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REVIEWS

The application of fluorescence spectroscopy to organic matter characterisation in drinking water treatment

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Abstract Key to effective disinfection byproduct (DBP) management is source water control and management, and more specifically, organic matter (OM) control and management. However, the content and character of OM in source waters is spatially and temporally variable, and the prediction of its composition is challenging. Water treatment companies require adequate analytical techniques for OM characterisation to maintain the operation of the water supply and treatment systems adjusted to constantly changing environmental conditions. There is a requirement, therefore, for an improved understanding of OM composition and character in source water, how that composition and character varies with flow conditions, and how this impacts on drinking water treatment. This paper demonstrates that fluorescence spectroscopy offers a potential alternative to other analytical methods of OM characterisation. The advantages of fluorescence include rapid, sensitive and selective characterisation of OM, no sample pre-treatment, small sample volume, and the potential for on-line monitoring incorporation. Fluorescence can provide useful information on OM reactivity and treatability together with an indication of the OM sources (allochthonous or autochthonous). The paper discusses a body of literature which has identified relationships between fluorescence spectra and OM physico-chemical properties (i.e. degree of hydrophobicity, microbial content), has applied fluorescence spectroscopy to characterise the changes in OM upon disinfection, and has related the fluorescence properties to DBP formation. Further work is required in the robust management of data arising from fluorescence spectroscopy analysis and, in particular, Excitation Emission Matrices. Consideration must be given as to how the data might best be employed to greatest effect on a routine basis at WTW.

Keywords Fluorescence spectroscopy · Excitation emission matrix (EEM) · Disinfection byproducts · Organic matter

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1 Introduction

Natural organic matter (NOM) is a complex mixture of pedogenic (soil-derived) and anthropogenic (water column) material derived from the contact of water with dead and living organic matter in the

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hydrological cycle (Kitis et al. 2001; Egeberg and Alberts 2002). NOM is spatially variable, is present in dissolved, particulate and colloidal forms, and has a number of functions in aquatic systems. These include a carbon source for metabolism of living things, ecological and geochemical functions such as proton binding, influencing biogeochemical processes and photochemical reactions, transportation of inorganic and organic substrates and aggregation and photochemical reactivity (Frimmel 1998; Xiaoying 2001; Egeberg and Alberts 2002; Gjessing et al. 1999; Maurice et al. 2002). Studies of NOM characterisation have identified its main components as carbohydrates, lipids, protein polymers, humic macromolecules, nucleic acids and phenolic compounds (Edzwald 1993; Wu et al. 2003a, b). However, as NOM is highly source dependant, only 25% is well characterised (Thomas 1997).

Natural organic matter is transported to surface waters by surface run-off and near surface lateral flow, and can increase dissolved organic carbon (DOC) concentrations by up to 40% (Hurst et al. 2004). Recent research also indicates a trend towards increasing DOC concentrations in surface waters in the majority of the UK, with a 77% upward trend in DOC concentration since 1961. Trends are attributed to climatic changes (temperature increases and the frequency of severe droughts) as well as land use changes and, most recently, a rise in deposition-driven rainwater and soil acidity, influencing organic matter solubility and potentially increasing DOC export to the sea (Chapman et al. 2008; Monteith et al. 2007; Worrall and Burt 2007).

Municipal water treatment works (WTWs) usually abstract water from multiple sources representing different, temporally variable NOM concentrations, physico-chemical properties, reactivity and treatability. Depending on the specific catchment characteristics and combination of hydrological and climatic factors, NOM undergoes significant seasonal changes (Fabris et al. 2008). The seasonal problems encountered by treatment processes such as coagulation, flocculation, sedimentation, flotation and filtration, associated with increased rainfall and DOC concentrations have been reported previously (Parsons et al. 2004).

In many countries, environmental regulation exists to protect water quality. The challenge to ensure microbial quality is addressed by water companies through disinfection. The most commonly used disinfectant in the developed world is chlorine. However, the use of chlorine, ozone, or chlorine dioxide as a disinfectant in water rich in NOM (which also acts as a microbial food source) can lead to the occurrence of potentially carcinogenic disinfection byproducts (DBPs), e.g. trihalomethanes (THMs) and haloacetic acids (HAAs), which are by-products of chlorination, formed by reactions between halogens and residual OM in water, particularly that resulting from the decomposition of natural and anthropogenic organic material. (Fabris et al. 2008). Inadequate removal of OM at WTWs gives rise to an increased formation of DBPs, and therefore a major challenge in water treatment is the efficient removal of NOM with typical removal efficiencies varying from 20 to 90% (Sharp et al. 2006). Consequently, water companies must manage the competing needs of biological and chemical compliance; i.e. the risk of DBP toxicity must be weighed against the certainty that water that has not been disinfected can cause illness and even death.

Key to effective DBP management is source water control and management, and more specifically, OM control and management. However, the formation and composition of NOM in surface waters can alter substantially between catchments due to spatial and temporal variation, and therefore there is no definitive representation of NOM. Characteristics such as size, structure and charge density are useful distinguishing parameters which have been utilised in recent characterisation investigations as a basis to further our understanding of NOM complexity (Frazier et al. 2003; Thomsen et al. 2002; Rodriguez-Zuniga et al. 2008). Thus, prediction of OM composition is challenging. To face those challenges and meet current regulations regarding the formation of DBPs, water treatment companies must have adequate analytical techniques for OM characterisation to keep the operation of the water systems adjusted to constantly changing environmental conditions.

Recent research into the influences of NOM composition and character have focused on the seasonal changes experienced in source waters, as THM formation is more prevalent in summer months due to higher temperatures causing increased THM formation (Courtis et al. 2009). There is a requirement, therefore, for an improved understanding of OM composition and character in source water, how

that composition and character varies with flow conditions and how this impacts on drinking water treatment.

2 Organic matter characterisation

A range of characterisation tools are available for the identification of NOM components and these can be split into four tiers of analysis; preliminary characterisation, size characterisation, chemical identification and behaviour and spectral signature. Preliminary characterisation tools include total and dissolved organic carbon (TOC and DOC respectively), suspended solids concentration and ultraviolet absorbance (UV). Preliminary analysis typically focuses on the dissolved fraction and indicates the amount of NOM available for isolation and can denote preferential techniques for isolation and characterisation. The complex nature of NOM composition therefore requires more sophisticated analytical techniques which differentiate physico-chemical properties. Such techniques are predominantly laboratory based, requiring extensive sample preparation. These include resin extraction, high performance size exclusion chromatography (HPSEC) and gas-chromatography mass-spectrometry (GC–MS). However, the selectivity of the fractionation techniques can be limited to particular OM fractions. For instance, in HPSEC analysis OM is separated according to its molecular weight but the detection of non-chromophores is relatively poor limiting the application of the technique to heterogeneous OM samples (Her et al. 2002). Furthermore, in fractionation studies the aquatic OM is decomposed into a set of different fractions and moieties whose reactivity and treatability can be entirely different from the bulk OM properties.

2.1 Spectroscopic techniques

Optical methods can provide an alternative to isolation techniques in OM characterisation (Kalbitz et al. 2000; Kitis et al. 2001; Baker 2002; Her et al. 2003; Jaffe et al. 2008). With the recent advances in spectroscopic techniques, ultraviolet–visible absorbance spectroscopy (UV–Vis) and fluorescence spectroscopy have become common OM characterisation tools, providing rapid, non-invasive and sensitive analysis of bulk OM properties.

Optical methods of OM characterisation are based on the measurement of several parameters describing absorption and emission of energy by OM molecules. When an OM molecule is exposed to an external source of light, a photon is absorbed by the molecule and the electron configuration changes. An electron from the ground state is promoted to an upper excited singlet state. The reverse process, in which a photon is emitted during transition of an electron from an excited energy level to the lower level (i.e. ground state), is called luminescence (Lakowicz 1999). Two types of luminescence are observed; the direct electron transition from the singlet state to lower energy level is called fluorescence, whereas the phosphorescence process involves an additional transition to a triplet state with electron spin change (Fig. 1) (Lakowicz 1999). Although fluorescence is the reverse of absorption, absorption (excitation) occurs at a shorter wavelength than the corresponding fluorescence emission due to energy loss in non-

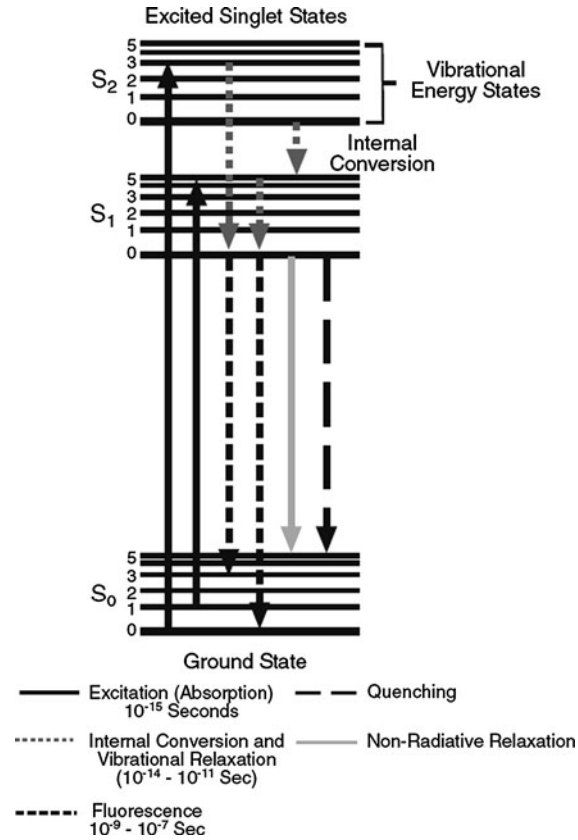


Fig. 1 Jablonski energy diagram (after Hudson et al. 2007; Lakowicz 1999)

radiative decay (the Stoke's shift) (Senesi 1990; Lakowicz 1999; Hudson et al. 2007). Both absorption and fluorescence wavelengths are specific to a particular molecule. The inherent optical properties of bulk OM samples result from the superposition of the optical properties of individual compounds and the intermolecular interactions.

The fraction of DOM that absorbs ultraviolet and visible light is often referred to as chromophoric or coloured DOM (CDOM), whereas the DOM fraction exhibiting fluorescence in both ultraviolet and visible range is described as fluorescent DOM (FDOM) (Helms et al. 2008). CDOM comprises 10–90% of the total DOM pool and therefore constitutes a significant DOM fraction in aquatic ecosystems controlling the photochemical reactions of the surface water and nutrient and light availability for aquatic organisms (Thurman 1985; Twardowski et al. 2004).

Spectroscopic techniques circumvent the limitations of the fractionation techniques. They provide rapid, non-invasive analysis of aquatic OM samples, with the potential for online monitoring assessment of OM reactivity and treatability. Several spectral properties of the OM absorbance spectra have been proposed as surrogate parameters for characterisation of OM reactivity and treatability, with UV measured at 254 nm (UV_{254}) and SUVA being the most commonly utilized.

To extract information about DOM properties from absorption and fluorescence spectra, several spectral parameters can be defined. In the absorption spectra analysis, a common approach is to record the absorption values at particular wavelengths. Absorption at 254 nm (or sometimes at 272 nm) has been widely used as an indicator of aromaticity and humification (Weishaar et al. 2003; Helms et al. 2008). Likewise, absorption at 254 nm normalized by DOC concentration and referred to as a specific UV absorbance (SUVA) has been shown to correlate strongly with the percentage of DOM aromaticity determined by ^{13}C-NMR of humic isolates ($R^2 = 0.97$) (Weishaar et al. 2003). By fitting the absorption values to an exponential function, a spectral slope parameter can be obtained (the exponent) describing the relative steepness of the spectra (Twardowski et al. 2004). The spectral slope is often used for characterisation of CDOM composition and molecular weight. Helms et al. (2008) used the absorption spectral slope ratio as an indicator of NOM from dissimilar water sources along the estuarine transect (correlating the ratio of absorption spectral slopes of 275–295 nm and 350–400 nm with molecular weight obtained from size-fractionation analyses), whilst Spencer et al. (2007) used spectral slope to identify diurnal fluctuations in riverine DOM.

However, absorbance-based techniques can only determine the content and properties of the aromatic functional groups in OM with no information provided on the presence and relative importance of non-chromophoric OM. Furthermore, absorbance techniques are prone to interference from various impediments present in water (i.e. UV-absorbing impurities, pH, iron, nitrate). Therefore, correlations between absorbance-derived surrogates and OM reactivity and treatability parameters (i.e. reactivity with coagulants and disinfectants, THMFP) may contain limited information and reflect only the site-specific OM composition. Thus, a direct comparison between the reactivity of different waters or OM fractions with absorbance techniques is limited. This limitation can be overcome to a large extent by the use of fluorescence spectroscopy.

Over the decades of research applying fluorescence spectroscopy to environmental studies, the technique of measuring the sample fluorescence has shifted from simple measurements of selected excitation or emission wavelengths to the simultaneous collection of fluorescence data over a wide range of different excitation and emission wavelengths (Baker 2002; Hudson et al. 2007). This latter technique is known as excitation-emission fluorescence spectroscopy and is considered as the best currently available fluorescence technique (Hudson et al. 2007).

A fluorescence excitation-emission matrix (EEM) contains a substantial amount of information on OM composition and structure. Fluorescence EEMs provide valuable information on the DOM composition that allows for ecological fingerprinting of aquatic samples. Each EEM demonstrates a specific combination of fluorescence intensities over a range of excitation and emission wavelengths; however, in freshwater three main fluorescence regions are typically present: humic-, fulvic-, and protein-like fluorescence. Coble (1996) introduced an alternative classification of fluorescence regions, where humic-like fluorescence is subdivided into peak A and peak C, and protein-like fluorescence collectively denotes peak B (tyrosine-like) and peak T (tryptophan-like)

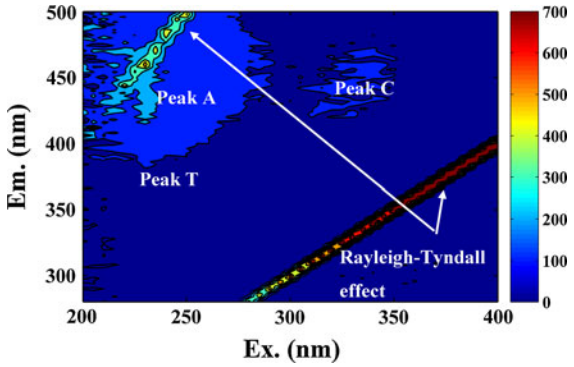


Fig. 2 Fluorescence excitation–emission matrix with typical fluorescence features (*peak A, C, T* and Rayleigh-Tyndall effect) (after Coble 1996)

Table 1 Excitation and emission wavelength pairs for principal peak fluorescence intensities

Peak		λ_{ex} (nm)	λ_{em} (nm)
Humic	A	237–260	400–500
Humic	C	300–370	400–500
(Highly coloured)	C_1	320–340	410–430
	C_2	370–390	460–480
Tyrosine	B_1	225–237	309–321
	B_2	275	310
Tryptophan	T_1	275	340
	T_2	225–237	340–381
Humic (marine)	M	290–310	370–410

(Fig. 2). Excitation and emission wavelength pairs for the principal peaks are shown in Table 1.

To retrieve the most important fluorescence information, a number of methods can be utilized, from simple visual inspection and recording of the peak maxima values to more advanced statistical methods. The simplest spectral parameter derived from an EEM for DOM characterisation is the wavelength-independent position of each of the fluorophores recorded as emission and excitation wavelengths of the fluorescence intensity maximum (Coble 1996). Ratios of certain fluorescence peaks have been found to be useful in distinguishing between OM sources in rivers (Baker and Spencer 2004), lakes (McKnight et al. 2001), marine ecosystems (Coble 1996). In particular the fluorescence index (defined as the ratio of fluorescence intensity at 450 nm emission wavelength to fluorescence intensity at 500 nm emission wavelength, both at 370 nm excitation wavelength) has been used to indicate the degree of aromaticity (McKnight et al. 2001).

Analysis of multi-dimensional fluorescence data is often coupled with the application of advanced statistical and computational techniques for exploration of the patterns within fluorescence spectra, for classification and calibration purposes. In particular, methods from multi-way analysis have become the most popular in modelling of fluorescence data, with parallel factor analysis (PARAFAC) being the current state-of-the-art technique (Bro 1997; Stedmon et al. 2003; Fellman et al. 2008). In the PARAFAC model fluorescence data are decomposed into a set of independent components reflecting the changes in composition and source material between water samples. Each component therefore represents a group of fluorophores of similar, specific fluorescence properties (Stedmon and Markager 2005). Combined EEM-PARAFAC modelling is often used as a tool to trace DOM fractions in different environments and in DOM mixing studies (Fellman et al. 2008; Engelen et al. 2009). Baghoth et al. (2011) used EEMs and PARAFAC to characterise NOM from WTW samples and then correlated maximum fluorescence intensities of the PARAFAC components with NOM fractions using liquid choromotography with organic carbon detection (LC-OCD). This work demonstrated that fluorophores derived from EEM/PARAFAC modelling could be related to previously defined NOM fractions. In an alternative approach, Bieroza et al. (2010, 2011) used EEMs successfully in conjunction with self-organising maps (a powerful pattern recognition algorithm based on a two-layered artificial neural network) for the determination of NOM removal efficiency in water treatment where patterns in the data were assigned to categories not known a priori.

In several studies, a combined application of several analytical techniques for comprehensive OM characterisation has been presented, i.e. absorption and spectrofluorometric techniques or HPSEC measurements with online fluorescence detection (del Castillo et al. 1999; Gjessing et al. 1999; Her et al. 2002; Nagao et al. 2003; Wu et al. 2003b; Fellman et al. 2008). Those comparative studies provide the opportunity to compare the efficacy of DOM characterisation techniques. DOM characterisation via HPSEC coupled with fluorescence EEM spectroscopy provides presentation of fluorescence properties as a

function of molecular weight (Her et al. 2003; Nagao et al. 2003; Wu et al. 2003a). However, research carried out by Wu et al. (2003a) suggested that HPSEC can underestimate the concentrations of humic- and protein-like fluorescence and larger molecular weights due to their hydrophobic character and adsorption onto HPSEC column.

2.2 Application of florescence spectroscopy in OM characterisation of water and wastewater quality

Previously, the application of fluorescence spectroscopy in the water industry has been focussed on water quality assessment, wastewater characterisation, and monitoring of biological activity in bioreactors (Reynolds and Ahmad 1995; Galapate et al. 1998; Ahmad and Reynolds 1999; Hudson et al. 2007; Wolf et al. 2007). However, more recently, some workers have considered how the technique might be utilised to assess water treatment process performance. In particular, Bieroza et al. (2009, 2010) successfully demonstrated the use of fluorescence spectroscopy in assessing the removal of organic matter across the combined coagulation/flocculation/clarification stages, and also across a complete treatment works.

Fluorescence EEM spectroscopy has been used for fingerprinting of wastewater sources due to distinctively increased levels of protein-like fluorescence found therein (Baker and Spencer 2004; Elliot et al. 2006; Hur et al. 2008; Nam and Amy 2008). Henderson et al. (2009) evaluated the application of fluorescence spectroscopy as a recycled water monitoring tool for process performance assessment, cross-connection detection and overall water quality in indirect potable reuse systems. Fluorescence spectroscopy can detect small changes in DOM/TOC concentration and characteristics (Henderson et al. 2009). With the potential for discrimination of water sources (e.g. drinking and recycled water, riverine water and wastewater), fluorescence spectroscopy can be a useful monitoring tool in water quality studies (Henderson et al. 2009). Likewise, Nam and Amy (2008) compared the fluorescence properties of wastewater effluent to NOM properties. The increased levels of protein-like fluorescence and humic-like fluorescence location at lower excitation and emission wavelengths were found to be the most important features discriminating between wastewater and freshwater OM. However, several compounds will fluoresce in the same region as proteins (e.g. naphthalene). Consequently, it is important to note that whilst fluorescence is extremely valuable in determining OM sources, caution must be exercised when attributing fluorescent components to specific compounds.

The application of fluorescence spectroscopy in bioprocess monitoring has been investigated by several authors (Li and Humprey 1991; Khoury et al. 1992; Wolf et al. 2007; Denkhaus et al. 2007; Wolf et al. 2007). Biofilms are populations of microorganisms that can accumulate at phase boundaries and biodegrade organic compounds from water i.e. biologically active carbon or in the distribution system (Hallam et al. 2001; Denkhaus et al. 2007; Velten et al. 2007; Simpson 2008). Organisms in the biofilm were found to contain natural intracellular fluorophores (tryptophan, pyridoxine, NAD(P)H, riboflavin) that can provide an indication of microbial activity, biomass development phase, and cell concentrations. For the exponential cell growth phase, tryptophan-like fluorescence intensity correlated with cell concentrations (Li and Humprey 1991; Khoury et al. 1992). More recently, fluorescence spectroscopy, coupled with chemometric techniques (i.e. partial least squares (PLS), principal components analysis (PCA), artificial neural networks (ANNs)), has been utilized for online monitoring of an anaerobic digestion process (Skibsted et al. 2001; Morel et al. 2004; Wolf et al. 2007). Morel et al. (2004) found a good correlation between synchronous fluorescence spectra characterising the digestion process with chemical oxygen demand, volatile fatty acids content, and methane production.

In algal monitoring, portable spectrophotometers have been commonly used for in situ assessment of algae composition and quantity. Algae do not pose a significant problem to water treatment processes provided the populations are relatively low. However, if present in high concentrations, algogenic OM is known to interfere with several drinking water treatment processes (i.e. coagulation, flocculation, filtration). Algogenic OM was found to contain a relatively high nitrogen content and low aromatic carbon and phenolic contents (Fabris et al. 2008; Henderson et al. 2008a, 2009). The fluorescence spectra of algogenic OM exhibit a predominance of tryptophan-like fluorescence of higher excitation

wavelengths and intensities in the exponential phase compared to the stationary phase (Determann et al. 1998; Smith et al. 2004; Nguyen et al. 2005; Elliot et al. 2006; Henderson et al. 2008a). A strong affinity of autochthonous OM for hydrophilic material (i.e. neutral polysaccharides, low-molecular weight mono- and di-carboxylic acids and acidic sugars; Edzwald 1993), has also been corroborated (Laabs et al. 2004; Henderson et al. 2008a).

Of special importance is the detection of cyanobacteria occurrence and dynamics in freshwater bodies used as drinking water supplies (Gregor et al. 2007; Henderson et al. 2008a, b) as many cyanobacteria are toxin-producing (the most common being *Planktothrix rubescens* and *Microcystis aeruginosa*) (Leboulanger et al. 2002). Standard methods of cyanobacteria detection, including monitoring of phytoplankton assemblages and direct chromatographic measurements of cyanotoxins are selective, expensive and time-consuming (Gregor et al. 2007). Fluorescence spectroscopy utilizes the excitation and emission signatures of chlorophyll *a* and other chloroplastic pigments, responsible for light absorption and conversion in autotrophs. Different phytoplankton groups, e.g. green algae and cyanobacteria, have a unique pattern of pigments (chlorophyll *a*, other accessory chlorophylls, carotenoid protein pigments, phycobilins) of intrinsic fluorescence properties, being the basis for the spectral differentiation of phytoplankton communities (Yentsch and Phinney 1985; Determann et al. 1998; Gregor and Maršálek 2005). For chlorophyll *a* the frequently reported emission wavelength value is 680 nm with the maximum chlorophyll *a* content at excitation wavelength 440 nm (430–530 nm). Cyanobacterial pigments are excited at higher wavelengths in the red and orange part of the spectrum, with maximum at 620 nm (550–680 nm) and with emission at 645 nm (640–680 nm). Chlorophyll fluorescence measurements can be carried out in a discrete way (water samples, in vivo fluorescence) or in situ, directly in a water column, with the possibility of online detection and quantification. In situ chlorophyll *a* fluorescence measurements have been performed by several authors (Asai et al. 2001; Pinto et al. 2001; Beutler et al. 2002; Leboulanger et al. 2002; Gregor and Maršálek 2005). However, in situ fluorescence measurements of cyanobacteria would be far more challenging as cyanobacteria demonstrate a high peak T response (Henderson et al. 2008b), similar to that of microbial activity, making it difficult to differentiate between the different fluorophores.

2.3 Application of florescence spectroscopy in OM characterisation in water treatment

Applications of fluorescence spectroscopy in drinking water treatment include studies of OM reactivity with disinfectant (Korshin et al. 1999; Westerhoff et al. 1999; Świetlik and Sikorska 2004), prediction of THM formation (Beggs et al. 2006; Johnstone and Miller 2009; Roccaro et al. 2009), correlation of fluorescence properties with SUVA and DBPs formation during chloramination (Yang et al. 2008), OM characterisation in membrane permeates (Peiris et al. 2008) and, more recently, assessments of OM removal in the coagulation, flocculation and clarification processes (Bieroza et al. 2009) and across complete water treatment works (Bieroza et al. 2010). Her et al. (2003) demonstrated a combined HPSEC-fluorescence approach for DOM characterisation in bulk water samples without fractionation.

Korshin et al. (1999) investigated the effect of chlorination on OM fluorescence. The presence of three groups of fluorophores of different reactivity with chlorine was ascertained (fast-, medium-, and slow-decaying fluorophores). It was observed that upon chlorination, fast-decaying sites were selectively eliminated, whereas the contribution of medium- and slow-decaying fluorophores increased with the chlorine dosage. Overall, the fluorescence emission bands were contracted and a shift towards lower wavelengths was discerned, indicating a breakdown of high molecular weight, aromatic compounds. The changes in structural composition of OM were also reflected in the increase of fluorescence intensity for Cl:DOC < 2.

Świetlik and Sikorska (2004) corroborated the results obtained by Korshin et al. (1999) and found that the observed changes in fluorescence spectra are oxidant dependent. While the fluorescence emission tends to shift to lower wavelengths upon disinfection with chlorine, ozonation produces the opposite effect and a relative increase in the emission wavelength can be observed. Thus, during ozonation, the structural composition of fluorophores is changed and carbonyl-, hydroxyl-, alkoxyl-containing moieties and amino groups become more predominant. A hypsochromic shift in the fluorescence spectra (towards shorter wavelengths) during chlorination

can be caused by a reduction of conjugated bonds in aromatic rings and transformation to more aliphatic conformation by elimination of particular functional groups (carbonyl, hydroxyl, and amine). Furthermore, ozonation increased the protein-like fluorescence intensity (comprising small molecular weight components, aromatic and aliphatic amines and amino acids), whereas the oxidation effect on humic-like and fulvic-like fluorescence was more equivocal. Oxidation leads to fractionation of OM into smaller chromophoric fractions enhancing fluorescence, whereas advanced oxidation produces structural changes in fluorophores decreasing fluorescence (Henderson et al. 2009).

Beggs et al. (2006) found that the reaction between chlorine and DOM resulted in a decrease in fluorescence intensity. A shift to shorter emission wavelengths was observed with chlorine addition, the shift being greater with higher chlorine dosages and reaction times.

Marhaba et al. (2000, 2003, 2009) introduced multiple regression models providing prediction of DOC, concentrations of OM isolates obtained from resin fractionation (Leenheer 1981) and THMFP from the fluorescence properties. The authors measured the fluorescence signal of different OM fractions (hydrophobic and hydrophilic acid, neutral, and base fraction) and defined the EEM areas of the highest intensity for each isolate. All fractions demonstrated increased emission intensities within UV excitation wavelengths, with hydrophobic fractions extending from 370 to 430 nm emission wavelength and hydrophilic fractions with emission wavelengths lower than 370 nm. Likewise, the optical properties of the hydrophobic and hydrophilic fractions were evaluated by Chen and Valentine (2007) and Yang et al. (2008). However, in each of those studies, the spectral location of the peaks reflecting the contribution of particular isolates was different and therefore the results are inconclusive.

Chen and Valentine (2007) conducted a comparative study of N-nitrosodimethylamine (NDMA) formation with monochloramine. Monochloramine as a disinfectant exhibits a lower oxidation strength compared to free chlorine and longer residuals in disinfected water. Wu et al. (2003b) showed that small amounts of free chlorine in equilibrium with monochloramine react preferentially with aromatic moieties in humic substances. Free chlorine during the chlorination was found to destroy more effectively cross-linking structures of fused-ring systems of humic acids compared to monochloramine. Thus, chlorination was demonstrated to create more active sites for subsequent oxidation or substitution reaction than chloramination and was hypothesized to form more THMs than HAAs (Wu et al. 2003b).

As a result of the reaction between NOM and disinfectant, fluorescence EEMs revealed changes in the location and relative intensity of fluorescence peaks. Overall, the changes indicated a breakdown of the peak C fluorophore and a shift towards lower emission wavelength suggesting a reduction in the degree of aromaticity. No relationship was found between NDMA formation potential and fluorescence intensity and hence the authors concluded that fluorescence is a poor indicator of NDMA formation. However, the study appears to contain several methodological inaccuracies regarding naming and identification of fluorescence EEM peaks and no attempts have been made to utilize the changes in spectral peak locations.

Yang et al. (2008) compared the application of SUVA and fluorescence OM properties to DBP formation during chloramination. Although OM-chlorine interactions in chloramination process are less intensive than in chlorination, they can yield significant information on the overall reactivity of OM with oxidants. Yang et al. (2008) found that SUVA performed better as a THMFP predictor than fluorescence. The poorer fluorescence performance was ascribed to the utilization of the fluorescence regional integration (FRI) model for integration of the fluorescence spectra instead of specific peak information. The FRI model provides an evaluation of relative distributions of different fluorophores. In this method, the EEM is divided into a small number of fluorescence regions notionally corresponding to particular OM constituents of distinctive spectrofluorometric properties (Chen et al. 2003) (Fig. 3). The relative importance of a particular fluorescence region can be quantified by determining the volume of fluorescence beneath a given region. It was found that the cumulative EEM volumes at regions II and IV (protein-like fluorescence) correlated with the yields of dichloroacetic acid (DCAA, $R^2 = 0.60$), chloroform ($R^2 = 0.42$), dichloroacetonitrile (DCAN, $R^2 = 0.53$), and TOX ($R^2 = 0.63$). The corresponding correlations with SUVA were higher (DCAA $R^2 = 0.82$, chloroform $R^2 = 0.73$, DCAN $R^2 = 0.88$ and TOX $R^2 = 0.80$). Thus, the authors concluded that the FRI could possibly have hindered the investigation of any

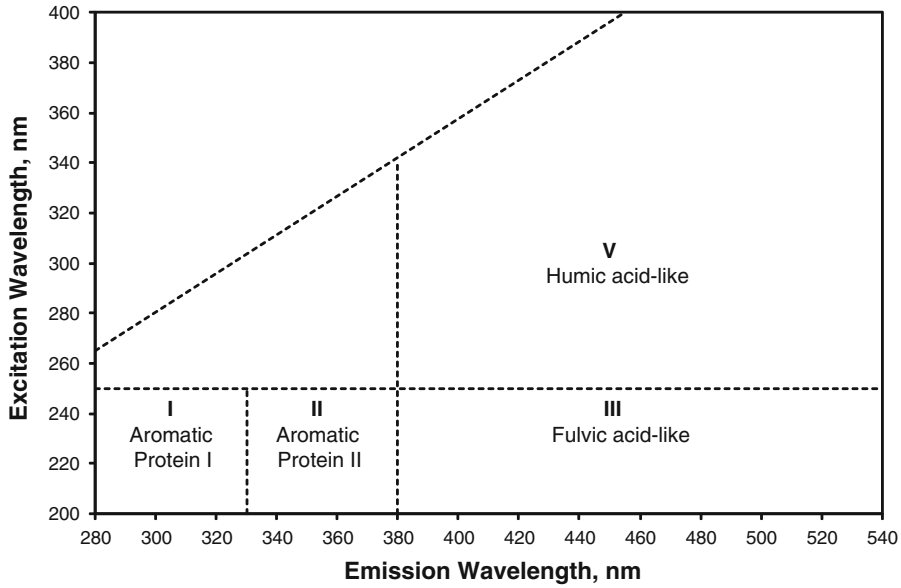
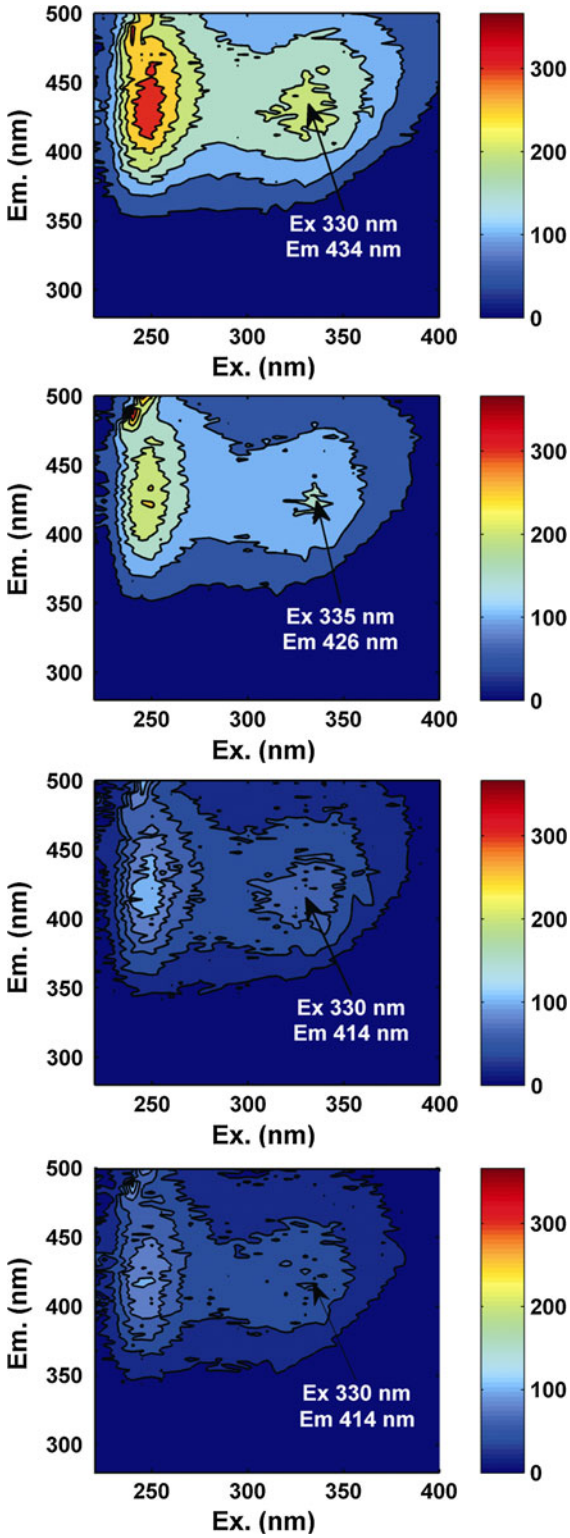


Fig. 3 Fluorescence regional integration, after Chen et al. 2003

relationship between fluorescence and DBP formation and recommended further investigation of the fluorescence peak information. It is the case that the FRI method can only provide quantitative information on the content of pre-defined groups of fluorophores located in the similar areas of the EEM. As different fluorophores can reflect substantially unlike OM properties, the use of FRI can lead to inadequate conclusions regarding OM reactivity and functionality. This can explain the poor correlations found between FRI-derived parameters and OM properties (e.g. THMFP). On the other hand, the PARAFAC model produces a unique, potentially chemically meaningful solution (Bro 1997).

Johnstone and Miller (2009) used the EEM technique coupled with FRI to determine DBP formation. DBP formation was related to changes in fluorescence properties and chlorine consumption using multifactor linear regression. The authors tested two models; the first model incorporating only FRI data and the second model with both fluorescence and chlorine consumption predictors. The prediction quality was dependent on the particular DBP species formed and was significantly improved for the more complex model (CL_3AA $R^2 = 0.59$ for the fluorescence model and 0.90 for fluorescence/chlorine consumption model, $CHCl_3$ $R^2 = 0.33$ and 0.82, Cl_2AA $R^2 = 0.38$ and 0.86 respectively). Furthermore, different fluorescence regions were found to be predictors of different DBP species. In particular, region 2 of simple aromatic proteins correlated with chloroform yield ($R^2 = 0.78$), region 4 of soluble microbial-like OM correlated with dichloroacetic acid ($R^2 = 0.79$), and the combination of regions 3 (humic-like fluorescence with UV emission) and 4 was found to correlate with trichloroacetic acid ($R^2 = 0.87$). Similar to the study of Yang et al. (2008), the results obtained by Johnstone and Miller (2009) refer to the arbitrary selected fluorescence regions, not to the real fluorophores. Therefore the true relationships between fluorophore properties and DBPs formation remained obscured.

Roccaro et al. (2009) attempted to correlate changes in synchronous fluorescence properties of OM with DBP formation. They defined a fluorescence index $\lambda_{0.5}^{em}$ as the position of the normalized emission band at its half-intensity for the fixed excitation wavelength of 320 nm. During chlorination, a blue-shift of the fluorescence index was observed with increasing reaction time or chlorine dosage. Similarly, the changes in the magnitude of fluorescence intensity ratio at 500 and 450 nm were examined. Both parameters were also found to correlate with the concentrations of several DBP species ($R^2 > 0.90$). The changes in the fluorescence parameters were hypothesized to reflect destruction of the reactive aromatic groups in OM. However, the effect of chlorine quenching on the applicability of fluorescence parameters presented in the chlorination study was not addressed by the authors.



◄ **Fig. 4** Fluorescence EEMs of raw, post-clarification, post-GAC, and final water at site 13 collected on August 5, 2007. The location of *peak C* fluorescence maximum indicated with *arrow*. Fluorescence emission intensity in arbitrary units

In the majority of studies discussed above, fluorescence measurements were carried out on OM fractions derived from other analytical techniques (i.e. HPSEC or resin fractionation) and no reference to bulk OM properties was made. To address this shortfall, Bieroza et al. (2009) investigated bulk OM removal across the coagulation/flocculation/clarification processes at 16 UK WTWs and found that peaks C and T characterized the OM in terms of hydrophobicity and microbial activity, and that fluorescence properties could be used to predict both removal and spatial and temporal variations of OM. The same authors subsequently presented EEMs of water at various stages of the treatment process (raw, clarified, post-GAC, final) (Bieroza et al. 2010), demonstrating clearly the reductions in Peaks C, A and T as the water quality improved (Fig. 4). In combination, this work demonstrated the real potential of using fluorescence spectroscopy at WTWs to predict both OM removal and therefore optimise coagulant dosage, and also to monitor unit process performance and so identify plant failure.

3 Summary

OM characterisation (including its reactivity with disinfectants and treatability with standard treatment processes) is of great importance to WTW managers and operators. Understanding the organic character of water can help operators to optimise coagulation and subsequent OM removal techniques and to estimate the required chlorine dose. Moreover, the rapid assessment of OM constituents can help in predicting DBP concentrations for the final water and in making adequate adjustments to the operation of the processes prior to disinfection. Therefore, knowledge about the structural and chemical properties of OM and their reactivity is a key factor in controlling DBP formation in drinking water treatment.

Two groups of methods for characterising OM in drinking water treatment are often employed; viz.

fractionation techniques (i.e. HPSEC, resin fractionation) and spectroscopic techniques based on several parameters derived from UV–Vis absorbance measurements. The first group of methods provides comprehensive OM characterisation in terms of molecular weight distribution and the degree of hydrophobicity, which are important characteristics of OM reactivity and treatability. However, the fractionation measurements are time-consuming, require intensive sample pre-treatment that can significantly change the OM properties, and are difficult to implement in the rapid assessment of OM at WTWs. Furthermore, the selectivity of the fractionation techniques can be limited to particular OM fractions.

Fluorescence spectroscopy is more sensitive compared to UV–Vis absorbance spectroscopy (10–1,000 times) with the possibility of single-molecule detection (Kalbitz et al. 2000; Henderson et al. 2009). Fluorescence measurements are more robust than absorbance at low DOM concentrations (del Castillo et al. 1999). Furthermore, fluorescence spectroscopy provides discrimination between chromophoric OM absorbing at similar wavelengths (McKnight et al. 2001; Stedmon and Markager 2005; Henderson et al. 2009).

To optimise OM removal and to minimise and control the formation of DBPs successfully, a suitable analytical technique for comprehensive OM characterisation should be employed at WTWs. Fluorescence spectroscopy offers a potential alternative to the existing analytical methods of OM characterisation. The advantages of fluorescence include rapid, sensitive and selective characterisation of aquatic OM, no sample pre-treatment including chemical alterations, small sample volume (a few ml), and the potential for on-line monitoring incorporation. Fluorescence enables the rapid assessment of OM constituents and can potentially provide useful information on OM reactivity and treatability. Additionally, fluorescence can provide an indication of the OM sources (allochthonous versus autochthonous). There now exists a body of literature which has identified relationships between fluorescence spectra and OM physico-chemical properties (i.e. degree of hydrophobicity, microbial content), has applied fluorescence spectroscopy to characterise the changes in OM upon disinfection, and related the fluorescence properties to DBP formation.

Further work is now required in the robust management of data arising from fluorescence spectroscopy analysis and, in particular, EEMs. Consideration must be given as to how the data might best be employed to greatest effect on a routine basis at WTW. It is believed that this could be achieved via minimization of expert user input and the automatic, continuous reporting and monitoring of fluorescence intensities at a small number of excitation (e.g. Peaks T1, T2, A and C).

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