macromolecules in seawater, and can be well predicted by a permeation model. The percentage of colloidal species in seawater can be estimated from the slope and intercept values derived from the relationship between $\log C_p$ and $\log CF$.

Based on the results from our laboratory experiments, high CFs (> 40) are recommended for quantifying marine colloids by ultrafiltration methods. We believe that results which lead to previous recommendations for low CFs are biased and therefore should be used with caution.

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