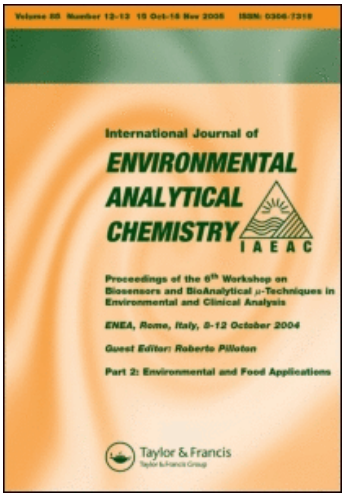


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

# Concentration of Hydrocarbons, Acyl Lipids, Ketones and Alcohols in Marine Colloids by Cross-flow Filtration

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**To cite this Article** Liu, Qingjun and Parrish, C. C.(1996) 'Concentration of Hydrocarbons, Acyl Lipids, Ketones and Alcohols in Marine Colloids by Cross-flow Filtration', International Journal of Environmental Analytical Chemistry, 64: 2, 135 — 145
**To link to this Article: DOI:** 10.1080/03067319608028342
**URL:** http://dx.doi.org/10.1080/03067319608028342

*Intern. J. Environ. Anal. Chem.*, Vol. 64, pp. 135–145
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# CONCENTRATION OF HYDROCARBONS, ACYL LIPIDS, KETONES AND ALCOHOLS IN MARINE COLLOIDS BY CROSS-FLOW FILTRATION

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*(Received, 28 December 1995)*



The use of cross-flow filtration (CFF) to concentrate hydrophobic material in marine colloids was investigated. The efficiencies of CFF to fractionate and concentrate colloids from water samples were evaluated by determining blanks and mass balances of hydrocarbons, acyl lipids, ketones and alcohols in marine colloids from algal cultures and seawater. It was possible to minimize artifact formation in the CFF system through the use of Teflon components, by not recycling retentate fractions, and by optimizing cleaning procedures and filtration conditions. The hydrocarbon and total lipid blanks in the CFF system were 0.5 μg and 7 μg respectively. For the major lipid classes in both algal culture and seawater samples, the mass balances on the 0.45 μm filter and the 10 K Dalton cut-off filter were 80–120%. This indicates that contamination from the CFF system and adsorption onto the components have been minimized.

**KEY WORDS:** Lipids, tangential path, blanks, mass balances.

## INTRODUCTION

The amount of dissolved organic carbon (DOC) and colloidal organic carbon (COC) in the ocean is at present controversial and difficult to compare because of differences in blank corrections, analytical methodology, spatial variability, and use of different filters, and also because of the flexible nature of colloids[1]. However, most independent investigations indicate that a significant fraction (10–40%) of the "dissolved" organic matter in seawater exists in colloidal forms[2–8], indicating that marine colloids play an important role in oceanic carbon cycling.

Studies assessing the quantities and composition of colloids in aquatic systems require an efficient collection of the colloidal/macromolecular materials. New ultrafiltration techniques such as cross-flow filtration (CFF) can overcome some shortcomings in traditional pass-through filtration methods and thus become a powerful tool in concentrating colloidal/macromolecular material from aquatic environments. Although CFF is being increasingly used to fractionate and concentrate colloids, studies on CFF itself are few. Also, few publications exist which investigate hydrocarbons and more polar lipid classes on marine colloids. The large numbers of sub-micrometre particles in the upper ocean provide a large solid/liquid interface[1] which may be an important site for

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adsorption of hydrophobic material including hydrophobic contaminants[9]. This study evaluates the use of the CFF system for studies of colloidal hydrophobic compounds through a detailed examination of blanks and recoveries.

## MATERIALS AND METHODS

### *Marine samples*

Laboratory algal culture samples with high colloid concentrations and seawater samples with relatively low colloid concentrations were used in this study. Laboratory cultures of two species of microalgae (*Isochrysis galbana* and *Chaetoceros muelleri*) were sampled. The microalgal cells were grown in nutrient-replete semi-continuous cultures. The density at which the samples were collected was 4–6 $\times$ $10^6$ cells/mL and the growth rate varied from 0.24 to 0.90 divisions/day. The sample volume was about 1–2 L for each sample. Before the culture samples were collected in a 1 L dark Teflon container, the containers were rinsed three times with culture. After sample collection, each sample was immediately filtered.

Four seawater samples were collected in a time course following the spring phytoplankton bloom in Conception Bay, on the east coast of Newfoundland. Conception Bay is about 70–100 km long and 20–40 km wide. It is a fjord-like embayment opening directly onto the Newfoundland Shelf. The bay has a 170 m deep sill at the mouth and a maximum depth of 300 m in the central basin. Over the sampling period, seawater temperatures ranged from 1–10°C, while salinites remained close to 35. The samples were taken from the surface mixed layer (10–28 m) at the Brigus-Long Pond (BRLP4) station (47°32.2′N, 53°06.0′W) by Niskin bottle or Teflon-lined Go-Flo sampler. The depth at the sampling site was 215 m. In order to remove larger particles, the sea water samples were passed through a 75 μm copper sieve. Then, about 15–35 L of seawater were collected in 20 L high density polyethylene containers. The containers were precleaned with distilled water for several days. Immediately before seawater samples were collected, the containers were rinsed with seawater three times. All of the seawater samples were transported to the laboratory within 2 h of collection and then immediately filtered.



### *Filtration and ultrafiltration*

Samples were filtered through glass-fibre filters (Whatman GF/C or Gelman A/E) and then ultrafiltered using a Millipore CFF apparatus to obtain different particle size fractions. Glass fibre filters were precombusted at 450°C for 20 h to remove organic impurities. Filtration with glass fibre filters was completed within 20 min by conventional flow through methods under slight vacuum and under a blanket of nitrogen.

The Millipore CFF system (Figure 1) consists of a Minitan® filtration unit (Millipore Corporation, Bedford, MA), a Millipore pump (XX 80 000 00), a pressure gauge, and vessels (glass or polyethylene). We connected the components with Teflon tubing, fittings and valves. The Minitan® filtration unit is a tangential flow device consisting of Minitan® filter plates, open channel retentate separators, an acrylic housing, and two stainless steel plates. Four Minitan® microporous filter plates (Cat. No. HVLP OMP 04) or four Minitan® ultrafiltration filter plates (Cat. No. PTGC OMP 04) were used. The

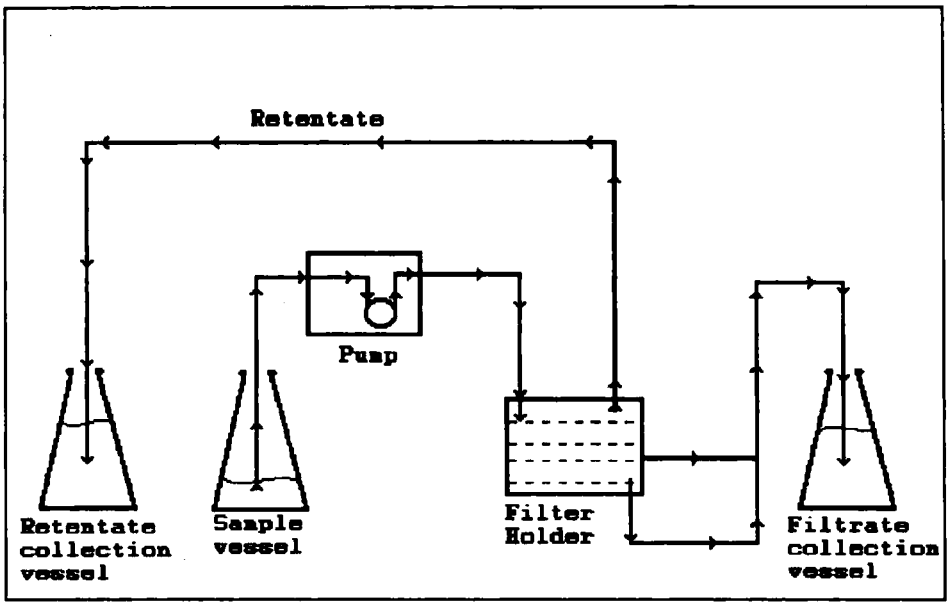



**Figure 1** Diagram of Millipore cross-flow filtration system.



microporous filter plates are constructed by heat-binding two hydrophillic Durapore® filter membranes to a polyvinylidene fluoride (PVDF) backing. The nominal pore size of the Durapore® filter membrane is 0.45 μm. Each ultrafiltration filter plate consists of two polysulfone filter membranes heat-bonded to a polypropylene backing. The ultrafiltration membrane has a nominal MW cut-off of 10,000. Flow rate and direction are adjusted with the Millipore pump.

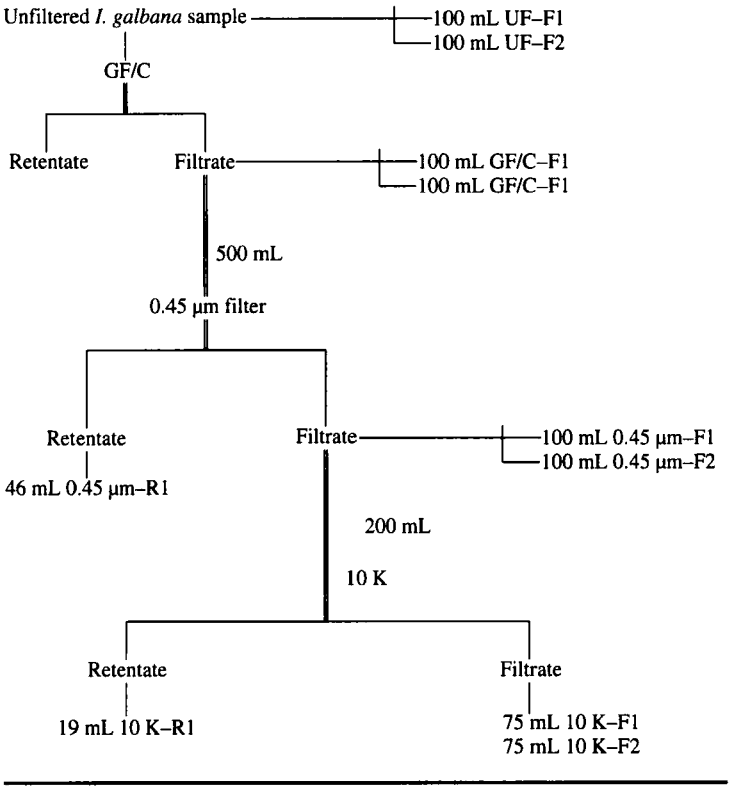
Cross-flow filtration was conducted in a covered chamber (180 L) in which the whole Millipore CFF system (Figure 1) was placed. When filtering, the temperature in the cold box was held at 5°C. A gentle nitrogen flow was delivered to the mouth of each vessel: the sample, retentate, and filtrate collection vessels. The chamber was kept dark. The filtration pressure was less than 1.5 $kg^2cm^{-1}$ and flow rate was ~3 L $h^{-1}$. CFF filtration was completed within 2–3 h for culture samples and 5–10 h for seawater samples.

The filtration flow diagram for a culture sample is shown in Table 1. The filtration procedure for other culture and seawater samples was similar to the sample in Table 1 except that large volumes of seawater samples were used.

### *Lipid extraction*

The filtration fractions of culture samples were extracted 4 times with chloroform in a 1 L separatory funnel. For each fraction (< 200 mL), 60 mL (20 mL, 20 mL, 10 mL, and 10 mL) of chloroform was used. The filtration fractions of seawater samples were extracted 4 times with chloroform in a 4 L amber glass bottle. The volume of chloroform used in the extraction depended on the volume of the seawater sample. Usually, 60 mL of chloroform was used to extract 1 L of seawater sample. Before extraction, 5 drops of

**Table 1** Filtration flow diagram for an *Isochrysis galbana* (T–ISO) culture sample.






methanol were added to each sample to help disrupt membranes and to lower enzyme activity. After the first extraction, 6 drops of sulphuric acid per L of sample was added to adjust the pH to pH 4–5 to facilitate the extraction of free fatty acids.

The resulting lipid extracts were concentrated to about 2 mL in a Rotavapor under vacuum at < 30°C. All the concentrated extracts were transferred to a small vial, concentrated to 2 mL under a gentle nitrogen flow, and then stored in a freezer until measurement of lipids.

### *Lipid analysis*

The extracts were analyzed[10] in an Iatroscan MK-5 TLC-FID Chromatographic analyzer (Iatron Laboratories, Inc. Tokyo, Japan). The relatively precise technique of thin-layer chromatography with flame ionization detection (TLC-FID) was selected to analyze the complete suite of lipid classes, hydrocarbons, acyl lipids, ketones, and alcohols, in the samples because it does not necessitate the preparation of volatile derivatives as with gas chromatography, thus simplifying the analytical procedures.

Extracts were spotted on Chromarods-SIII using a Hamilton syringe fitted into a Hamilton repeating dispenser with divisions of 0.5 μL. The volume of sample usually

was in the range of 1 to 20 μL. When spotting samples or standards on the rods, the rack holding the rod was placed on a warm hot-plate and nitrogen was blown over the rods so that the chloroform evaporated quickly.

After spotting was finished, the rack holding the rods was put in a constant humidity (~30%) chamber. Before each development, the rods were conditioned for 5 min. Before separation of lipid classes, the rods were put into a developing tank containing acetone to narrow and align the spotted band. The rods were then conditioned for 5 min. Usually, four development solvent systems were used to separate the lipids on the rods and the rods were scanned three times. The first solvent system was hexane/diethyl ether/formic acid (99:1:0.5 v/v/v). After a 25 min and then a second 20 min development, the rods were scanned to the position behind the ketone peak to obtain the first chromatogram. The scanner may be programmed to scan a part of a rod, and then the remaining unscanned lipids can be well separated in the next development solvent system. The second chromatogram was obtained after a 40 min development in the second solvent system, hexane/diethyl ether/formic acid (80:20:0.1 v/v/v), and scanning to the lowest point behind the sterol peak. The remaining lipids were developed twice in acetone for 15 min and then twice in chloroform/methanol/water (50:40:10 v/v/v). The third chromatogram was obtained by scanning the entire Chromarod. The three chromatograms obtained by development in the four different solvent systems were combined by computer to form a single chromatogram using T Data Scan software (RSS Inc., CA).



*Millipore CFF system blank*

Lipid-free water was prepared by extracting distilled water with chloroform. The extracted water was bubbled with nitrogen until no chloroform could be detected by smell. After the Millipore system was cleaned with distilled and lipid-free water, 500 mL of lipid-free water was filtered through the CFF system 5 times. The lipid-free water that had been filtered was then extracted with chloroform, and the extracts were concentrated under nitrogen. The lipid class composition in the extracts was measured using the TLC-FID method. These analytical results were compared with the Chromarod-Iatroscan system blank and the extracting solvent blank. These two blanks were subtracted from the results to obtain the Millipore CFF system blank. The Chromarod-Iatroscan system blank was the value obtained after development when no sample was applied to the rod. The solvent blank was obtained by concentrating a known amount of solvent and spotting it on the rod.

*Mass balance on filters*

Before the seawater or culture samples were filtered, the Millipore CFF system was thoroughly cleaned with distilled water and lipid-free water and then a small volume of sample, usually about 40 mL for cultures and about 150 mL for seawater samples, was filtered and discarded. The microporous filters with 0.45 μm rated pore size were usually used first in the CFF system. The filtrate of the 0.45 μm filter was then filtered through the ultrafiltration membrane with a 10,000 Da cut-off (pore size ~3 nm). In this way, different size fractions were obtained and then analyzed. In order to calculate the mass balance (%), the volume in all fractions including prefiltered, retentate, and filtrate fractions was measured and recorded (e.g. Table 1).

## RESULTS AND DISCUSSION

### *Characteristics of CFF*

CFF differs from traditional flow-through filtration in that the flow is parallel rather than perpendicular to the filter. The tangential flow flushes particles from the surface of the filter, reducing clogging, increasing filtration rates, and making particle cut-offs consistent with nominal pore sizes. Whitehouse *et al.*[11] confirmed that colloids do not accumulate on the filter membrane when CFF was employed. They found a very good linear relation between flux and increasing pressure when seawater was filtered by CFF. The advantages of CFF have lead it to be widely used to fractionate and concentrate marine colloids (Table 2).

This study differs from others in that we chose to use a system designed for smaller volumes, and we examined its application in the complete suite of lipid classes. In using the Minitan CFF system we were able to operate with small dead volumes (< 10 mL), and to reduce possibilities for absorptive loss of lipids on the components of the system.

### *Optimization of the CFF system*



In order to optimize CFF for hydrophobic compounds and to avoid contamination from system materials, Teflon fittings, valves and tubing were used in the system. Various solutions including 50% methanol in water, 0.1 M NaOH, 500 ppm NaOCl, 3.5% NaCl, and purified water were used to clean the CFF system. The solutions did not show obvious advantages in decreasing the value of the lipid blank by comparison with purified water, so they were not employed to avoid the risk of changing the pore size of the ultrafilter. Purified water was found to be sufficient if used continuously for 4 days. After the CFF system was cleaned with distilled water and then lipid-free water, the samples were filtered. In order to avoid chemical transformations of organic matter in the filtration fraction, the whole CFF system was placed to a 180 L covered box which contained a fan and temperature control unit. The CFF operation was conducted in the dark, at 5°C, and under nitrogen. To eliminate any chance of filter clogging, the retentate fractions were collected and not recycled through the filters.

### *CFF system blank*

After optimizing the CFF system, the total lipid in the CFF system (Table 3) for both filters (0.45 μm and 10 kDa cut-off) was approximately 6–8 μg (0.5 μM C). Hydrocarbon, triacylglycerol and acetone-mobile polar lipids contributed about 6–7%, 60–70%, and 20–25% of the total lipids found in the CFF blank, respectively. The pattern of the lipid class distribution in the CFF system blank for both filters is almost the same. Since both filters were made of different materials, it seems unlikely that triacylglycerol and acetone-mobile polar lipids came from the filter materials. These 2 contaminants may have come from other components of the CFF system. Phthalate esters have previously been identified in the Millipore Pellicon system blank[11,12]. These esters, which elute close to triacylglycerols on Chromarods[13], may have contributed to triacylglycerol apparently being the major lipid class in the CFF system blank.

Direct measurement of organic carbon in CFF filtrates without extracting and concentrating procedures, also indicated the contamination in the CFF system had been

**Table 2** Cross—flow filtration (CFF) in marine application.

| *Reference* | *CFF appratus & filter* | *Particle size range* | *Volume* | *Research area* |
|---|---|---|---|---|
| Whitehouse *et al.*[12] | Millipore Pellicon CFF Polysulfone PTGC filter | 10 kD–1 μm | 200 L | Organic carbon (OC) and aliphatic hydrocarbons in the Baltic Sea |
| Moran and Moore[3] | Millipore Pellicon CFF Polysulfone PTGC filter | 10 kD–0.45 μm | 70–100 L | OC and trace metals in coastal and open ocean waters off Nova Scotia |
| Whitehouse *et al.*[14] | Osmonics Inc. OSMO/AN filter | 10 kD–0.2 μm | – | OC in seawater and river water |
| Whitehouse *et al.*[11] | Millipore Pellicon CFF Polysulfone PTGC filter | 10 kD–1 μm | 6–102 L | OC and trace metals in seawater and river water |
| Baskaran *et al.*[15] | Amicon CFF Amicon H10P10–20 filter | 10 kD–0.4 μm | 200 L | Th–232 and Th–234 in surface water from the Gulf of Mexico |
| Benner *et al.*[4] | Amicon DC10L CFF spiral—wound polysulfone filter | 1 kD–0.2 μm | 200 L | OC and carbohydrates in the North Pacific Ocean |
| Moran and Buesseler[16] | Millipore Pellicon CFF 10 k NMW cut—off filter | 10 kD–0.2 μm | 80–100 L | Residence time of colloids in western North Atlantic seawater |
| Kepkay *et al.*[17] | Millipore Pellicon CFF Polysulfone PTGC filter | 10 kD–0.2 μm | 100 L | OC produced during a spring bloom in a marine inlet in Nova Scotia |
| Guo *et al.*[7,18] | Amicon DC10L CFF Amicon S10N1 & H10P10—20 filter (1k & 10 k NMW cut—off) | 1 kD–10 kD | 200 L | OC in the Gulf of Mexico and off Cape Hatter as |
| Santschi *et al.*[19] | Amicon DC10L & DC30L CFF 1 k & 10 k NMW cut—off filter | 10 kD–0.2 μm | 200–1,000 L | Residence time of colloidal matter in seawater and mechanism for colloid turnover |
| Benoit *et al.*[20] | Amicon CFF Amicon H10P10–20 filter | 10 kD–0.5 μm | 100–200 L | Trace metals in Texas estuaries |
| This study | Millipore Minitan CFF Polysulfone PTGC filter | 10 kD–1 μm | 1–35 L | Hydrocarbons and more polar lipid classes in Newfoundland seawater and algal cultures |

minimized. Organic carbon in the filtrates of artificial seawater (inorganic salts and distilled water) was measured with a new solid state NDIR detector by Dr. Kepkay in Bedford Institute of Oceanography, NS. The DOC concentrations in the filtrates for both 0.45 μm and 10 kDa filters in the CFF system were less than 14.5 μM C, approaching the detection limit.

**Table 3** Contributions to the lipid class blank (μg) by each step in the procedure: the Chromarod-Iatroscan system, the extracting solvents, and the cross-flow filtration unit.

| *Lipid classes* | *Chromarod & Iatroscan (n = 6–16)* | *Extracting solvent (n = 10–20)* | *Cross-flow filtration* | |
|---|---|---|---|---|
| | | | *0.45 μm (n = 5–9)* | *10 kDa (n = 2–9)* |
| HC | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.4 ± 0.6 | 0.5 ± 0.2 |
| SE | N.D. | 0.5 ± 0.0 | 0.0 ± 0.1 | 0.2 ± 0.3 |
| KET | N.D. | 0.2 ± 0.0 | N.D. | 0.1 ± 0.1 |
| TAG | 0.4 ± 0.0 | 2.9 ± 2.0 | 3.8 ± 7.3 | 4.7 ± 5.4 |
| FFA | 0.2 ± 0.0 | 0.3 ± 0.2 | 0.1 ± 0.6 | –0.2 ± 0.6 |
| ALC | N.D. | 0.5 ± 0.1 | 0.1 ± 0.2 | –0.2 ± 0.1 |
| ST | N.D. | 0.6 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.2 |
| AMPL | 1.4 ± 0.3 | 1.0 ± 0.5 | 1.4 ± 1.5 | 1.8 ± 1.1 |
| PL | 1.5 ± 0.3 | –0.2 ± 0.4 | 0.2 ± 0.4 | 0.2 ± 0.4 |
| Total | 3.6 ± 0.4 | 6.0 ± 2.1 | 6.1 ± 7.5 | 7.2 ± 5.6 |

HC: hydrocarbon; SE: sterol ester;
KET: ketone;
TAG: triacylglycerol;
FFA: free fatty acid;
ALC: aliphatic alcohol;
ST: sterol;
AMPL: acetone-mobile polar lipid;
PL: phospholipid



## *Mass balance*

In order to determine mass balances, the concentration factor (F) was first calculated according to the formula $F = V_i/V_r$ where V is volume, and i, r, indicate initial and retentate fractions. Because the filtrate is closely related to a specific filter, the initial fraction was defined as the prefiltered fraction for a specific filter. In a sequential (stepwise) filtration, the initial fraction in a second filtration is the filtrate fraction of the first step filtration. The detailed filtration sequence is shown in a filtration diagram (Table 1). The concentration factors (F) in these studies varied from 3.4 to 69 for seawater samples and from 7.0 to 16.7 for culture samples.

The concentrations (μg $L^{-1}$) of lipid classes in initial ($C_i$), retentate ($C_r$) and filtrate ($C_f$) fractions were measured by TLC-FID, and then lipid blanks in the Chromarod-Iatroscan system, the extracting solvents and the CFF system were subtracted. The concentration (μg $L^{-1}$) of lipid classes on particles ($C_p$) was obtained by applying the formula $C_p = (C_r–C_f)/F$. Mass balance, or percent recovery, was calculated as $R(\%) = 100\ (C_p + C_f)/C_i$. Recoveries greater than 100% indicate contamination from the CFF system. Recoveries less than 100% indicate lost of materials during cross-flow filtration.

From the mass balances obtained in the culture samples (Table 4), the recovery of most lipid classes is between 80% and 120%. The coefficient of variation (CV) for lipid classes varies from 5% to 50% for both the 0.45 μm filter and the 10 kDa cut-off filter. Some of the high CV values can be attributed to imprecisions in the measurement of trace materials. Individual lipid classes such as hydrocarbons sometimes have higher uncertainties in recovery because of their low concentrations and large CVs in measurement near the detection limit. The recoveries for the major lipid classes approach 100% with relatively low CVs. Therefore, the recovery data obtained suggest contamination and adsorption from the CFF system and chemical transformation of lipid

**Table 4** Mass balances (mean ± S.D. n = 6–10) of hydrocarbon and more polar lipid classes on both filters in cross-flow filtration of laboratory culture samples.

| *Samples* | *I. galbana* ($5.8 \times 10^6$ cells $mL^{-1}$) | | *I. galbana* ($4.2 \times 10^6$ cells $mL^{-1}$) | | *C. muelleri* ($4.2 \times 10^6$ cells $mL^{-1}$) |
|---|---|---|---|---|---|
| *Filters* | *0.45 μm* | *10 kDa* | *0.45 μm* | *10 kDa* | *10 kDa* |
| HC | 93 ± 46 | 105 ± 278 | 119 ± 14 | 135 ± 18 | 64 ± 15 |
| SE | 123 ± 14 | 104 ± 10 | 104 ± 9 | 36 ± 10 | 99 ± 61 |
| KET | 106 ± 33 | 101 ± 19 | 100 ± 10 | 102 ± 34 | 120 ± 4 |
| TAG | 93 ± 32 | 84 ± 48 | 114 ± 28 | 113 ± 13 | 79 ± 11 |
| FFA | 111 ± 30 | 117 ± 46 | 102 ± 12 | 106 ± 30 | 127 ± 24 |
| ALC | 111 ± 12 | 112 ± 13 | 86 ± 17 | 74 ± 15 | 105 ± 15 |
| ST | 113 ± 10 | 109 ± 6 | 93 ± 45 | 117 ± 33 | 98 ± 13 |
| AMPL | 94 ± 27 | 103 ± 37 | 96 ± 39 | 116 ± 26 | 98 ± 20 |
| PL | 106 ± 75 | 106 ± 79 | 91 ± 7 | 106 ± 6 | 71 ± 22 |
| Total | 100 ± 15 | 99 ± 21 | 104 ± 39 | 111 ± 40 | 94 ± 9 |

classes have been minimized for filtering cultures having high concentrations of colloids. There were no obvious differences in recoveries between the two kinds of filters.

Recoveries obtained from seawater samples usually varied from 75% to 130% for most lipid classes with no obvious relationship between recovery and concentration factor (Figure 2). These results are consistent with the results of Whitehouse *et al.*[11] who obtained recoveries of 71–132% for organic carbon and trace metals in seawater and river water by CFF. However, abnormally high recoveries (> 175%) were sometimes obtained for triacylglycerol, free fatty acid and alcohol. These abnormal values probably result from contamination or possible decomposition of other lipid classes during sample handling. It is less likely that this massive contamination comes from the CFF system. If it did, then all recoveries of triacylglycerol, free fatty acid, and alcohol in all samples would be much larger than 100%. In fact, only a few recovery values were abnormally high. Low recoveries of hydrocarbon with the 0.45 μm filter appear to result from loss of hydrocarbon during CFF or absorption.

Seawater samples are more prone than cultures to contamination because of very low lipid concentrations; however, the CV of the culture samples is usually larger than that of seawater. This may be attributed to the higher biological activity in the algal samples.

### *Seawater concentrations of colloidal hydrocarbons and lipids*

CFF was applied to fractionate and concentrate colloids from 15–35 L seawater samples for lipid measurements. Samples were collected in the surface mixed layer (~25 m) in the centre of Conception Bay from May to October, 1993. Colloidal (0.45 μm–10 kDa) hydrocarbon concentrations and total lipid concentrations were, respectively, 0.1–0.4 μg $L^{-1}$ and 0.9–8.7 μg $L^{-1}$ in the coastal surface water (Figure 3). The major colloidal lipid classes were triacylglycerol, free fatty acid and phospholipid.

These data indicate that colloidal lipid concentrations increase after the spring diatom bloom, presumably as a result of zooplankton feeding. The maximum in colloidal hydrocarbon concentrations occurred after the maximum in total lipids perhaps because of absorption of dissolved hydrocarbons onto the more hydrophobic particles produced after the bloom.

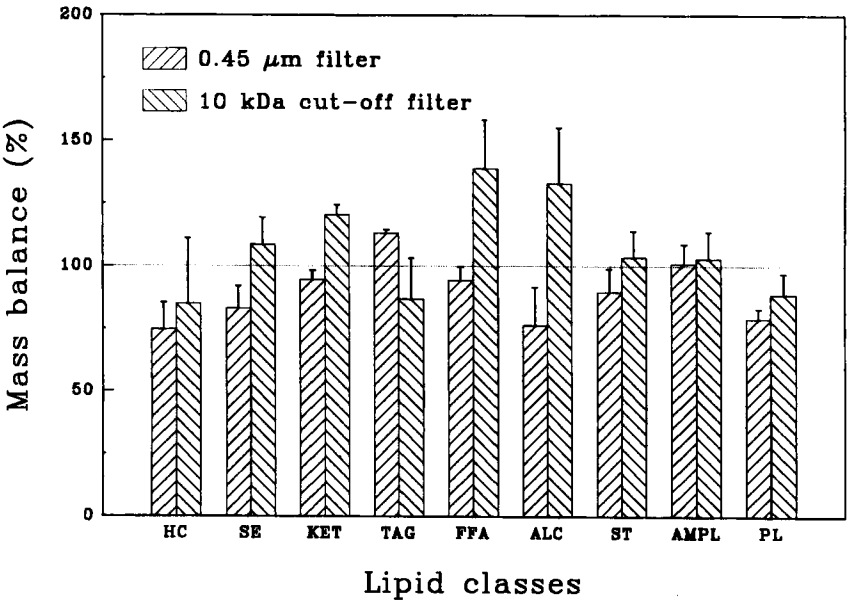



**Figure 2** Mass balances (%, mean ± S.E.M, n = 3–4) of lipid classes on both filters in cross-flow filtration of seawater samples collected from Conception Bay, Newfoundland.

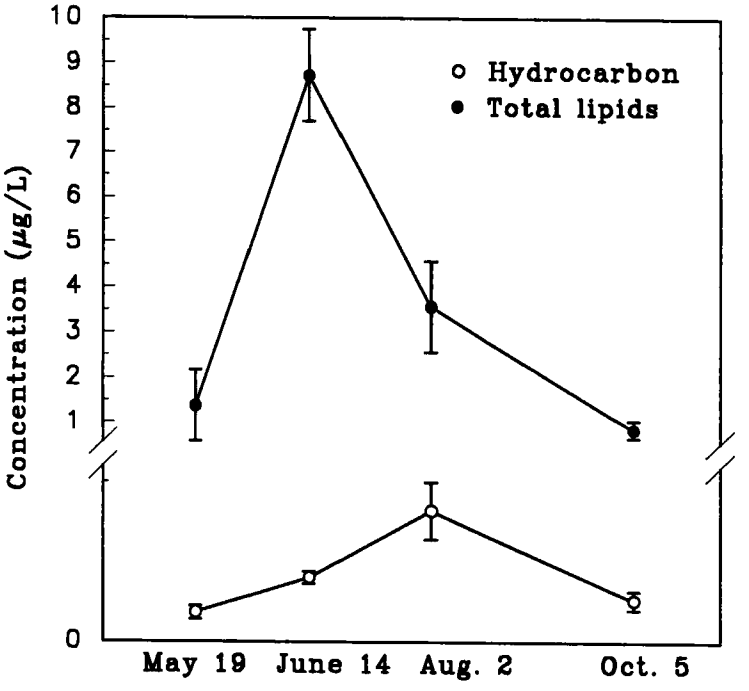



**Figure 3** Colloidal (0.45 μm–3 nm) hydrocarbon and total lipid concentrations (μg $L^{-1}$, mean ± S.D., n = 4–6) in seawater samples collected from Conception Bay, Newfoundland.

## CONCLUSION

It is possible to minimize artifacts by cleaning the CFF system in a large volume (> 40 L) of distilled water for four days and by optimizing operating procedures such as the use of Teflon tubing and filtration in the dark, at low temperature and under nitrogen. The hydrocarbon and total lipid blank in the CFF system was 0.5 μg and 7 μg respectively. For the major lipid classes in both culture and seawater samples, the mass balances on the 0.45 μm filter and the 10 kDa cut-off filter approach 100%. This indicates that contamination from the CFF system and adsorption on the components have been minimized.

*Acknowledgements*

We thank Dr. Robert Helleur for valuable technical support and advice, Dr. Paul Kepkay for DOC measurements, Dr. Richard Rivkin for help in collecting seawater samples, and Ms. Qing Wei for technical assistance. This research was supported by the Natural Sciences and Engineering Council of Canada and a Department of Fisheries and Oceans subvention. O.S.C. contribution 268.



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