

THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

New applications of fluorescence spectroscopy for
monitoring drinking water disinfection and
distribution

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Fluorescence excitation-emission matrix of drinking water

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Abstract

The task of providing safe drinking water requires proper monitoring of water quality and treatment performance from source to tap. Accordingly, the demand for online monitoring is increasing both at treatment plants and within distribution networks. Some of the available techniques use correlations between the optical properties of dissolved organic matter (DOM), mainly absorbance, and other water quality parameters. Fluorescence spectroscopy is significantly more sensitive than absorbance and gives comprehensive information about the composition and concentration of organic matter, so has a strong potential for online monitoring applications.

In this thesis, the application of fluorescence spectroscopy was investigated at two locations in the drinking water treatment system: the distribution network and the ultraviolet (UV) disinfection chambers. In the distribution network, the sensitivity of fluorescence to detect contamination caused by entrainment was compared to the sensitivity of other common water quality parameters including several trace elements and microbial indicator species abundances. Of these, fluorescence was the most sensitive tracer for distinguishing contamination from natural variation, followed by absorbance. The relationship between fluorescence and microbial regrowth was also examined, however, no correlation was observed. Following UV disinfection, the application of fluorescence as a proxy of the UV dose was examined. Fluorescence was sensitive enough to detect changes in the fluorescent fraction of organic matter due to UV irradiation at disinfection doses. A linear relationship was observed between UV dose and changes in humic-like fluorescence intensities at doses up to 350 mJ/cm^2 .

Keywords: Fluorescence spectroscopy, drinking water, distribution network, UV disinfection, dissolved organic matter.

LIST OF PAPERS

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- i. Heibati, M., Stedmon, C.A, Stenroth, K., Rauch, S, Toljander, J, Säve-Söderbergh, M. and Murphy, K.R. Assessment of drinking water quality at the tap using fluorescence spectroscopy. Submitted manuscript.
- ii. Heibati, M., Stedmon, C.A, Bergstedt, O., and Murphy, K.R. Identifying a fluorescent residual following UV disinfection of drinking water. Manuscript.

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Mohanna

LIST OF NOTATIONS

UV	Ultraviolet
UV254	Ultraviolet (irradiation at 254 nm)
TOC	Total Organic Carbon
BOD	Biochemical Oxygen Demand
DOM	Dissolved Organic Matter
DOC	Dissolved Organic Carbon
SUVA	Specific Ultra Violet Absorbance
WTP	Water Treatment Plant
FDOM	Fluorescent Dissolved Organic Matter
CDOM	Chromophoric (coloured) Dissolved Organic Matter
NOM	Natural Organic Mat

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1 Introduction

Monitoring and control technologies are essential for the production and delivery of safe drinking water to costumers. They are the tools providing information on source water quality and defining the boundary conditions and operational parameters for treatment processes, and consequently the efficiencies of each treatment step. Not only are they mandatory for ensuring the success of treatment processes, but they also are indispensable for detecting changes in water quality within the distribution network and at the consumers' taps (Hargesheimer et al. 2002).

Existing methods for monitoring the microbial and chemical quality of water and the performance of treatment processes are either time consuming or not sensitive enough. Common parameters such as pH, turbidity, flow and ultraviolet (UV) absorbance can be monitored in real time during the treatment processes and the distribution (Storey et al. 2011, van der Gaag et al. 2008). However, they only provide general information about the bulk properties of water. Microbial parameters such as microbial indictors are measured in the laboratory in a process that usually takes 1-7 days, therefore, not able to detect subtle changes in water quality rapidly and differentiate systematic variation from normal variation in real time.

Additional online monitoring techniques could deliver early warning systems for monitoring water quality (Storey et al. 2011) and the performance of treatment processes (Hargesheimer et al. 2002). Some of these techniques use the correlation between physical properties, in particular the optical properties (mainly absorbance) of organic matter, and other water quality parameters, such as biochemical oxygen demand (BOD), TOC and turbidity (Storey et al. 2011). The potential of fluorescence spectroscopy as an online monitoring tool for water quality has been investigated in previous studies (Ahmad and Reynolds 1999, Baker et al. 2015, Hambly et al. 2010, Shutova et al. 2014). Due to significantly the higher sensitivity of fluorescence compared to absorbance, there is great interest in fluorescence spectroscopy for monitoring drinking water treatment systems and distribution networks. However, before implementing fluorescence as a proxy of any other water quality parameter, its sensitivity and reproducibility must be understood.

Disinfection by UV irradiation and chlorination are implemented in the final stages of drinking water treatment. After water has been treated to remove organic and inorganic matters (suspended or dissolved) and microorganisms, typically by coagulation/flocculation, sedimentation and filtration, disinfection is applied to inactivate any remaining pathogenic microorganisms. UV disinfection is often considered the primary disinfection method due to its higher efficacy against *Giardia lamblia* cysts and *Cryptosporidium* oocysts compared to chlorination (Hijnen et al. 2006). UV irradiation can modify the structure of organic matter pool in drinking water (Del Vecchio and Blough 2002), leading to direct or indirect disinfection by products and formation of assimilable organic matter. However, the composition and concentration of organic matter varies among source waters, affecting the degree of organic matter modification due to UV irradiation. In addition, the source and dose of the applied UV

light are important factors defining the extent to which UV disinfection alters the structure of organic matter. Although some studies reported an effect of UV irradiation on organic matter composition at UV doses applied during drinking water disinfection (Liu et al. 2002, Magnuson et al. 2002), the relationship between UV dose and organic matter properties is poorly understood, as is the effect on this of a change in water source. A better understanding of this relationship might assist us to predict the effectiveness of UV disinfection by its impact rate on organic matter rather than by performing time-consuming microbial analyses.

After leaving the treatment plant, drinking water enters the distribution network and resides there until it is delivered to the costumers' tap. Water quality can quickly deteriorate within the distribution network due to e.g. microbial regrowth, corrosion or entrainment of untreated water through damaged pipes, leading to serious health risks to consumers (WHO 2014). High organic matter concentrations can provoke excessive regrowth, resulting in declining water quality including poor odour, taste and turbidity (Van Der Kooij 2000). Changes to water quality within distribution networks need to be detected before the water reaches the customer. Fast and sensitive monitoring methods are needed that can distinguish unsafe conditions from natural variation.

1.1 Research hypotheses and aims of this thesis

The aim of the study was to answer the following research questions:

1. What is the fluorescence composition of treated drinking water?
2. Is organic matter fluorescence a sensitive proxy to detect drinking water quantity/quality changes?

Paper I:

- 2.1. How much does organic matter fluorescence intensity vary in distribution networks compared to other parameters?
- 2.2. Is the protein-like fluorescence intensity (short wavelength excitation) a proxy of microbial regrowth in the distribution network?

Paper II:

- 2.3. Do the fluorescence intensities of organic matter components in treated water respond to UV disinfection in the same way?
 - 2.4. Does UV disinfection cause any fluorescence components to be produced?
 - 2.5. Is there a relationship between UV dose and fluorescence intensity? If there is such a relationship, is it predictable?
3. What artefacts interfere with quantifying fluorescence in treated drinking water?

Paper I:

- 3.1. What artefacts might affect fluorescence measurements at the tap?

Paper II:

- 3.2. Is fluorescence the same if measuring in-situ compared to measuring grab samples?

2 Background

2.1 Dissolved organic matter (DOM)

Natural organic matter (NOM) is a complex, heterogeneous mixture of organic compounds present in all natural surface and ground waters. It is composed of a continuum of sizes between two operationally-defined groups: dissolved and particulate DOM. DOM is defined as the fraction of organic substances which passes through a filter (typically 0.45 - 1.0 μm) whereas the particulate fraction does not (Hansell and Carlson 2002). DOM derives from a range of origins including terrestrial (exogenous) or aquatic (autochthonous, *in situ*) sources and is one of the largest reservoirs of organic matter in natural waters. The dominant fraction of DOM comprises of humic substances (humic and fulvic acids). Among the other fractions of DOM, there are carbohydrates, amino acids, proteins and phenols with various molecular sizes and properties (Rodriguez-Zuniga et al. 2008).

DOM in natural water plays multiple functions in photo-induced, chemical, microbial and geochemical processes (Corin et al. 1996, Ogawa and Tanoue 2003). For example, it interacts with metal ions and controls their speciation and bioavailability (Cuss and Gueguen 2014, Yamashita and Jaffe 2008). By absorbing light irradiation, DOM has a substantial impact upon the amount of light available to aquatic microorganisms for photosynthesis (Del Vecchio and Blough 2004). Through photochemical reactions, the bioavailability of DOM is altered and photochemistry may destroy or generate the substrates for microorganisms (Mopper and Kieber 2002, Rodriguez-Zuniga et al. 2008).

The chemical characterization of DOM components is hampered by their chemical complexity. DOM is often quantified only as total organic carbon (TOC) or dissolved organic carbon (DOC) which represent the overall quantity of organic matter in the particulate and dissolved pools (Hansell and Carlson 2002). To study the composition and sources of additional DOM pools in water, various analytical technologies and methods have emerged. Measuring the spectroscopic properties of the optically-active fraction of DOM, including UV- visible spectra and fluorescence spectra, is one of the most important developments in the water sciences for gaining information about the concentration, composition and source of DOM (Coble et al. 2014a). Advantages of these spectroscopic approaches include high sensitivity and simplicity of their techniques. They also provide rapid and non-invasive methods using a small volume of sample with little need for preparation prior to analyses.

2.2 DOM Optical properties

2.2.1 Absorbance

The optically-active fraction of DOM, called chromophoric or coloured dissolved organic matter (CDOM), determines the optical properties of DOM (Green and Blough 1994, Stedmon et al. 2000). CDOM consists of a mixture of components which have different optical properties. They absorb light in visible wavelengths and also ultra violet UV-A (315 - 400 nm) and UV-B (280 - 315 nm), producing the yellow to brown colour of natural waters (Blough and

Del Vecchio 2002). CDOM light absorption decreases in an approximately exponential fashion with increasing irradiation wavelength across the visible-UV spectrum (Figure 1) (Coble 2007) (eq. 1).

$$a(\lambda) = a(\lambda_0) + e^{-S(\lambda - \lambda_0)} \quad \text{eq. 1}$$

where, $a(\lambda)$ and $a(\lambda_0)$ are the absorption coefficient at wavelength λ and reference wavelength λ_0 , respectively and S is the spectral slope parameter. The absorption coefficient, a , is calculated as according to eq. 2:

$$a = 2.2302 A/l \quad \text{eq. 2}$$

where, l is the path length and A is absorbance, calculated as:

$$A = \log(I/I_0) \quad \text{eq. 3}$$

where, I and I_0 are the incident and transmitted light, respectively.

According to the Beer-Lambert law, the absorbent molecules act independently and do not interact with each other, so that the absorption spectrum of a mixture of DOM components is a simple superposition of the spectra from the individual chromophores (Coble et al. 2014a). However, some researchers believe that interactions between molecules commonly influence the shape of absorbance spectra in DOM mixtures, especially at longer wavelengths (Del Vecchio and Blough 2004).

The UV- visible absorbance spectra of water samples normally appear to be featureless, yet are still informative. Absorption ratios are correlated to other properties such as molecular size, aromaticity, atom ratios of oxygen to carbon (O:C) and carbon to nitrogen (C:N) (Chen et al. 1977). Absorbance at 254 nm divided by DOC concentration (SUVA) is correlated with the concentration of aromatic components (Helms et al. 2008, Weishaar et al. 2003).

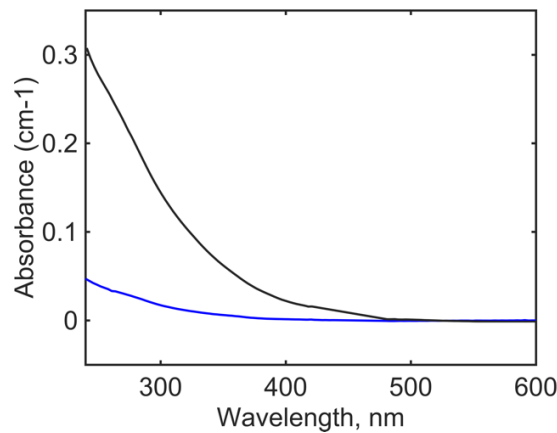


Figure 1. UV- visible absorption spectra of a drinking water sample at the tap (blue) and an untreated water sample from treatment plant (black) (Paper II)

2.2.2 Fluorescence

A subset of CDOM compounds emit some absorbed light as fluorescence and belong to a pool known as fluorescent dissolved organic matter (FDOM). Fluorescence is the phenomenon occurring following light absorption by fluorophores (Figure 2). The electron of the fluorophore molecule is excited to a higher energy level after absorbing the light (photon). This is followed by vibrational relaxation to the lowest level of the excited state. Finally, the electron returns to the ground state by emitting the energy as fluorescence. Sir G. G. Stokes first explained that the energy of light emitted by a fluorophore is always less than the energy of absorption (i.e. longer wavelength) because of the loss of energy at the vibrational state. This phenomenon is called the Stokes shift (Lakowicz 1983) (Figure 3).

The intensity and the wavelengths at which each fluorophore fluoresces is controlled by its molecular structure. For instance, aromatic molecules containing freely rotating functional groups lose energy through these functional groups, thus aromatic molecules with functional groups fluoresce less intensely compared to those without. Conjugated molecules fluoresce at longer energy wavelengths (lower energy) due to the small energy gaps between the excited and ground levels. The emission spectra of CDOM are broad and featureless. By increasing the excitation wavelength, the emission spectra of CDOM shift to longer wavelengths (red shift) and decrease in intensity (Coble et al. 2014b). Figure 1 shows the absorption (excitation) and emission spectra of a FDOM component identified in Paper II of this study.

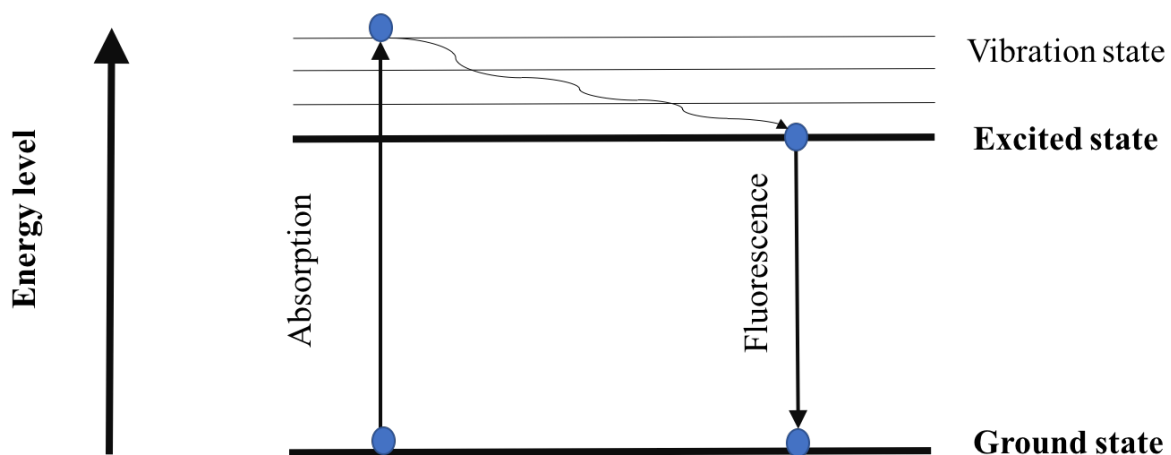


Figure 2. Simplified Jablonski diagram describing the fluorescence phenomenon.

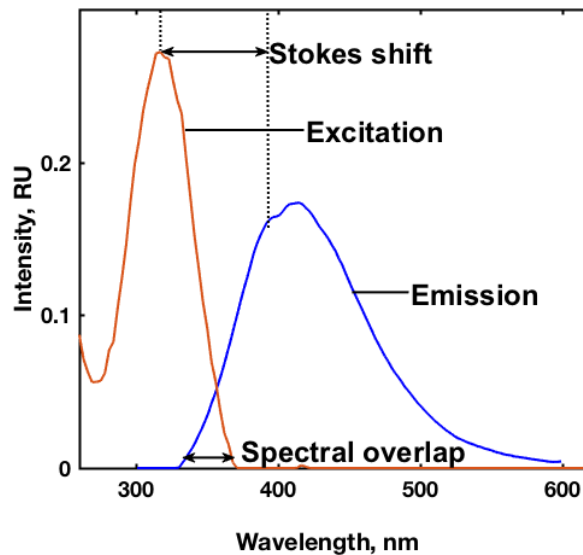


Figure 3. Excitation and emission spectra of a FDOM component, identified using parallel factor analysis in Paper II.

The most comprehensive method of measuring FDOM is to measure fluorescence emission over a range of excitation wavelengths, which produces excitation-emission matrices (EEMs, Figure 4) (Baker 2001, Coble et al. 2014a). The EEMs can be collected together in a 3-dimensional dataset of fluorescence intensities for different samples and excitation-emission pairs. EEMs can provide considerable information about the DOM pool. A commonly-used parameter calculated from fluorescence EEMs is the so-called fluorescence index (FI), which is the ratio of fluorescence intensity measured at 470 to 520 nm at excitation wavelength of 370 nm (Cory and McKnight 2005). The FI is sometimes used to estimate the relative contribution of microbial and terrestrially derived FDOM in DOM pool, although its sensitivity for this purpose has been questioned (Lavonen et al. 2015, Murphy et al. 2008). Another parameter in frequent use is the freshness index (emission intensity at 380 nm divided by the maximum intensity between 420 and 435 nm at 310 nm excitation) which is used to estimate DOM age (Lavonen et al. 2015). Protein-like fluorescence (tryptophan- and tyrosine-like) is attributed to proteins or aromatic amino acids; therefore, is used as a proxy of biological activity (Determann et al. 1998, Sorensen et al. 2015).

Previously-reported fluorescence components extracted from EEM datasets, and their reported sources, are listed in Table 1 (Coble 2007, Ishii and Boyer 2012, Murphy et al. 2008). The fluorescence components that have been observed in drinking water samples are similar to those observed in diverse aquatic samples. They include humic-like components with either a terrestrial or autochthonous origin, and protein-like components containing tyrosine-like or tryptophan-like fluorophores.

Table 1. Fluorescence properties of reoccurring CDOM components

Component	Peak name (Ref. 1, Ref. 2, Ref. 3) ¹	Peak position (Ex/Em) ²	Peak Source
Tyrosine-like, protein-like	C1 ¹ , B ²	270/300	Autochthonous
Tryptophan-like, protein-like	C6 ¹ , T ²	275/328 (340)	Autochthonous
UVC humic-like	C3 ¹ , C8 ¹ , A ² , C1 ³	260/400-460	Terrestrial, allochthonous
UVA humic-like	C3 ¹ , C8 ¹ , C ²	320-360/420-460	Terrestrial, anthropogenic
Marine humic-like	C2 ¹ , M ²	290-310/370-410	Terrestrial, agriculture
UVA humic-like + UVA humic-like	C2 ³ , C2 ⁵	<240-275(295-380)/375-450	Terrestrial, anthropogenic ¹

¹ Ref. 1 (Murphy et.al. 2008); Ref. 2 (Coble 2007); Ref. 3 (Ishii and Boyer 2012)

² Wavelength given in parenthesis represents the secondary peak.

Parallel factor analysis

If the Beer-Lambert law applies, then fluorescence EEMs datasets can be separated into chemically and mathematically independent fluorescence components using parallel factor analysis (PARAFAC). PARAFAC decomposes multi-way data of EEMs (sample x excitation x emission) into independently-varying components using alternating least squares algorithm. The results of a PARAFAC model are represented as the excitation and emission spectra of the identified independent fluorescence signals termed as ‘components’ and the calculated relative intensity of each component in each sample termed as ‘score’ (Bro 1997). The trilinear terms and residual array of a PARAFAC model are presented as:

$$x_{ijk} = \sum_{f=1}^F a_{if} b_{jf} c_{kf} + e_{ijk} \quad \text{eq. 4}$$

In eq. 4, x_{ijk} is the fluorescence data of the i^{th} sample at the j^{th} and k^{th} excitation and emission wavelengths, respectively. f represents the number of underlying component and e_{ijk} is the residual of data which is not explained by the model.

Figure 4 illustrates the decomposition of drinking water EEMs into underlying fluorescence components using PARAFAC.

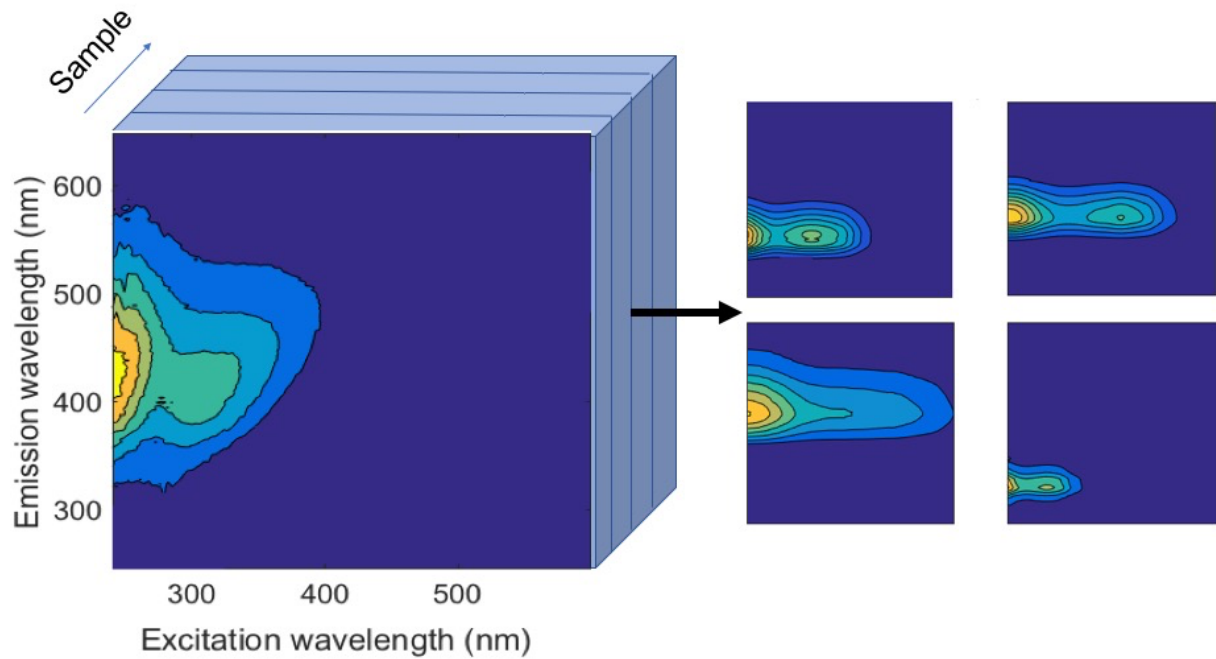


Figure 4. A drinking water EEM dataset can be decomposed into underlying fluorescence components using PARAFAC (Paper I)

2.2.3 Parameters and processes affecting DOM fluorescence

In practice, the fluorescence properties of DOM, including the intensity and position of spectral peaks, do not only depend on fluorophore type and concentration. They are also a function of temperature and pH together with chemical interactions and processes. At low concentrations and under constant measurement conditions (pH, temperature, metal ion concentration, etc.), it can be assumed from the Beer-Lambert law that the fluorescence intensity of each component is directly proportional to its concentration (Lakowicz 1983). However, at high concentrations, and under changed measurement conditions, fluorescence properties vary as described below.

Temperature and pH

DOM fluorescence is influenced by pH due to protonation or deprotonation of acidic (-COOH and -OH) and basic (-NH₂) functional groups of aromatic fluorophores. Protonation (adding H⁺) has different effects on various acidic and basic functional groups. Fluorescence of the fluorophores containing electron-withdrawing functional groups (-COOH) shifts to longer wavelengths by protonation and those with electron-donating groups (-NH₂ and -OH) shift to shorter wavelengths (Coble et al. 2014a). The scale of pH influence on fluorescence intensity varies between fluorophores. In aquatic systems when the pH is increased from 5 to 9, fluorescence intensity has been reported to change limited (Spencer et al. 2007). Tyrosine-like fluorescence has been reported to be more sensitive to changes in pH than humic-like fluorophores (Reynolds 2003).

Fluorescence intensity decreases with increasing temperature due collisional quenching at high temperature (Baker 2005, Hudson et al. 2007). Different fluorophores show different dependencies on temperature. The effect of temperature is greater for tryptophan-like fluorescence than for humic-like fluorescence (Baker 2005).

Quenching

Fluorescence quenching is a phenomenon which involves a chemical interaction between a fluorophore and a quenching agent resulting in reduced fluorescence intensity. Depending on whether the interaction between the fluorophore and quencher occurs in the excited state or the ground state of the fluorescing molecule, there are dynamic and static quenching processes, respectively (Lakowicz 1983). In dynamic quenching, the interaction between the fluorophore and quenching species occurs in the excited state of the potentially fluorescing species. Static quenching is due to chemical bonding between a fluorophore in ground state and a quenching species, resulting in the formation of non-fluorescing complexes. One of the most well-known examples of static quenching is the interaction between trace metal ions in aquatic environments such as iron and copper and DOM ligands (Yamashita and Jaffe 2008). The quenching or suppression of DOM fluorescence intensity by copper ions in drinking water system was investigated in Paper I of this thesis.

To estimate the binding parameters of metal-ligand complexation using fluorescence data, the modified Stern-Volmer model can be used (Hays et al. 2004). The two binding parameters including the quenching constant (K_M) and initial fraction of fluorescence contributing to quenching (f) are calculated according to eq. 5:

$$\frac{F_0}{F_0-F} = \frac{1}{f \cdot K_M \cdot C_M} + \frac{1}{f} \quad \text{eq. 5}$$

where, C_M is the measured metal concentration. F and F_0 are fluorescence intensities corresponding to the sample with metal concentration of C_M , or in the absence of metal, respectively. K_M and f values were determined by plotting the relative fluorescence intensity of each component ($\frac{F_0}{F_0-F}$) against the inverse concentration of metal ($\frac{1}{C_M}$).

Inner filter effects

Fluorescence quenching can also occur in the absence of chemical interactions with other species (Coble et al. 2014a). Self-quenching, also called the “inner filter effect” (IFE), needs to be considered while collecting fluorescence data. IFE occurs when the molecules absorb a fraction of either excited light (primary IFE) or emitted light by the fluorophore, leading to reduced fluorescence intensity (Tucker et al. 1992). IFE is function of absorbance and light path length. Since absorbance increases in exponential fashion by decreasing light wavelength, IFE is greater at shorter wavelengths (Kothawala et al. 2013, Lakowicz 1983).

2.3 Photochemistry

CDOM is the main visible- and UV-light absorbing component of aquatic systems. Light absorption by the organic molecules in CDOM is followed by structural alteration and gradual degradation. Photochemical reactions can produce intermediate products which also destroy CDOM, ultimately leading to changes in CDOM optical properties including UV-absorbance and fluorescence intensity. This is called photobleaching (Del Vecchio and Blough 2002, Helms et al. 2014, Moran et al. 2000).

Fluorescence intensity (Moran et al. 2000, Waiser and Robarts 2004) and UV-absorbance (Ferrari et al. 1996) by CDOM both decrease as a result of solar and UV irradiation. Additionally, molecular size, aromaticity and DOC concentration decrease after irradiation (Benner and Ziegler 1999). The loss rates of various DOM fractions (DOC, CDOM and FDOM) are different following irradiation. The photodegradation rate of DOC is lower than for CDOM photobleaching, and the removal of FDOM outpaces the removal of CDOM (Moran et al. 2000, Spencer et al. 2009).

The extent of alteration of DOM fluorescence by irradiation differs for different fluorophores (Moran et al. 2000). In response to UV and solar irradiation, protein-like fluorescence decreases less than humic-like fluorescence (Moran et al. 2000). The wavelengths where fluorescence peaks are observed is also prone to alteration. Photoirradiation destroys conjugated bonds producing smaller molecules and fluorescence shifts to shorter wavelengths (Helms et al. 2013).

In drinking water systems, UV irradiation is applied as the primary disinfection process to inactivate pathogens. It is most common to use low-pressure (LP, monochromatic at 254 nm) or medium-pressure UV (MP, polychromatic) light sources (Hijnen et al. 2006). The light dose employed for disinfection under normal operating conditions is often considered to be too low to alter DOM structure or change its optical properties. However, in Paper II of this thesis, UV₂₅₄ irradiation of drinking water is shown to produce measurable changes to DOM fluorescence (Paper II).

2.4 Applications of fluorescence in aquatic systems

For more than 60 years, fluorescence spectroscopy has been used to characterise DOM in various aquatic systems (Hudson et al. 2007). The large quantity of carbon in oceanic DOM gives it an important role in balancing the global carbon budget. Extensive studies have been made of marine ecosystems, using DOM fluorescence to characterize composition, concentration and dynamic of DOM derived from different sources (Coble 1996, Jaffe et al. 2004, Yamashita and Tanoue 2003). Fluorescence spectroscopy has also been used extensively to study the DOM distribution in marine systems due to its significant role in the microbial loop and in determining nutrient fluxes to microorganisms.

In rivers and lakes, to lesser extent than in marine systems, fluorescence spectroscopy has been used to characterize DOM composition and source (Hautala et al. 2000, Her et al. 2003, Newson et al. 2001). The spatial changes of humic-like and tryptophan-like DOM fluorescence has been

used to trace the source of DOM in upstream and downstream waters (Baker 2002, Galapate et al. 1998). DOM fluorescence has also been used to identify the pollutants in urban catchments affected by agriculture (Baker et al. 2004, Graeber et al. 2015). A strong correlation between protein-like fluorescence and biodegradable DOM is reported for freshwater ecosystems, therefore fluorescence has been used to evaluate biological activity in rivers and lakes (Fellman et al. 2010).

In wastewater systems, fluorescence spectroscopy has been used for monitoring the treatment processes and water quality (Ahmad and Reynolds 1999). Due to the strong fluorescence signals in sewage-derived DOM, fluorescence has been investigated as a surrogate for various water quality parameters commonly used to monitor river pollution (Goldman et al. 2012) and wastewater treatment. Strong correlations have been found between tryptophan-like fluorescence intensity and phosphate (PO_4^{3-}) and nitrate (NO_3^-) in the catchment area of the river water (Baker and Inverarity 2004). Correlations between bulk organic matter parameters including TOC, DOC and chemical oxygen demand (COD) and fluorescence are inconsistent between studies. However, when sewage-derived DOM components are at high concentrations, the correlation between COD and fluorescence is improved (Baker and Inverarity 2004, Wu et al. 2006). There are positive correlations between tryptophan-like fluorescence and biochemical oxygen demand (BOD) in settled sewage (Ahmad and Reynolds 1999). Protein-like fluorescence has been used as an indicator of the presence /absence of faecal coliforms (Sorensen et al. 2015).

2.5 Fluorescence spectroscopy in drinking water

DOM optical properties, mainly involving UV absorbance, have been used to monitor water quality and assess treatment performance in drinking water systems (Bridgeman et al. 2011). UV absorbance parameters, particularly SUVA (UV₂₅₄ absorbance normalised to DOC concentration) has been used as a surrogate for water quality during various treatment processes such as coagulation and filtration (Archer and Singer 2006). In drinking water, SUVA correlates positively with aromaticity, hydrophobicity and the molecular weight of organic matter (Ates et al. 2007, Weishaar et al. 2003). SUVA and UV₂₅₄ are also useful for estimating the formation of disinfection by products (DBPs) (Ates et al. 2007). Fluorescence spectroscopy has greater sensitivity than absorbance (UV-Visible) spectroscopy (Coble 1996). However, it has been used in drinking water systems to a lesser extent than absorbance due to higher instrument costs, and difficulties related to artefacts caused by light scattering by particles, and calibration (Guilbault 1990).

The application of fluorescence spectroscopy to drinking water system has been mainly focused on monitoring water quality. DOM fluorescence can be used as surrogate parameter for other water quality parameters such as DOC and *E-Coli* (Baghoth et al. 2011a, Baker et al. 2015). Strong correlations have been observed between tryptophan-like fluorescence and the abundances of coliforms (Sorensen et al. 2015), the biopolymer fraction (Baghoth et al. 2011b) and bacteria extracted from drinking water, such as *Xenophilus* sp and *Rhodococcus* sp (Bridgeman et al. 2015).

Fluorescence could potentially be used to trace contamination in drinking water distribution networks, as attempted in Paper I of this thesis. Fluorescence is expected to fluctuate when the intrusion of untreated water or microbial regrowth happens within the network and causes probable changes in the CDOM composition or concentration. A high organic matter concentration is a risk factor for microbial regrowth since it provides a source of nutrients and energy for microorganisms. Strong correlations have been reported between humic-like fluorescence (Ex/Em: 300-360/400-480 nm) and DOC concentration of the water samples at taps connected to drinking water network using in-situ fluorometers (Bridgeman et al. 2015). However, the low sensitivity of portable fluorometers makes it difficult to detect small changes in fluorescence due to low-level contamination. In Paper I of this thesis, a benchtop fluorometer is used to examine the limits of fluorescence intensity for detecting contaminated drinking water at the tap. In addition, due to the reported positive correlations between *E. coli* concentrations and tryptophan-like fluorescence in environmental samples (Baker et al. 2015), the correlation between fluorescence intensity and abundance of microbial indicator was assessed.

Fluorescence spectroscopy has also been used to monitor the performance of drinking water treatment processes (Baghoth et al. 2011b, Bieroza et al. 2009a, Bieroza et al. 2009b, Shutova et al. 2014). A few studies used fluorescence to assess the performance of TOC removal during the treatment processes (Bieroza et al. 2009a, Bieroza et al. 2010), but the main application has been to predict the formation of disinfection by products (DBPs) during chlor(am)ination processes (Beggs et al. 2009). Owing to its high sensitivity and ability to give information about the activated aromatics, fluorescence has been used to study the reactivity of organic matter with disinfectants (Korshin et al. 1999).

UV disinfection is used for primary disinfection due its high efficacy against *Giardia lamblia* cysts and *Cryptosporidium* oocysts at many water utilities (Hijnen et al. 2006). UV irradiation during disinfection could potentially modify organic matter structures, leading to changes in their optical properties (absorbance and fluorescence) (Liu et al. 2002). Being able to detect subtle changes to organic matter structure or optical properties following low doses of UV irradiation could be useful for assessing disinfection efficiency. In Paper II of this thesis, DOM fluorescence is used to study changes to the fluorescent fraction of organic matter following UV₂₅₄ irradiation as a proxy of UV dose as a validation method to estimate the UV dose applied during disinfection process.

3 Materials and methods

3.1 Experimental design

3.1.1 Paper I: distribution network

Drinking water samples were collected at 87 locations (taps of houses and public buildings) connected to the municipal drinking water distribution network in Gävle, Sweden in December, 2015. The sampling locations were chosen to encompass the entire range of water residence time (0.5-50 h) within the network.

Concentrations of trace metals (Al, Cu, Fe) were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo Scientific iCAP Q spectrometer. Microbial analyses were performed according to standard methods for quantifying culturable microorganisms (Pour plate technique, incubation at 22°C for 3 days) and slow-growing bacteria (incubation at 22°C for 7 days).

To compare the precision of measured variables in distribution network study, relative standard deviations (RSD = standard deviation/mean) of the replicate samples were determined for each of the chemical and microbial parameters. The averaged measurements of the replicate samples were used in all further analyses.

To determine the threshold at which a significant elevation in the chemical and microbial parameters could be diagnosed, trigger thresholds (TT) were calculated according to eq. 6:

$$TT = \bar{x} + 3s \quad \text{eq. 6}$$

where, \bar{x} and s are the average and standard deviation of measurements from samples collected across the whole network.

3.1.2 Paper II: UV disinfection

The effect of UV_{254} irradiation on DOM fluorescence of drinking water was studied in both field and laboratory experiments. In the field experiment, UV disinfection system at a drinking water treatment plant was studied. The aim was to determine whether the UV disinfection process at the dose applied in treatment plant has a residual on DOM fluorescence or not. Changes in fluorescence intensities of pre- and post- disinfection water were measured during two operational modes: ‘normal flow mode’ and ‘standby mode’.

In the laboratory experiments, water sample was subjected to UV_{254} irradiation at the low range of UV_{254} doses corresponding to UV disinfection and higher UV doses, while DOM fluorescence was measured continuously. The aim was to investigate the relationship between radiant UV_{254} dose and DOM fluorescence intensities. In addition, the effect of DOC concentration of the irradiated sample on fluorescence change was assessed.

The irradiation experiments in the lab were conducted using a customized flow through system (Figure 5). During each experiment, the sample was circulated using a peristaltic pump from the sample vial to the irradiation cell where it was exposed to the UV₂₅₄ irradiation. From the irradiation cell, the sample flowed to the fluorescence cell placed in spectrofluorometer where the fluorescence/absorbance were measured and then recirculated back to the samples vial. The sample temperature during measurements was set at 20°C using a water bath circulated past the fluorescence cell.

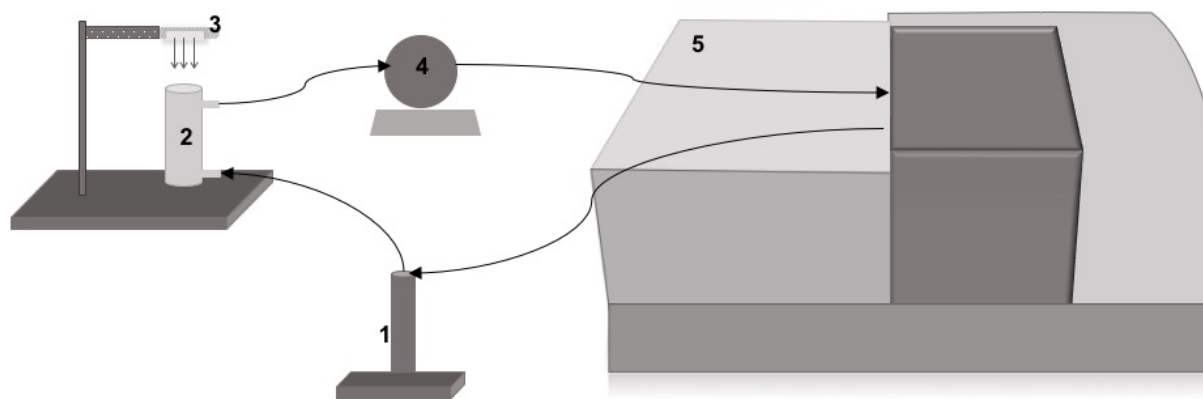


Figure 5. Irradiation experimental set up. 1) sample vial; 2) irradiation cell; 3) UV light source; 4) peristaltic pump; 5) Horiba Aqualog fluorometer. (Paper II)

The UV light source used in the irradiation experiments was a mercury Pen-Ray lamp (UVP, Cambridge, UK) which emits the mercury spectrum with the primary radiant wavelength at 254 nm. To adjust the UV dose, the light intensity or irradiation time were changed. Dose was calculated according to equation 7:

$$\text{UV dose (J/m}^2\text{)} = \text{UV intensity (W/m}^2\text{)} \times \text{Exposure time (s)} \quad \text{eq. 7}$$

The applied UV dose ranged from low (10 - 350 mJ/cm²) to very high (up to 20000 mJ/cm²). The low doses are similar to the range of doses employed for drinking water disinfection (Lyon et al. 2014).

3.2 Equipment and analyses

3.2.1 Optical properties

DOM fluorescence and absorbance were measured using an Aqualog spectrofluorometer (Horiba Scientific) in a 1-cm quartz cuvette (Paper I) or a 1-cm quartz through-flow cell (Paper II). Excitation and emission matrices (EEMs) were obtained at 20°C with 2-3 s integration time. The emission wavelengths spanned from 240 to 800 nm at 2.33 nm increments, and the

excitation wavelengths spanned from 220 or 240 to 600 or 700 nm at 3 nm increments. Blank EEMs were obtained from ultra-pure water sealed in a quartz fluorometer cell.

The measured EEMs were imported into MATLAB for post-processing. Fluorescence intensities were converted to Raman unit (RU) by dividing to the Raman area of the blank within the emission wavelength of 378 - 424 nm at excitation wavelength of 350 nm (Lawaetz and Stedmon 2009, Murphy et al. 2010). Prior to modelling, the scatter bands (Raman and Rayleigh) were removed and data were corrected for concentration biases (inner filter effects) (Andersen and Bro 2003, Lakowicz 1983).

PARAFAC modelling was implemented using the *N-way* and drEEM toolboxes for MATLAB (Andersson and Bro 2000, Murphy et al. 2013). The results of the PARAFAC modelling are presented as the maximum fluorescence intensity (F_{\max}) for each component which were calculated by multiplying the maximum loadings of excitation and emission for each component by its score (Murphy et al. 2013).

3.2.2 Dissolved organic carbon (DOC)

DOC was measured using a Shimadzu TOC-V_{CPH} carbon analyser, using the non-purgeable organic carbon (NPOC) method (EN 1484: 1997). Samples were acidified to pH = 2 using HCl (37% v/v) prior to the analysis. The dissolved inorganic carbon was converted to carbon dioxide after sparging the sample for five minutes with purified air before injection into the analyser.

4 Results and discussion

4.1 The fluorescence composition of drinking water

The fluorescence components identified in three different Swedish drinking water systems were similar to each other (Paper I and Paper II). The PARAFAC components identified in drinking water network samples (Paper I, Model G) and two different treatment plants (Paper II, Models L and M) are listed in Table 2. An additional component was identified in Model M (C3) compared to Model L and Model G. This might be because Model M was calculated from samples exposed to UV₂₅₄ irradiation for a long period of time. The UV irradiation experiments created dynamic changes in the fluorescence components resulting in more complexity in the dataset. Four of PARAFAC components found in this study have been reported from various aquatic systems (Ishii and Boyer 2012, Murphy et al. 2008).

Table 2. Fluorescence components in three PARAFAC models of treated drinking water. The samples in each model were collected from the Gävle distribution network (Model G), the Lilla Edet treatment plant (Model L), or the Mölndal treatment plant (Model M)

Component name	Peak position (Ex/Em)	Model G. (Paper I)	Model L. (Paper II)	Model M. (Paper II)	Other studies	Potential Source
UVA humic-like + UVA humic-like	< 280 (310-320) / 410	F1	C1	C2	C2: (Ishii and Boyer 2012)	Terrestrial, anthropogenic
UVC humic-like+ Marine humic-like	< 280 (320-350)/440 - 450	F2	C2	C1	C3: (Ishii and Boyer 2012)	Terrestrial, Anthropogenic, agriculture
Tryptophan-like, protein-like	< 280/350- 387	F4	C3	C5	C6: (Murphy et al. 2008), T: (Coble 2007)	Autochthonous
humic-like	<280(400)/500)	F3	C4	C4		
humic-like	310 / 388	-	-	C3	C2: (Murphy et al. 2008), M: (Coble 2007)	Autochthonous

4.2 Is fluorescence a useful proxy of changes to treated drinking water quality?

4.2.1 Paper I: Assessing water quality at the tap

Across the Gävle distribution network, the abundances of microbial indicators and the concentrations of chemical parameters other than CDOM were generally low (Table 3). Abundances of microbial indicators were always below the detection limits or lower than the regulated limits for drinking water. Concentrations of all trace metals were within the health limits recommended by the World Health Organization (WHO 2011). These results indicated that there were no microbial or chemical issues in the network.

Stability is a requisite criterion for a water quality tracer if it is to be used to distinguish significant deviations from natural variation. The more stable the concentration of a water quality tracer within a distribution network, the easier it is to detect contamination caused by entrainment of untreated water or caused by microbial regrowth. To estimate the threshold at which each parameter is able to distinguish potential contamination from natural variation, trigger thresholds (TT) were used (eq. 6). The lower the value of TT_{rel} (TT divided by the mean) the higher the sensitivity of a parameter for detecting contamination.

Natural variability affects the sensitivity of trigger thresholds by defining the extent of the deviation in the concentration of each parameter compared to the network mean. The relative standard deviation (RSD) of slow growing bacteria abundances was 100 %, due to the relatively low precision of heterotrophic plate counts. Different trace metals had relative standard deviations ranging between 14% and 170%. In contrast, fluorescence intensities were stable across the network compared to other parameters (Figure 6). The lowest variability (RSD = 4%) occurred at the long excitation and emission wavelengths (F1, F2, F3 in Table 2) where humic-like components fluoresce. Absorbance was more variable than humic-like fluorescence (RSD = 10% for A_{254}) and almost as variable as tryptophan-like fluorescence (RSD = 11%) (Table 3).

Measurement precision also affects the sensitivity of trigger thresholds by its effect on the experimental variance. The less variance among the concentrations of replicate samples, the smaller the fraction of the standard deviation in eq. 6 (s) that is devoted to measurement variance, and the lower the trigger threshold. Fluorescence intensities of the humic-like components varied less than other parameters in replicate samples due to very high measurement precision (Table 3).

Fluorescence was the most sensitive tracer for detecting contamination among the parameters measured in Gävle. This sensitivity improved further when copper was taken into account (Section 4.3.1.). The low variability in fluorescence intensities (standard deviation = 4 - 11%) across the network and its high measurement precision (averaged standard deviation between replicates = 1 - 4%) made it the most sensitive tracer among all measured parameters. Absorbance was the second most sensitive tracer. A 20% increase in absorbance at 254 nm

(Abs₂₅₄) relative to the system average would be needed to trigger an outlier, compared to a 10% increase needed for fluorescence.

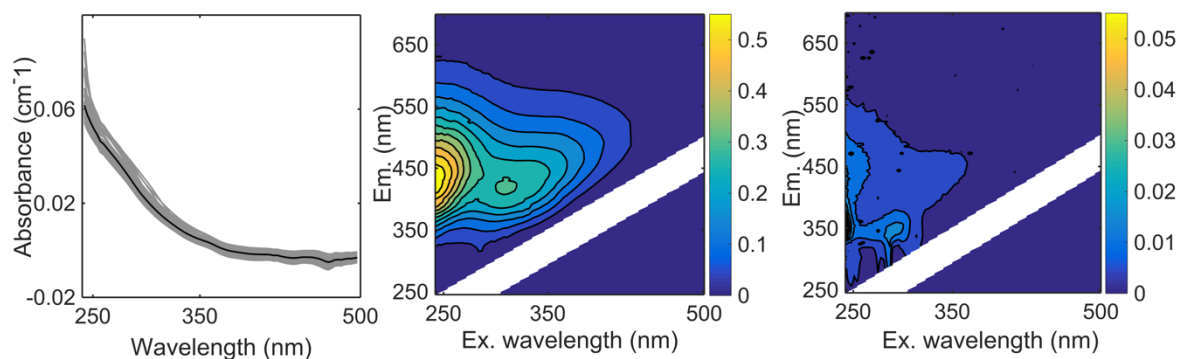


Figure 6. Variation in optical properties across the distribution network. (a) Absorbance spectra (grey lines) compared to the average spectrum (black line); (b) Average fluorescence; (c) standard deviation of fluorescence. (Paper I)

Table 3. Water quality parameters measured in households on the Gävle drinking water distribution network (modified from Paper I). ¹Analytical limits of detection (LoD) were determined from procedural blanks. ^{un}Uncorrected data. ^cCorrected data. TT= Trigger threshold.

Parameter	Min	Max	Median	RSD across sites (%)	RSD between replicates (%)		LoD ¹	TT	TT _{rel} (TT/mean)
					Median	Max			
Fe (ppb)	1	20	4	75	7	17	0.74	17.7	3.3
Al (ppb)	0.50	8	2	47	14	79	1.72	6.7	2.4
Cu (ppb)	10	500	50	87	3	14	0.07	253	3.7
F 2 (RU)	0.25	0.31	0.29	4	1	1.4	0.01	0.32 ^{uc}	1.1 ^{uc}
F 4 (RU)	0.13	0.23	0.14	11	4	14	1.2	0.35 ^c	1.06 ^c
DOC (ppm)	2.5	8.9	3.5	41	8	13	0.16	10.34	2.25
A ₂₅₄ (cm ⁻¹)	0.04	0.08	0.05	10	2	19	0.001	0.06	1.20
Slow-growing bacteria (cfu/ml)	< 1	110	17	100	25	94	1	120.3	3.9

Fluorescence was not a useful proxy of microbial regrowth in this study. There was no correlation between any of the microbial indicator abundances and tryptophan-like fluorescence. A weak correlation was observed between slow growing bacteria and four chemical parameters (F_4 , A_{254} , Fe and Pb) in a subset of network locations where water residence time and microbial abundances were highest. The lack of a strong correlation between the tryptophan-like fluorescence and the abundance of slow growing bacteria was possibly due to the fact that the distribution network under study was 'healthy', with little systematic variation among the response variables (slow growing bacteria) that could be modelled using the measured chemical parameters.

4.2.2 Paper II: Fluorescence as a proxy of UV dose

UV₂₅₄ irradiation had a measurable impact on the fluorescence intensity of drinking water, even at low UV doses similar to doses normally applied during drinking water treatment. The fluorescence intensities of all but one of the underlying fluorescence components (Model M in Table 1) decreased monotonically due to UV₂₅₄ irradiation. Component C4 followed a different trend and increased due to irradiation. The different behaviour of this component may be explained by its excitation (absorption) spectra. The excitation maxima of components C1, C2, C3 and C5 components (280-340 nm) are located closer to the monochromatic output of the light source (254 nm) than the C4 excitation peak (385 nm). Therefore, UV₂₅₄ irradiation caused the fluorescence intensities of C1, C2, C3 and C5 to behave differently compared to C4 at low UV doses.

The raw fluorescence measurements followed the same trend as the underlying PARAFAC components. At low doses of UV₂₅₄ irradiation ($< 350 \text{ mJ/cm}^2$), fluorescence intensities at 320/420 nm (corresponding to PARAFAC component C2) decreased linearly, while fluorescence at 400/500 nm (corresponding to PARAFAC component C4) increased linearly. The ratio of their intensities (C4:C2) also increased linearly at UV doses of 10-350 mJ/cm^2 (Figure 7). The results suggest that changes to fluorescence intensities could be useful proxies of the UV dose applied during drinking water disinfection.

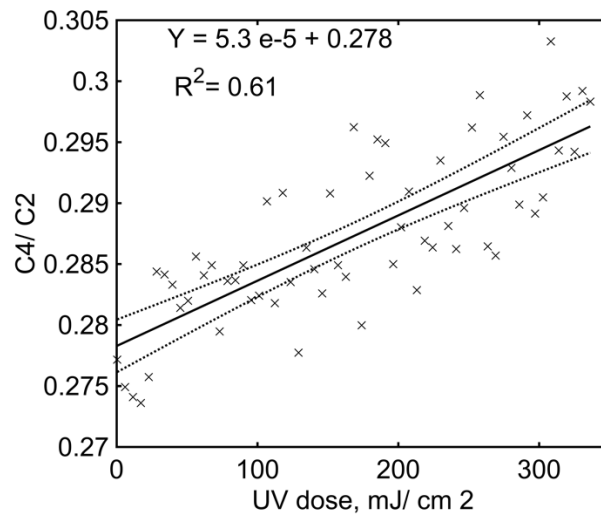


Figure 7. Changes in the ratios of raw fluorescence intensities (RU) at two specific wavelength pairs at low UV_{254} doses. C4: (Ex/Em: 400/500 nm) / C2 (Ex/Em: 320/420 nm). (Paper II)

In a full-scale treatment plant, UV disinfection caused significant changes in measured fluorescence intensities. Fluorescence was measured pre- and post-disinfection during two operational modes. During normal flow mode, when the water was subjected to UV irradiation at 35 mJ/cm^2 , fluorescence intensities changed by 1-8% (Model L in Table 1). Figure 8 shows the changes in fluorescence intensities of components C3 (Ex/Em: <280 / 350) and C4 (Ex/Em: <280 (400) / 500) of the pre- and post-disinfection samples. In agreement with the results of the irradiation experiments performed in laboratory, the fluorescence intensity of long-wavelength component C4 increased after disinfection.

During the standby mode, when water was stagnated in the UV chamber but the light was still on, dramatic changes were observed in fluorescence intensities of components C3 and C4 in the post-disinfection samples. The maximum fluorescence intensities (F_{max}) of C3 and C4 in post-disinfection samples during the standby mode were respectively 23% lower and 37% higher than in pre-disinfection samples (data points on the right side of the Figures 8a and 8b). The higher changes in fluorescence intensities during the standby mode reflects the higher doses experienced by water stagnated in, or passing slowly through, the UV chamber.

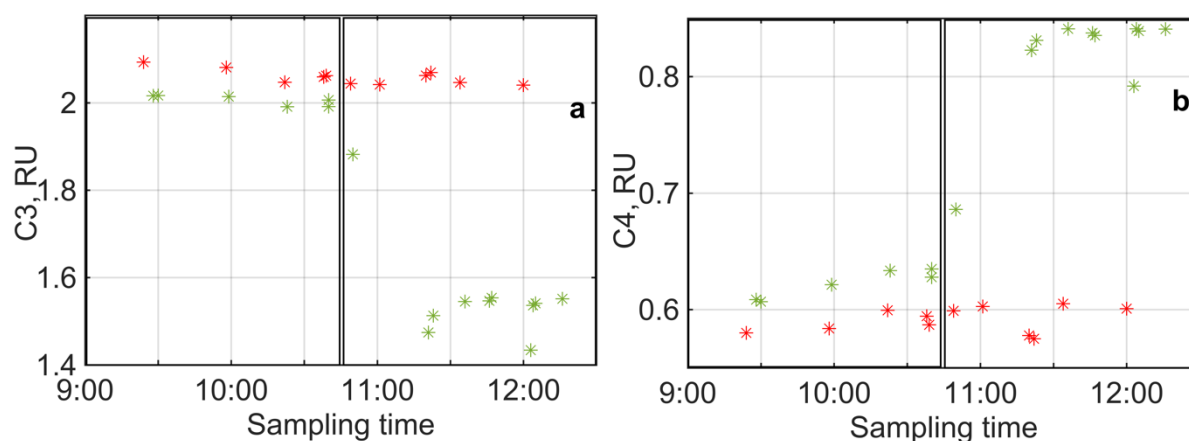


Figure 8. Changes in fluorescence intensity of C3 and C4 during the UV disinfection at WTP. (before UV disinfection: red asterisks; after UV disinfection: green circles). The data on the left side of each figure belongs to the 'normal flow mode' and the data on the side belongs to 'standby mode'.

4.3 Parameters and processes interfering with fluorescence measurements in drinking water systems

4.3.1 Copper and fluorescence/absorbance interaction

In the distribution network, copper interfered with the fluorescence intensities of humic-like components. Strong negative correlations were observed between copper concentration and the fluorescence intensities of three humic-like components (F_1 , F_2 and F_3 in Model G, Table 1) with Pearson correlation coefficients of 77-78%. Protein-like fluorescence (F_4) did not correlate with copper. There was also a positive correlation between UV absorbance (A_{254}) and copper concentration (Figure 9b). The correlation between copper concentrations and humic-like fluorescence were modelled using modified Stern-Volmer model ($R^2 = 37 - 49\%$). The interaction parameter calculated using modified Stern-Volmer model ($\log K_m$ in eq. 5) was comparable with the values reported in previous studies (Yamashita and Jaffe 2008).

The strong correlations between copper concentration and fluorescence intensities indicate that some measured variability in humic-like fluorescence intensities is due to the presence of copper. The fluorescence data were corrected for copper quenching using the Stern-Volmer model, by calculating the fluorescence intensities in the absence of copper (i.e. at the intercept $[Cu] = 0$) (Figure 9a). After correcting the fluorescence intensities and eliminating their variability due to the copper quenching, the coefficients of variation of fluorescence intensities reduced by 39- 47%.

The main source of copper is likely to have been the corrosion of interior copper plumbing in the buildings (WHO 2011). In addition, copper represented 0.7% of the pipe materials in the

distribution system. Therefore, to investigate the organic matter fluorescence as contamination indicator in new systems, trace metal measurements should be taken into consideration at the point-of-use.

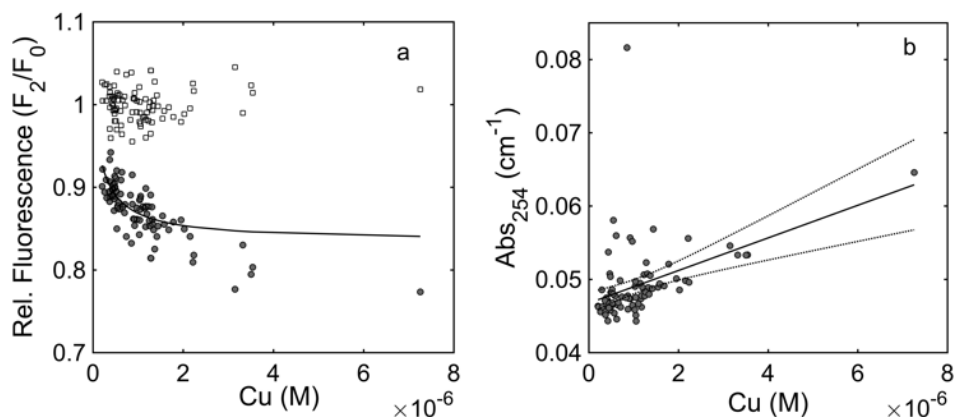


Figure 9. Correlations between DOM optical measurements and copper. (a) Relative fluorescence intensity of component F2 versus copper concentration. Solid line: modified Stern-Volmer model ($R^2 = 0.6$); Closed circles: uncorrected data; Open squares: corrected data 1. (b) Abs_{254} versus copper concentration, $R^2 = 0.2$. (Paper I).

4.3.2 Dark recovery of irradiated drinking water samples

Fluorescence intensities in drinking water samples that were subjected to UV_{254} irradiation increased slightly after irradiation was stopped and the samples were stored in the dark. To investigate the effect of a time delay between sampling an irradiated sample at a treatment plant and its analysis at the laboratory, experiments were conducted in which samples were subjected to alternating periods of irradiation and the dark. Figure 10 shows the changes in the fluorescence intensities of PARAFAC components (Model M in Table 1) during 1h of UV_{254} irradiation at 20 W/m^2 (green markers) followed by 1h of the dark (red markers). The response of fluorescence signals is shown as the change in normalized F_{\max} values versus time. To better illustrate the changes in the fluorescence intensities during the dark period, the insert in Figure 10 also shows the change in F_{\max} normalized to the first measurements of the dark period. Overall, the fluorescence intensities of all components increased in the dark by 1-5%.

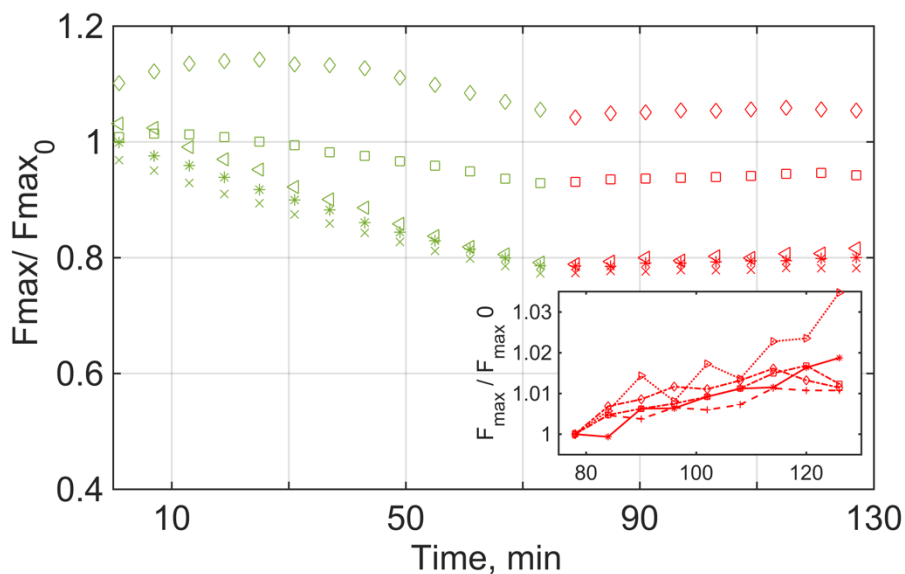


Figure 10. Fluorescence changes in drinking water from Mölndal WTP during UV_{254} irradiation followed by period of darkness (Paper II). Relative fluorescence intensities (normalized to the first measurement) are plotted separately for each PARAFAC component (C1: asterisks, C2: crosses, C3: squares, C4: diamonds, C5: triangles). Green markers: data obtained during irradiation; red markers: data obtained during subsequent darkness. The insert shows the same fluorescence intensities normalized to the first dark measurement.

Although the magnitude of fluorescence dark recovery was low compared to the changes that occurred as a result of irradiation in the laboratory, it was of similar magnitude to the changes measured following the comparatively low UV doses used for disinfection at treatment plants. Therefore, dark recovery should be taken into consideration when there is a delay between the sampling and measurement of irradiated samples. In future, it should be examined whether the dark recovery is predictable or whether it differs between various source waters.

5 Conclusions

- Similar underlying fluorescent organic matter components were identified in three different drinking water systems in Sweden.
- CDOM fluorescence measurements in a healthy drinking water distribution network showed high measurement precision and low variability across the network.
- CDOM fluorescence would be a sensitive tracer of contamination caused by entrainment in the Gävle distribution network. An outlier would be triggered when the fluorescence intensity of humic-like components (emission at visible light wavelengths) exceeds the network average by 10%.
- Tryptophan-like (UVA) fluorescence was not a useful proxy of microbial regrowth in the Gävle distribution network.
- Fluorescence spectroscopy was sensitive enough to detect small changes in organic matter fluorescence intensities due to UV₂₅₄ irradiation (35 mJ/cm²) during full-scale drinking water disinfection.
- CDOM fluorescence intensity had a linear response to low doses of UV₂₅₄ irradiation, and therefore is a potential proxy of the applied UV dose during UV disinfection of drinking water. To fully examine this hypothesis, irradiation experiments are needed on a broader range of water samples from various treatment plants.
- Spectroscopic measurements are affected by trace metals present in drinking water. Interference by copper and other pipe materials can influence the thresholds for detecting changes in organic matter quantity and quality in distribution networks.
- In samples that have been irradiated, dark recovery of the fluorescence signal can occur between sampling at the treatment plant and measuring fluorescence at the laboratory. This should be considered when interpreting fluorescence intensities for irradiated samples.

6 Future research directions

- The change in fluorescence intensity of organic matter due to UV₂₅₄ irradiation is in part the result of the alteration in composition of DOM pool and destruction of dissolved organic carbon components to molecules with smaller size. These smaller molecules might serve as nutrients for microbial regrowth following disinfection. Future research will assess the bioavailability of organic matter in the irradiated water.
- To assess whether fluorescence spectroscopy can be applied in real-time at treatment plant using the available online fluorometers, the sensitivity of in-situ fluorometers to detect the small changes in fluorescence intensities due to UV disinfection will be investigated.

- To derive a robust relationship between UV dose and DOM fluorescence, irradiation experiment will be performed on water samples from other treatment plants.

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Paper I

Identifying a fluorescent disinfection residual following UV treatment of drinking water

Masoumeh Heibati, Colin A. Stedmon, Olof Bergstedt and Kathleen R. Murphy
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Manuscript

Paper II

