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ScienceDirect
Chemosphere 68 (2007) 1304–1311
CHEMOSPHERE
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Fluorescence characterization of cross flow ultrafiltration derived freshwater colloidal and dissolved organic matter

Ruixia Liu, Jamie R. Lead *, Andy Baker

Division of Environmental Health and Risk Management, School of Geography, Earth and Environmental Science, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Received 24 October 2006; received in revised form 15 January 2007; accepted 17 January 2007
Available online 9 March 2007

Abstract

3-D fluorescence excitation-emission matrix (EEM) spectrophotometry was applied to investigate the fluorescence characterization of colloidal organic matter (COM) and truly dissolved organic matter (DOM) from an urban lake and a rural river fractionated by the cross flow ultrafiltration (CFUF) process with a 1 kDa membrane. Relatively high tryptophan-like fluorescence intensity is found in the urban water, although the fluorescence of both water samples is mainly dominated by humic/fulvic-like fluorophores. During CFUF processing, the fluorescence intensities of humic/fulvic-like materials in the retentate increased rapidly, but a slight increase is also observed in the permeate fluorescence intensity. Very different ultrafiltration behaviour occurred with respect to the tryptophan-like fluorophore, where both permeate and retentate fluorescence intensities increase substantially at the beginning of the CFUF process, then tend to remain constant at high concentration factor (cf) values. Comparison with tryptophan standards demonstrates that freshwater tryptophan-like fluorescence is not dissolved and 'free', but is, in part, colloidal and related to the ultrafiltration behaviour of fulvic/humic-like matter. A good linear relationship between the retentate humic/fulvic-like fluorescence intensity and organic carbon concentration further reveals that fluorescent humic/fulvic-like substances are the dominant contributors to colloidal organic carbon, mainly in the colloidal fraction.

Keywords: Colloidal and dissolved organic matter; Cross flow ultrafiltration; Fluorescence; Tryptophan

1. Introduction

Organic matter (OM) in natural waters is a diverse mixture of organic material containing low molecular weight compounds, *e.g.* simple organic acids, short-chained hydrocarbons, and macromolecules such as humic substances with size ranging from a few hundred to several hundred thousand Dalton (Thurman, 1986; Amy et al., 1987; Wagoner et al., 1997; Jerry and Jean-Philippe, 2003). When associated with other colloidal material, OM can be in the size range of tens to hundreds of nm (Lead and Wilkinson, 2006). It is known that this organic matter plays a critical role in controlling the biogeochemical cycling of trace elements and water quality (Buffle, 1990; Guo et al., 2001; Wetzel, 2001). Historically, organic matter has been divided into dissolved (DOM) and particulate organic matter (POM) (Amy et al., 1987; Raymond and Bauer, 2001). In many instances, DOM is the larger pool of organic matter and can contain more than 90% of total organic matter (TOM) (Thurman, 1986; Kececioglu et al., 1997). Nevertheless, the historical definition and determination of particulate and dissolved fractions are not sufficient to understand element biogeochemical cycling and the mobility and bioavailability of trace pollutants in the aquatic environment. Therefore, fractionation and analysis of the colloidal phase from the bulk water is crucial. In recent years, numerous studies have indicated the importance of the colloidal fraction in aquatic systems. As an intermediary, the colloidal organic matter (COM) governs the

* Corresponding author. Tel.: +44 121 4148147; fax: +44 121 4145528.
E-mail address: j.r.lead@bham.ac.uk (J.R. Lead).

0045-6535/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2007.01.048

speciation, bioavailability, transport and ultimate fate of trace pollutants (Hodge et al., 1993; Lead et al., 1999; Wang and Guo, 2000; Gustafsson et al., 2001; Guéguen et al., 2002; Guo et al., 2003), makes up a substantial fraction of freshwater OM, has a potentially large surface area and a large number of binding sites which are capable of interacting with trace pollutants. Understanding the structure and speciation of dissolved and colloidal organic carbon is thus a research problem of major importance in aquatic chemistry and techniques for this purpose have been developed and used in the last decade or so. Cross flow ultrafiltration (CFUF) is currently an effective technique for the separation and concentration of colloids from large amounts of bulk natural waters and a significant improvement on traditional filtration, with reduced concentration polarisation and clogging (Dai et al., 1998; Larsson et al., 2002; Wilding et al., 2004; Liu et al., 2005; Liu and Lead, 2006) although alteration of colloidal conformation is possible.

OM has distinctive spectrophotometric properties in both absorption and fluorescence and can be characterized by appropriate methods and is usually described in terms of 'tryptophan-like', 'fulvic-like' and 'humic-like' fluorescence, although other terminology has been used in the literature (Coble, 1996; Baker, 2001). The relationship of these components to the chemical form of the OM under investigation, including whether the tryptophan-like fluorescence is free or bound and the relationship of fulvic-like and humic-like fluorescence to extracted humic substances (HS), is not known. With recent improvements of the technique, fluorescence spectroscopy has been used extensively in aquatic systems. In particular, since the mid 1990s, the 3-D fluorescence excitation-emission matrix (EEM) spectrophotometric technique has been developed for the investigation of DOM from freshwater, coastal waters and wastewaters (Coble, 1996; Baker, 2001; Baker et al., 2004; Baker and Inverarity, 2004). In this technique, fluorescence spectra are obtained by acquiring emission spectra at a series of successively longer excitation wavelengths, and the fluorescence intensity is presented as the function of excitation and emission wavelengths. By comparison with the traditional single-scan technique, EEM spectra provide a visualization of a range of fluorophores and has a greater information content (Coble, 1996; Baker, 2001). Fluorescent 'DOM' (based on single filtration through a membrane of between 0.2 and 1.0 μm) characteristics have been determined by the EEM technique in a range of aquatic systems (Clark et al., 2002; Baker and Spencer, 2004; Boehme et al., 2004) and the fluorescent properties of colloidal and dissolved OM within CFUF processes has also been investigated by single-scan technique (Mopper et al., 1996; Guéguen et al., 2002; Wilding et al., 2005; Belzile and Guo, 2006), but combination of CFUF with 3D EEM fluorescence has not been performed to investigate colloidal and dissolved fluorescent material. For instance, it is not known if the tryptophan-like fluorescence is free or bound to other solid-phase material. In this study, we combined CFUF with 3D EEM fluorescence techniques in different types of freshwater samples in order to answer these questions. Specifically we determined the physical speciation of fluorescent organic matter (humic-, fulvic- and tryptohan-like).

2. Experimental

2.1. Study sites and sampling

Two sample sites, an urban lake water and a rural river water, were chosen for this study to provide different types of waters. The urban lake water was collected from the Vale Lake near University of Birmingham, Birmingham, England. The Vale Lake is an artificial lake along the Chad Brook, part of the Bourn Brook tributary of the River Tame. The Bourn Brook is regularly monitored for water quality by the UK Environment Agency and is graded 'fairly good' in chemical water quality, suffering from high Biochemical Oxygen Demand, 'fair' biological quality, and 'high' phosphorous concentrations. The rural catchment sample was taken from River Tern, located in Norton in Hales, Shropshire, England. This site, free of large urban area, is on a groundwater fed river with extensive riparian wetlands. The River Tern is also graded 'fairly good' for chemical water quality, suffering from high Biochemical Oxygen demand, 'good' biological quality and a 'very high' phosphorous concentration (Environment Agency, 1998).

The waters were measured for pH, conductivity, temperature and dissolved oxygen in the field, and the results are presented in Table 1. All the water samples were collected in 25-l plastic bottles that had been soaked with 5% lipsol detergent overnight and thoroughly rinsed, first with deionised, then pure water ($R = 18.2\ \Omega$ cm). Samples were transported to the laboratory and filtered immediately through an ashed (500 °C for 5 h) Whatman GF/F (0.7 μm) membrane to remove larger particles.

2.2. Isolation of colloidal fraction

A CFUF system, containing standard Millipore Pellicon 2 acrylic filter holder and regenerated cellulose membrane with 0.5 m^2 surface area and 1-kDa (~1 nm) nominal pore size, was used for the colloid separation and concentration from bulk waters. An easy-load peristaltic tubing pump was connected to the system by silicone tubing with 12.7 mm internal diameter. Sampling mode was used to process the waters under the following conditions: feed inlet pressure 15–20 psi, retentate outlet pressure 10 psi, the retentate flow rate 1–1.5 l min^{-1}, and permeate flow rate 30–40 ml min^{-1}. The ultrafiltration system was cleaned before each experiment firstly with 2% lipsol detergent, then recirculated with 0.01 M of NaOH and HCl solution respectively for 1–2 h. Between each washing cycle, the system was thoroughly rinsed with 20 l nanopure water.

In CFUF operation, a constant volume in the retentate flask of 4 l was maintained by re-supply from a larger feed container of an identical initial concentration (Buesseler

Table 1
Fluorescence intensity and TOC at different cf values for the two sampling sites

	Retentate					Permeate				
	TOC ($mg\,l^{-1}$)	Tryptophan-like intensity (U)	Fulvic-like intensity (U)	Fulvic/tryptophan-like intensity	Humic-like intensity (U)	Humic/tryptophan-like intensity	TOC ($mg\,l^{-1}$)	Tryptophan-like intensity (U)	Fulvic-like intensity (U)	Fulvic/tryptophan-like intensity	Humic-like intensity (U)	Humic/tryptophan-like intensity
ex/em (nm)		275/350	330/420		380/470			275/350	330/420		380/470	
River Tern: pH 7.9, conductivity 387 ($\mu s\,C^{-1}$), dissolved oxygen 116 (%), temperature 5.7 (°C), sampling date 13/01/2006												
cf = 1	10.7	43	257	6.0	156	3.6	4.0	20	97	4.9	44	2.2
cf = 5	24.8	74	658	8.9	453	6.1	7.4	37	186	5.0	88	2.4
cf = 20	60.8	62	1330	21.5	1045	16.9	7.7	55	315	5.7	164	3.0
Mass balance[a] (%)	82.3	73.5	80.6		80.7							
Vale Lake: pH 7.2, conductivity 350 ($\mu s\,C^{-1}$), dissolved oxygen 31 (%), temperature 5.0 (°C), sampling date 07/12/2005												
cf = 1	9.9	87	165	1.9	87	1.0	7.3	34	67	2.0	29	0.85
cf = 5	20.9	83	390	4.7	231	2.8	6.7	64	126	2.0	62	0.97
cf = 20	51.7	92	565	6.1	423	4.6	10.2	89	208	2.3	99	1.1
Mass balance[a] (%)	99.8	76.6	77.2		88.4							

[a] Mass balance: comparison by total mass of fluorescent material or TOC at the beginning and end of CFUF operation.

et al., 1996; Dai et al., 1998). The permeate flow was directed to a container to be collected, allowing the total permeate to be collected and thus mass balance calculations to be carried out. Over time, colloidal materials became concentrated in the retentate flask. In this case, concentration factor (cf), one of the key parameters for ultrafiltration evaluation, was calculated as follows:

$$\mathrm{cf} = \frac{V_\mathrm{p} + V_\mathrm{r}}{V_\mathrm{r}}$$

where V_p and V_r are the permeate and retentate volumes respectively.

Prior to use in natural water, thorough validation and calibration of the CFUF membrane was performed by quantifying the retention and permeation of various sized molecular probes, such as 0.5-kDa rhodamine, 1.3-kDa vitamin B12 and 3-kDa dextran. In addition, quantification of the size fractionation was performed by atomic force microscopy (AFM) and the results reported by Liu and Lead (2006). Based on the definition of the filter cut-off as the molar mass (MM) corresponding to a retention coefficient of 90%, the predicted cut-off value in our CFUF system was ~2.0 kDa. Operationally we thus define our colloidal component as material between 2 kDa and 0.7 μm.

In the ultrafiltration process of natural water, 30 ml aliquots were collected in 40-ml amber glass bottles at different cf values from both permeates and retentates for the analysis of fluorescence and total organic carbon (TOC). Prior to sampling, the bottles were soaked in 5% detergent overnight and thoroughly cleaned with 10% HCl and nanopure water to ensure the removal of any residues. Appropriate blanks were used under the same experimental conditions.

2.3. TOC measurement

TOC concentration was measured by a high temperature combustion analyzer (Shimadzu TOC-V). Under high temperatures, total carbon (TC) was oxidised into carbon dioxide and analysed using an infrared gas detector. TIC was determined by acidifying the sample with phosphoric acid to produce carbon dioxide and followed by analysis. TOC was obtained by subtracting TIC from TC. Each sample was injected 2–3 times, and the coefficient of variation (CV) of replicated injections was lower than 2%. Fresh nanopure water blanks showed acceptable levels of TOC (less than 0.3 $mg\,l^{-1}$).

2.4. Fluorescence analysis

Fluorescence spectra of waters were recorded on a Varian Cary Eclipse spectrofluorometer using a 4 ml, 1 cm path length cuvette equipped with a water-cooled Peltier temperature controller. Following published methods (Baker, 2001), fluorescence EEMs were generated by scanning and recording emission spectra from 300 to 500 nm at

0.5-nm steps with 5 nm increments of excitation wavelength between 250 and 400. The slits for excitation and emission were 5-nm; the temperature of analysis and PMT voltage were set at 20 ± 0.1 °C and 770 V. The spectrophotometer was calibrated by detecting the Raman intensity at 395 nm emission using a sealed water cell and emission intensity averaged 21.0 ± 0.7 units with no drift during the analytical period. Highly fluorescent concentration samples were diluted prior to analysis to limit any reabsorption effects.

3. Results and discussion

3.1. 3-D fluorescence spectra

For a typical river water, the major fluorescence peaks, described as tryptophan-like, fulvic-like and humic-like fluorophores have been previously identified (Baker and Genty, 1999), with maximum emission peaks at 350, 410–430 and 460–480 nm excited by wavelengths of 275, 320–340 and 370–390 nm respectively. Fig. 1 shows fluorescence EEM data for raw River Tern water, filtered (0.7 μm) River Tern water and Vale Lake water. Both raw and filtered River Tern water exhibit similar fluorescent properties and no difference is observed between Fig. 1a and b, indicating that the fluorescence signature of OM is mainly formed by colloidal and dissolved material, and that particulate matter has no significant effect on the fluorescence properties of water. Obviously, there are three peaks on the EEM of two site waters, attributed to humic/fulvic-like materials and the substance located at 220–250 nm excitation and 400–460 nm emission although each of the two site waters has distinctive and different fluorescence properties. Due to the interference of the second order Rayleigh Tyndall scatter line, we do not look at the peak at 220–250 nm excitation and 400–460 nm emission here. In the case of tryptophan-like fluorophores however, the tryptophan-like fluorophore centre is partially obscured in both water samples by the humic/fulvic-like fluorescence when compared to the tryptophan standard (Fig. 1d). As shown in Fig. 1b and c, Vale Lake water has humic/fulvic-like fluorescence intensities which are approximately 50–60% those in the River Tern. For tryptophan-like fluorophores, the intensity of Vale Lake water is twice as high as the River Tern water. This result suggests that the fluorescence EEMs of both water samples are mainly controlled by humic and fulvic substances, with high tryptophan-like fluorescence intensity in the Vale Lake water as may be expected as it is associated with the pollution from human and animal wastes (Baker et al., 2004).

Prior to quantitative discussion of the results, it is essential to ensure excellent data quality. The CFUF system used has been fully validated elsewhere (Liu and Lead, 2006). However, mass balance data of fluorescence was also collected here. Mass balances, presented in Table 1, are 81–88% for humic-like materials from both waters and 77–81% for fulvic-like compounds, which are similar to other reported values in the literature for organic carbon

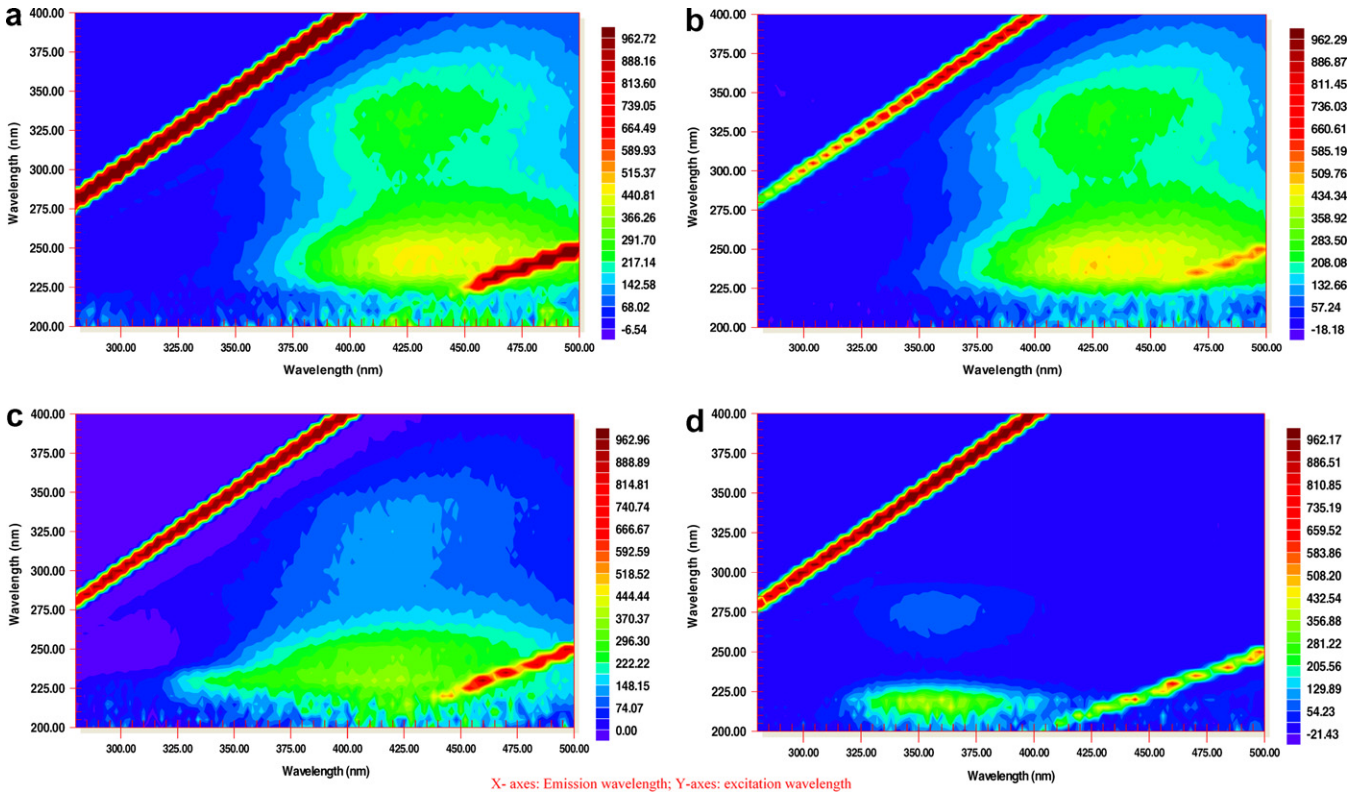

Fig. 1. Fluorescence EEMs for the raw River Tern water (a), filtered River Tern water (b), filtered Vale Lake water (c) and tryptophan standard (d).

(Mopper et al., 1996; Wilding et al., 2005; Belzile and Guo, 2006). For tryptophan-like fluorescence, lower mass balances (74–77%) were observed. These are the first reports to our knowledge of CFUF mass balances for these fluorophores and the results are promising. The small losses are most likely due to membrane sorption and dead volumes within the system. Measurement error of tryptophan at low fluorescence intensities and overlap with the humic-like peaks are also potential problems. In addition, the blank experiment pure water shows that, at all cf values, fluorescence intensities were less than 5% of the sample intensities, indicating a lack of contamination from the membranes.

The investigation into the fluorescence characterization of water samples processed through the CFUF system provides important information on the distribution of different NOM types between colloidal and dissolved phases in the freshwater environment. Through CFUF operation, high MM (HMM) OM in the retentate becomes more concentrated as CFUF proceeds. Fig. S1 presents the fluorescence features in the permeate and retentate at three cf values. It is apparent that, for both sites, the intensities of the fulvic-like and humic-like fluorophores that dominate the EEMs dramatically increase with increasing cf. Due to this increase in fluorescence intensity the fluorescence peaks broaden substantially. In the case of the permeate, fulvic-like and humic-like fluorescence also increases in intensity at high cf values due to increased permeation of low MM (LMM) fluorescent material. To quantify these results, the fluorescence intensities of three fluorophores and the ratios of fulvic-like and humic-like to tryptophan-like fluorescence intensity are presented in Table 1. In the River Tern water, fulvic-like and humic-like fluorescence intensities in the retentate rapidly increase at cf 20 due to the retention of HMM materials by the ultrafiltration membrane. For the permeate, the increases of fulvic-like and humic-like fluorescence intensities are also observed although the rate of increase is slower. Presumably the slower increase in the permeate is due to the majority of the humic- and fulvic-like fluorescence being present in the >2 kDa fraction. A similar trend was found in the Vale Lake water although at different overall intensity values. For both sites, tryptophan-like fluorescence intensities are all less than 100 units at all cf values, in both cases of permeate and retentate, even though slight increases of fluorescence intensity are observed.

Ratios of fulvic/humic-like to tryptophan-like fluorescence are also quantified (Table 1). In the retentate of the River Tern water, the ratios of fulvic-like and humic-like to tryptophan-like fluorescence increase strongly from 6.0 to 21.5 and 3.6 to 16.9, whereas in the permeate, these ratios exhibit little variability from 4.9 to 5.7 in the ratio of fulvic-like to tryptophan-like fluorescence, and 2.2–3.0 in the ratio of humic-like to tryptophan-like fluorescence. However, the intensity of each fluorescence centre increases substantially with increasing cf as noted earlier. Similar fluorescence behaviour is also found in the samples analysed from the Vale Lake. This result indicates that the permeation of tryptophan-like material is related to the ultrafiltration behaviour of fulvic/humic-like matter, possibly due to a physico-chemical association between the tryptophan and the humic-like fluorescence.

3.2. Ultrafiltration of fluorescent tryptophan-like compound

To further investigate the filtration properties, the ultrafiltration behaviour of fluorescent tryptophan-like material from River Tern as a function of cf was observed and is shown in Fig. 2. Similar results were obtained for Vale Lake (see Fig. S2). It appears that ultrafiltration behaviour of the tryptophan-like fluorophore follows two steps (see Fig. 2a). Permeate fluorescence intensity increases with increasing cf up to a value of cf 15, after which it remains constant, indicating the retention of LMM compounds containing tryptophan-like fluorescence, consistent with CFUF results demonstrated in molecular probe experiments (Guo et al., 2000; Wilding et al., 2004; Liu and Lead, 2006). In addition, the intensity of tryptophan-like fluorescence in the retentate exhibits an increase until cf 5–10,

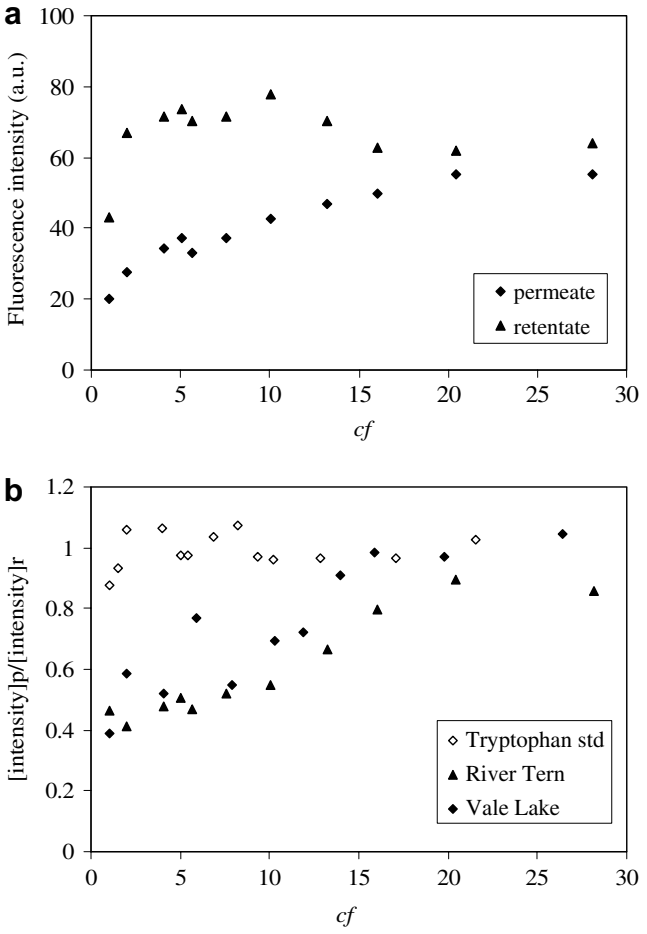

Fig. 2. The retention and permeation of tryptophan-like fluorescent compounds, presented as (a) the absolute intensity for River Tern, and (b) the ratio of permeate fluorescence intensity to retentate fluorescence intensity.

after which a slight decline or consistent intensity can be found after a cf of about 15. The slight decline in fluorescence intensity is likely attributed to the sorption, concentration polarization and membrane fouling (Van der Bruggen et al., 1999; Schafer et al., 2000), although tryptophan-like fluorescence levels are low and some noise is apparent. Intensity ratios are shown in Fig. 2b. The intensity ratio for tryptophan standards (molar mass 204) as the function of cf values are also plotted in Fig. 2b. The tryptophan standard, as expected for dissolved material, is unaffected by the membrane and the ratio is 1 at all cf values. For the river water signals, the values approach 1 at high cfs, indicating that the tryptophan-like fluorescence from these natural waters is essentially in the LMM fraction. Previous studies (Guo et al., 2000; Wilding et al., 2004; Liu and Lead, 2006) have indicated greater permeation of low molar mass material occurred at higher cf values. At lower cf values the ratios decrease to values of ca 0.4, indicating that the material is not dissolved and 'free', as with the standard, but is associated with other material, perhaps the smallest fraction of the polydisperse humic substances.

3.3. Ultrafiltration of humic-like and fulvic-like materials

Humic and fulvic-like fluorescence shows a different trend compared to fluorescent tryptophan-like compound as shown in Figs. 2 and 3. The results are consistent with a picture in which humic and fulvic substances are the cause of the humic-like fluorescence. Literature values report HS as having a molar mass within the range of a few hundred to a few thousand (Thurman, 1986; Ronald et al., 1987; Lead et al., 2000), indicating that most material is in the colloidal domain, while other HS molecules are in the dissolved size range. Similarly, the ratios of fluorescence intensity in the permeate to that in the retentate derived from Fig. 3, with cf, are shown in Fig. S3. The initial ratios of approximately 0.4 decline gradually until a cf of about 10. Subsequently, the ratios of humic-like and fulvic-like fluorescence remain reasonably stable at values of 0.15–0.18 (humic-like) and 0.25–0.31 (fulvic-like) for River Tern water, and 0.20–0.23 (humic-like) and 0.32–0.34 (fulvic-like) for Vale Lake water. The lower ratios of humic-like substance than those of fulvic-like compound are likely due to the lower molar mass of the fulvic-like fluorescence compared with the humic-like fluorescence. This is the first published CFUF data to indicate the size and structural characteristics of the two different fluorophores.

Taken together, the results indicate that tryptophan-like fluorescence is present as material which is somewhat polydisperse and is roughly 0.5–2 kDa (free tryptophan has a molar mass of 0.2 kDa), while the humic-like and fulvic-like fluorescence is larger and mainly within the colloidal fraction. The results agree with a recent study on estuarine systems using flow field fractionation (Boehme and Wells, 2006), where tryptophan-like fluorescence was found mainly in the 1–5 kDa fraction and humic-like and fulvic-like fluorescence was found in the 13–150 kDa fraction. The very good agreement, despite the differences in technique and sample site, indicates that similar fluorescence structures may be fairly consistent in different aquatic systems, although further work would be needed to validate this.

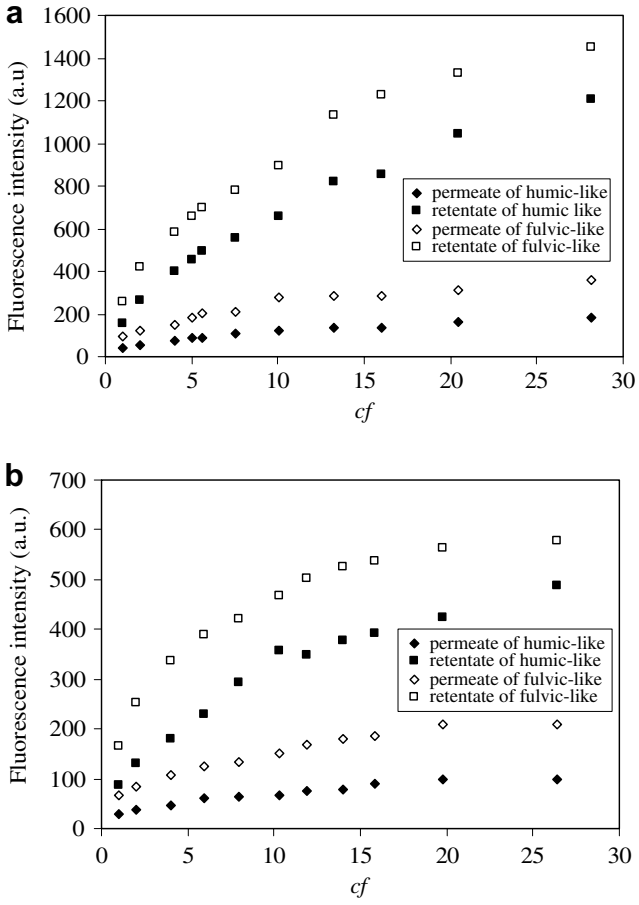

Fig. 3. The retention and permeation of fluorescent fulvic/humic-like materials from two sample sites (a) River Tern and (b) Vale Lake.

3.4. Correlation between fluorescence intensity and OC

Fig. 4 plots the intensity of three fluorophores against OC concentration in both permeate and retentate from Rive Tern. There is a strong linear relationship between the fluorescence intensity of humic/fulvic-like materials and OC concentration in the retentate, with correlation coefficients from 0.97 to 0.99, which indicates that fluorescent humic-type materials are a proxy of OC in the CFUF system. No relationship is shown between OC and tryptophan-like fluorescence, most likely because the tryptophan-like fluorophore is a highly efficient fluorophore but represents only a minor fraction of the organic carbon concentration. In the permeate, although more scatter is observed due to the lower fluorescence intensities, positive sometimes non-linear associations are also observed for all fluorophores. Most likely this relates to the greater

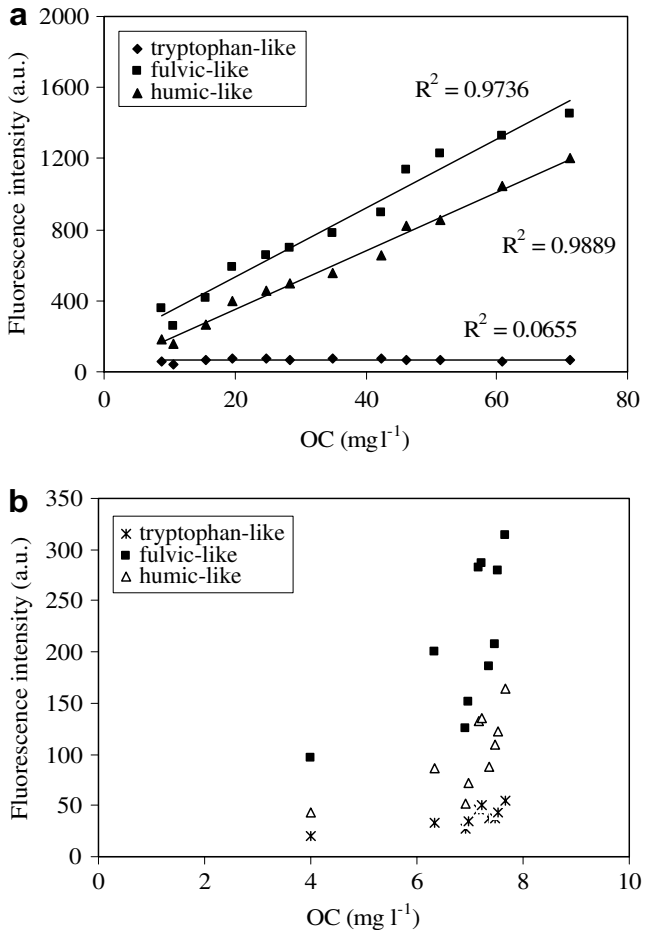

Fig. 4. Correlation between fluorescence intensity and OC from River Tern (a) retentate and (b) permeate.

concentrations of LMM material at higher OC concentrations. Similar results are also found in Vale Lake water, as shown in Fig. S4.

4. Conclusion

In summary, we have used CFUF to attempt to better characterize the physical properties associated with the organic material responsible for freshwater fluorescence. Based on the operational CFUF process, the permeate, i.e. the so-called dissolved phase, is mainly composed of tryptophan-like fluorescence and a fraction of the smaller fulvic-like fluorophores, while the fluorescence signature of retentate is dominated by HMM humic/fulvic-like materials. We have produced the data on unperturbed natural systems to conclusively indicate that tryptophan-like fluorescence is not truly dissolved i.e. hydrated, but exists as LMM material, perhaps bound to the smallest fractions of humic substances. Humic and fulvic-type fluorescence clearly are higher molar mass moieties and fulvic-like fluorophores at 320–340 nm excitation and 410–430 nm emission are related to material that is smaller than associated with humic-like fluorophores at 370–390 nm excitation and 460–480 nm emission. The strong correlation between these fluorescence centres and organic carbon concentration confirms that these moieties are indeed in some respects similar to extracted humic and fulvic acids that make up the bulk of freshwater organic carbon.

Acknowledgement

The authors thank L. Guo for helpful discussions prior to this work commencing. Funding was provided by the Natural Environment Research Council (NE/C510532/1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2007.01.048.

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