Separation of natural organic colloids with a PALL tangential flow filtration system
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ABSTRACT

The applicability of a PALL tangential flow filtration (TFF) system for size fractionation of natural dissolved organic matter was investigated. The performance of polyethersulfone membranes with nominal molecular weight cut-off of 1 kDa, 5 kDa and 50 kDa was examined for isolation of low and high molecular weight compounds in fresh and estuarine waters with diverse physico-chemical properties. Detailed protocols for operating the TFF system and for membrane cleaning are proposed. The ultrafiltration membranes can be efficiently cleaned to provide low carbon blanks (<0.09 mg/l). Standard colloid tests confirmed that the higher molecular weight compounds were isolated in the retentate and the lower molecular weight compounds remain in the permeate. Mass balance of fractionated natural samples showed good recoveries for dissolved organic carbon (DOC) (99 ± 13% (1 kDa); 103 ± 20% (5 kDa); 94 ± 14% (50 kDa) (n = 9). Moreover, high ionic strength or high DOC content did not enhance either fouling or contamination of the membrane. These findings demonstrate that the PALL TFF system is reliable for natural organic colloids fractionation in aquatic systems across both salinity and DOC gradients.

Key words | colloids, estuary, freshwater, organic carbon, tangential flow filtration

INTRODUCTION

Organic Matter (OM) has traditionally been subdivided into dissolved and particulate fractions, separated by filtration in the cut-off range of 0.2–1.0 µm (Gustafsson et al. 1996). The dissolved fraction excludes particulate material and bacteria (Guéguen et al. 2002) and can be further subdivided into a colloidal fraction with high molecular weight (HMW) and the ‘truly’ dissolved fraction with low molecular weight (LMW) organic compounds. Realistic contaminant transport modelling requires that colloids are considered as a third, separate phase, distinct from the ‘dissolved’ and ‘particulate’ phases (Gschwend & Wu 1985). Aquatic colloids are entities with supramolecular structure and properties, but small enough to remain in suspension (Amon & Benner 1996; Buffle et al. 1998) and they are widespread in the aquatic environment. The boundary between the LMW and colloidal phase is defined operationally as a given membrane’s molecular weight cut-off (MWCO) in an ultrafiltration (UF)/tangential flow filtration (TFF) system (Guo & Santschi 2006). Typically, membranes used for natural organic matter separations have MWCOs in the range from 1,000–10,000 Dalton, and the 1,000 Dalton size exclusion is widely used to separate ‘dissolved’ from ‘colloidal’ matter (Guo et al. 1994; Buesseler et al. 1996 and references therein).

OM is the most important component of natural colloids in aquatic systems (Dai & Benitez-Nelson 2001; Wilding et al. 2005). Colloid abundance is considerably influenced by the aquatic environment: marine 10–40% (Guo et al. 1994; Dai & Benitez-Nelson 2001), estuarine (4–45%), riverine (36–86%) or lake (~50%) (Guéguen et al. 2002). However, ~50% of colloidal organic matter (COM) is still poorly characterized (Guo & Santschi 1996).
Relatively little is known about the molecular size distribution and chemical composition within the COM fraction. Electron microscopy images showed three morphologies in colloids: fibrils, globules and amorphous matter (Buffel & Leppard 1995; Wilkinson et al. 1999), suggesting that COM is composed of many different components.

In recent years, TFF has become one of the most commonly used techniques for fractionating freshwater and marine colloids. TFF can process greater volumes per unit area of membrane surface compared to standard filtration. It allows preparative isolation of the desired amount of colloids for further studies, despite their low concentration (Doucet et al. 2004). As well, crossflow minimises build up of molecules at the membrane surface avoiding fouling as the sample solution flows through the feed channel, tangentially to the surface of the membrane as well as through the membrane. In spite of increased TFF use, only a few controlled laboratory studies aiming at the implementation of stringent TFF experimental protocols and operational procedures have been done (Guo & Santschi 2006). Size class separations of natural fresh and marine water dissolved OM have been performed using varying types and properties of ultrafiltration membranes (e.g. Chin & Gschwend 1991; Amon & Benner 1996; Guo & Santschi 1996) and may explain disagreements between reported data.

Also, few detailed analyses of molecular weight distribution within the COM fraction have been performed on natural samples with different ionic strengths and organic matter content (see review by Guo & Santschi 2006), because of the necessity of multiple performance checks when using more than one ultrafiltration membrane. Furthermore, larger volumes of initial samples are required for the necessary conditioning of each membrane.

In our study the PALL Centramate TFF system and three low protein binding Omega membranes with different MWCO were tested for fresh and estuarine water colloid characterisation. This system although mainly used in lab scale process development and production applications (Schwartz & Seeley 2009), has also been utilized in environmental colloid studies (Fine et al. 2002; Minor et al. 2002; Powell et al. 2005; Waiser & Roberts 2005). Details on the TFF operational protocol and membrane characteristics in these studies, however, were not given and consequently direct comparison of their colloid data could not be done. The TFF system, originally developed for industrial and biochemical purposes, necessitates additional and specific TFF integrity studies before environmental application, because of the stark contrast between industrial fluids and the dilute and heterogeneous colloid suspension of natural waters. Therefore, we not only tested the applicability of the PALL Centramate system to fractionate and concentrate natural dissolved OM samples with different properties, but also checked the membrane retention capacity and the TFF system blank of each membrane. We further established an efficient cleaning procedure for application to natural samples. The resulting information should prove useful for further studies on the qualitative and quantitative characteristics of molecular weight size fractionated natural COM.

MATERIALS AND METHODS

The TFF system

The TFF system consists of a PALL Centramate membrane holder with an Omega membrane, a cogwheel pump with control unit (Gather), tubing, valves, clamps, two pressure gauges, and a sample reservoir and permeate flask. The membranes with an area of 0.09 m² are made of low protein-binding modified polyethersulfone (PES). The membrane holders, luer fitting, tie rods and washers are made of stainless steel. The O-ring for the luer fitting consisted of ethylene propylene diene monomer (EPDM). All tubing is of inert tygon material (R 3603, Saint-Gobain) with an inner diameter of 9 mm and thickness of 3 mm for the connections with the pump and an inner diameter of 7 mm and thickness 1.5 mm for the connections to the reservoir and permeate flask.

The sample reservoir consists of a conical bottomed glass flask (2.5 l) with a centred outlet and a side mounted permeate inlet 5 cm above the outlet, while permeate is collected in a glass flask. Pump, reservoir, permeate flask and membrane holder are connected with tygon tubing. Pressure gauges are installed at the feed and retentate ports, to monitor and control the pressure for more consistent results.
In all experiments three Centramate Omega PES cassette membranes with different nominal cut-off sizes: 1 kDa, 5 kDa and 50 kDa, were used individually to separate very high molecular weight (VHMW) (>50 kDa), from HMW (>5 or > 1 kDa) and LMW organic compounds (<1 kDa).

**Fractionation by TFF**

The following steps are necessary to operate the Centramate system:
1. Rinsing the TFF system before use to remove the storage agent.
2. Conditioning the system with the sample (or buffer in some lab scale applications such as protein concentration). This step helps to remove air from the system, to adjust a similar temperature of sample and system which should be than constant throughout the experiment (25°C) and to prevent possible precipitation or denaturation of biomolecules resulting from contact with flushing solution.
3. Sample processing (concentration/fractionation).
4. System cleaning and determining the cleaning efficiency.
5. Storing TFF membranes.

The terms permeate and retentate used in this text are defined as followed: permeate is the fraction passing through TFF membranes while retentate is defined as the fraction retained by TFF membranes.

TFF can be carried out in two modes of operation: recirculation and concentration.

During recirculation mode, both, permeate and retentate flow were directed back into the reservoir flask and thus the reservoir sample volume remained constant. This mode is used for cleaning the membrane and for preconditioning it with natural sample. In the concentration mode, however, the permeate flow is collected in the permeate flask, while the retentate flow is recycled back into the reservoir. The concentration of colloids in the sample reservoir increases with time in direct proportion to the decrease in sample volume and enables its use for colloid isolation and concentration (Larsson et al. 2002; Wilding et al. 2004).

A parallel filtration scheme as used by Guo et al. (1994) and Amon & Benner (1996) was applied, to avoid carryover of contamination or sample losses from former membranes.

**TFF process variables**

Important variables involved in TFF are transmembrane pressure (TMP) and crossflow velocity (CFV) (Guo & Santschi 2006). The TMP is the force that drives fluid through the membrane, carrying along the permeable molecules. The CFV is the rate of the solution flow through the feed channel and across the membrane. The crossflow sweeps away larger molecules and aggregates that are retained on the surface of the membrane, preventing the formation of a concentrated biomolecule layer on the membrane surface that can foul or plug the membrane.

Samples flowing through the narrow feed channel create a pressure drop between the feed and retentate ports. This pressure, which is applied to the membrane, can be further increased by increasing the CFV or by restricting the tubing at the retentate port valve. Using TFF effectively means to regulate both the TMP and the CFV to prevent membrane fouling and restriction of the filtrate flow, thus allowing a greater volume of product to be processed in the least possible time.

Earlier studies indicate the importance of the cross flow ratio (CFR) for colloid recovery, as higher CFR resulted in increasing recoveries (Gustafsson et al. 1996; Larsson et al. 2002). The CFR is the retentate to permeate flow ratio, calculated as:

\[
\text{CFR} = \frac{\psi_{\text{Ret}}}{\psi_{\text{Perm}}}
\]

where \(\psi_{\text{Ret}}\) and \(\psi_{\text{Perm}}\) are the flow rate (ml min\(^{-1}\)) of the retentate and permeate, respectively. A CFR > 15 appears necessary to obtain good colloid recoveries (Larsson et al. 2002). The CFR were hence adjusted to > 25 for the 1 kDa and > 15 for the 5 kDa membrane, whereby flux restrictions on the retentate tubing were applied. The restriction influenced the feed pressure in the TFF system. Membranes with high MWCO have high transmembrane fluxes and hence CFR > 15 cannot be reached with the 50 kDa membrane (Larsson et al. 2002; Kottelat et al. 2008) and thus was adjusted to ~ 2.

The maximum operating pressure of Omega membranes is rated at 5 bar (500 kPa, 75 psi). The Centramate system was operated with a TMP of 1.1–1.8 Bar (depending on the sample flow) yielding a permeate flow rate of...
10–25 ml min⁻¹ (1 kDa), 40–80 ml min⁻¹ (5 kDa), 350–700 ml min⁻¹ (50 kDa) and a retentate flow rate/CFV ranging from 900–1,000 (1 kDa), 850–1,050 (5 kDa) and 200–700 ml min⁻¹ (50 kDa).

The concentration factor (cf), defined as the ratio of the initial sample volume to the retentate volume, was 20. The same operating conditions were used in all experiments, as consistent cf and CFR are critical in order to ensure reproducible and comparable colloid data. High cf (10–20) have been widely used in recent studies to minimise retention of LMW molecules that would otherwise lead to overestimation of the concentration in the colloidal fraction (Larsson et al. 2002; Wilding et al. 2004; Guo & Santschi 2006). Further increasing the cf could possibly cause breakthrough of HMW compounds into the permeate, even if this is reported to be minimal during ultrafiltration (Guo & Santschi 2006).

**Cleaning procedure and sample concentration**

Prior to use, membranes were stored in 0.1 N NaOH (99%, p.a.; Carl Roth, Germany) to avoid bio fouling and crystallisation on the membrane surface. Before each use, membranes were cleaned with distilled water (to remove NaOH storage solution) until neutral pH was achieved in the permeate and retentate. During this process, pH decreased linearly (0.97) with increasing distilled water volume. After flushing with 21 of distilled water, permeate and retentate were free of noticeable organic carbon residues. Reservoir flask of permeate/retentate and tubing were thoroughly rinsed with distilled water. The cleaning step was followed by membrane preconditioning using a natural prefiltered (cut-off 0.7 μm) sample (500–1,000 ml) in the recirculation mode. This preconditioning reduced system contamination (reservoir flasks, tubing) and minimised sorptive losses to the membrane and other surfaces (Buesseler et al. 1996). At the end of this process the sample was discarded. Colloid isolation samples (2,000 ml) were then run in concentration mode until ~100 ml was left in the retentate reservoir.

The established cleaning procedure after TFF of a sample was the same for each membrane:

1. 1–21 of distilled water was passed in concentration mode to remove the sample from the system.
2. To remove inorganic salts (especially iron) from the membrane surface, the system was flushed with 4% citric acid (≥ 99%; Alfa Aesar) and recirculated for 15 minutes afterwards.
3. Distilled water was passed in concentration mode to remove citric acid from the system (until neutral pH).
4. 0.2 N NaOH was used in recirculation mode for at least 15 minutes to remove biomolecules such as fats, proteins, starches, polysaccharides, and organic colloids from the membrane surface.
5. NaOH was discarded and the system flushed with clean 0.1 N NaOH to prevent bio fouling during storage.
6. Membranes were stored in 0.1 N NaOH at 4°C in an air and water tight box following the manufacturers’ recommendation.

**Membrane retention test/size cut-off**

Membrane choice is usually guided by its nominal molecular weight cut-off (NMWCO), typically defined as the equivalent molecular weight of the smallest molecule that would exhibit 90% rejection by the filter (Guo & Santschi 2006).

In order to establish the retention performance of used membranes, we examined their ability to retain standard molecules of known MW. The retention coefficient (RC) can be expressed as:

\[
RC = 1 - \left( \frac{C_{Perm}}{C_{Ret}} \right)²
\]

where \(C_{Perm}\) and \(C_{Ret}\) are the concentrations of a standard molecule in permeate and retentate, respectively.

A range of standard organic colloids used to assess the membrane retention included: Polyethylene glycol (600, 1,000, 1,500, 3,000, 4,000, 6,000, 10,000, 35,000, 40,000, 116,000 Da; synthesis grade; Merck, Germany); sucrose, alpha-D-raffinose, α-cyclodextrin (342, 595, 994 Da, respectively; research grade; Serva Feinbiochemica, Germany); L-glutamic acid (147 Da; ≥ 98%; Merck, Germany); aspartic acid (133 Da; 99%; Aldrich-Chemicals, Germany).

These standard organic colloids have shown wide stability and applicability in various size exclusion studies (Gustafsson et al. 1996; Guo & Santschi 2006).

Membrane retention tests were carried out in recirculation mode under sample operating conditions. Diluted standard molecules had a dissolved organic carbon (DOC) concentration of 20 mg C/l. These solutions were processed...
for 1 hour to establish steady-state conditions in both permeate and retentate. At the end retentate and permeate samples were collected for further DOC measurements.

Recovery of organic carbon in natural samples

To address losses of substances during TFF, organic carbon recovery (Recov) was calculated as:

\[
\text{% Recov} = 100 \times \left( \frac{C_{\text{Ret}} + C_{\text{Perm}}}{C_{\text{PFW}}} \right)
\]

(3)

where \(C_{\text{Perm}}\) and \(C_{\text{Ret}}\) are the organic carbon concentrations in permeate and retentate, respectively and \(C_{\text{PFW}}\) is the organic carbon concentration of the prefiltered water which was used as sample feed. This approach used the OM in natural water itself and provided a useful initial indicator of gross contamination.

Sample collection and storage

Water samples from fresh to coastal waters were collected in the tropical Manguaba lagoon complex (Maceió, NE-Brazil) and from a pond in the temperate zone (Hamburg, N-Germany). The samples had a broad range of salinities (0–35 psu) and DOC concentration (Table 1) as well as dissolved OM sources (freshwater river and pond; brackish phytoplankton and marine dissolved OM).

The pond sample was freshwater with high DOC concentration (22.5 mg/l). Fresh and low salinity lagoon DOC concentrations ranged from 3.6–11.5 mg/l. To obtain a sample with medium salinity and high DOC, pond water was mixed 1:1 with low Arctic DOC water (Kara Sea, Russia). Medium and high salinity water samples with low DOC (1.5–4.1 mg/l) were obtained in the estuary of the lagoon complex.

Water from each site was collected and placed into 20 l carboys, brought to the laboratory and immediately filtered through a precombusted GF/F filter (nominal pore size 0.7 μm - Whatman). To minimise contamination, the first 3 l of the filtrate were discarded. Filtered samples were analysed for bulk DOC (see below) and further processed using TFF. All aqueous samples were acidified to pH ~ 2 with 85% H₃PO₄ to avoid any biological activity and stored frozen until analysis (within one month).

DOC concentrations of all water samples were measured using a high temperature combustion analyzer (Shimadzu TOC 5050) with a Pt catalyst at 680°C. Samples were sparged for 5 min immediately prior to analysis with the same ultra-high purity synthetic air that was used as a carrier gas in the TOC analyzer. Standards (potassium hydrogen phthalate) were analyzed immediately prior to and after sample analysis. Water for standards was prepared by a Millipore Q-Pod system (total organic carbon, 5 ppm), producing a total DOC blank (including water and instrument) of approximately 0.02 mg/l.

All samples were analysed in triplicate. Precision, in terms of the relative standard deviation was ≤ 2%.

Table 1 | Different types of natural samples (Salinity, initial DOC concentration of prefiltered water) and their mass balance (Recovery) when fractionated with a Centramate tangential flow filtration system with Omega membranes of different nominal molecular weight cut-offs: 50 kDa, 5 kDa and 1 kDa

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sal</th>
<th>PFW</th>
<th>50 kDa</th>
<th>5 kDa</th>
<th>1 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond</td>
<td>0</td>
<td>22.5</td>
<td>89.9</td>
<td>80.0</td>
<td>108.3</td>
</tr>
<tr>
<td>River</td>
<td>0</td>
<td>6.1</td>
<td>81.0</td>
<td>129.8</td>
<td>106.3</td>
</tr>
<tr>
<td>River</td>
<td>0</td>
<td>3.6</td>
<td>78.4</td>
<td>100.7</td>
<td>76.6</td>
</tr>
<tr>
<td>Estuary</td>
<td>1.1</td>
<td>4.1</td>
<td>83.9</td>
<td>107.4</td>
<td>103.0</td>
</tr>
<tr>
<td>Estuary</td>
<td>2.4</td>
<td>11.5</td>
<td>82.9</td>
<td>70.2</td>
<td>97.4</td>
</tr>
<tr>
<td>Mix pond &amp; SW</td>
<td>18</td>
<td>10.6</td>
<td>103.5</td>
<td>93.6</td>
<td>83.1</td>
</tr>
<tr>
<td>Estuary</td>
<td>23.2</td>
<td>3.8</td>
<td>105.6</td>
<td>111.2</td>
<td>117.5</td>
</tr>
<tr>
<td>Estuary</td>
<td>23.5</td>
<td>4.1</td>
<td>108.5</td>
<td>102.0</td>
<td>91.6</td>
</tr>
<tr>
<td>Estuary</td>
<td>35.4</td>
<td>1.5</td>
<td>115.6</td>
<td>132.2</td>
<td>107.5</td>
</tr>
</tbody>
</table>

NMWCO = Nominal molecular weight cut-off; PFW = prefiltered water; SW = seawater.

RESULTS AND DISCUSSION

Integrity of the TFF membranes

In order to check membrane integrity, different standard molecules that spanned the colloidal size range were used. As expected, RC values increased with the size of the standard. Since a broad range of standard sizes was used, obtained data could be applied to calculate the cut-off size of the TFF membranes.
The rejection rate is not necessarily the same for other molecules having the same MW but different molecular properties and configurations (Guo & Santschi 2006). The MWCO, defined as the majority of pores that retain 90% of the molecules was deduced by fitting a sigmoidal equation to the retention profiles (Figure 1). The MWCO was then calculated by the equation given in Figure 1. The calculated cut-off of the different TFF membranes was found to be 1.4 kDa (1 kDa), 3.2 kDa (5 kDa) and 35.2 kDa (50 kDa); manufacturers specification in parentheses. We have to keep in mind that a small portion of HMW molecules may pass through the membrane and a portion of LMW molecules could be retained by the membrane and thus the determined MWCO is not static. In general the actual cut-offs agreed well with the cut-offs specified by the manufacturers. The operating cut-off for the 1 kDa membrane is slightly greater than the factory cut-off resulting in a slightly lower retention than specified. 5 kDa and 50 kDa membranes retain more molecules relative to manufacturers’ specification, as their operating cut-off is lower, when using globular molecules and polysaccharides. These small differences can occur because the cut-off characteristic of the membrane is affected by tertiary shape, electrostatic attraction or repulsion and other physico-chemical interactions of the compound in solution with the membrane and so retention is not simply a function of molecular weight (Buesseler et al. 1996; Buffle et al. 1998).

**Fractionation system blanks**

TFF fractionation of natural colloids requires correspondingly low system blanks, thus construction material must minimally affect OC of the sample. Blank tests are also essential performance checks for the ultrafiltration system, as the membrane can be a major source of contamination.

System blanks were determined by treating distilled water in strictly the same way as a sample. Permeate and retentate were analysed for DOC. The cleanliness of the membranes and the TFF system was judged according to the differences in DOC levels between blank distilled water and permeate/retentate. DOC concentration in the permeate was the same as in distilled water before TFF process. In the retentate blank an equal amount of DOC (as in the distilled water) was found in the 1 kDa system and 0.06–0.09 mg/l were found in the 5 and 50 kDa systems, which is 3 to 4 times greater than in distilled water (0.02 mg/l). Results presented here show that this cleaning procedure can be applied to obtain negligible DOC levels in permeate and retentate and that the membranes are not a source of contamination. Moreover these blanks were negligible compared to natural freshwater DOC concentrations. For natural waters with low DOC concentrations such as seawater, however, cleaning procedures must be performed with great care as blanks are of special concern and can explain poor recoveries.

![Figure 1](https://iwaponline.com/ies/article-pdf/9/5/583/417090/583.pdf)
Mass balance of DOC in TFF experiments with natural samples

For natural samples, when DOC recoveries are < 100% losses are greater than contamination, while for recoveries of > 100% contamination are greater than losses to the system (Gustafsson et al. 1996). For all experiments in this study, organic carbon recoveries of natural samples using the Centramate TFF system were 70 to 132% (Table 1). Recoveries of all samples independent of ionic strength or DOC concentration differed, on average, by less than ±15% when using 50 or 1 kDa membrane and by ±20% using 5 kDa membrane. A similar range of recoveries has been noted in other TFF studies (Guéguen et al. 2002; Wilding et al. 2005; Kottelat et al. 2008).

While the 50 kDa membrane showed a positive linear correlation between recovery and salinity ($R^2 = 0.94$), there was no linear correlation between recovery and organic carbon content of the sample ($R^2 = 0.07$). The recovery date from different freshwater and low salinity samples showed that the higher the DOC content the better the OC recovery. All these samples had OC recoveries < 100%. In comparison, samples with high salinity had OC recoveries > 100%. High salinity samples with low DOC showed higher recoveries than high salinity samples with high DOC ($R^2 = 0.63$). Membrane interaction was obviously dependent on the ionic strength of the sample, whereby samples with high ionic strength (estuarine samples) showed poorer efficiencies, as noted in other studies (Guéguen et al. 2002). The system blank is another factor for the poor recovery, especially at low DOC concentrations (1.5 mg/l).

The 5 kDa membrane also showed a clear correlation between recovery and salinity in high salinity samples ($R^2 = 0.94$). High correlations were also shown in DOC and OC recoveries of high salinity samples ($R^2 = 0.71$) similar to that seen in the 50 kDa system. In fresh and low salinity water samples, OC recoveries ranged from 70 to 130%. Fresh and low salinity water samples with low DOC content were very close to optimal recovery whereas losses of organic carbon were noted in samples with more than 10 mg/l DOC. Low salinity and high DOC waters appeared to enhance material adsorption to TFF membrane. One exception was the freshwater sample with a DOC of 6.1 mg/l where the high OC recovery was possibly a result of system contamination.

5 and 1 kDa membranes retained the natural colloidal OC in a fairly similar manner as their specific membrane cut-off is very close. The overall performance of the 1 kDa was better than the 5 kDa membrane. The 1 kDa membrane showed a great range of OC recoveries from 77 to 108% in the fresh and low salinity water samples and from 83 to 118% in the high salinity samples.

In principle, losses of colloids to the TFF system could occur either through a hydrodynamic effect, such as concentration polarisation, or through chemical interaction between the macromolecule and the membrane surface (sorptive losses). Thus, a high CFR is a preferable mode of operation to enable a substantial tangential flow ‘self-cleaning’, as higher CFR imply lower linear permeate flow velocity against which colloids need to diffuse (Larsson et al. 2002). Even though the CFR was much lower than 15 when using the 50 kDa system, recoveries were acceptable.

Membrane fouling and cake formation occur, especially at high colloid concentration and high cf, when retained particles build up on the membrane surface and pores clog. Indicators of such fouling are a relation between recovery and organic carbon in the retentate fractions and a decreasing permeate flow. In our experiments with natural samples, the permeate flow was relatively constant during TFF processing, suggesting proper functioning of the Centramate membranes. Hence, the established TFF process variables and cleaning protocol for the Centramate system (though time consuming—45 minutes for the 50 kDa, 75 min for the 5 and 1 kDa membranes) were very efficient (low blank) and provided good separation and low contamination.

CONCLUSIONS

Currently, it is difficult to directly compare colloid fraction results between different TFF studies due to the absence of standardised operating conditions. In this study we successfully established an operating protocol to enable future inter-study comparison of colloids across a salinity and DOC gradient within aquatic environments. The performance of TFF membranes was tested on natural samples with
a large range of DOC (1.5–22.5 mg/l) and ionic strength (salinity 0–35 psu). Results from this study indicate that these physico-chemical parameters can alter TFF recoveries. The TFF protocol used in this study (cleaning, conditioning, concentration factor, cross flow rate) was reliable and efficient for separating natural organic colloids in water from fresh and estuarine ecosystems. System cleaning, however, requires time and large volumes of reagents and clean water in order to prevent cross-contamination. Therefore, the length of cleaning time has to be considered before application in the field, especially when more than one membrane for size fractionation per sample will be used. This study also indicated that membranes should be periodically verified for integrity and cut-off with standard colloids as the actual membrane MWCO does not necessarily confirm the nominal cut-off provided by manufacturers.

REFERENCES


