Effect of Size Distribution and Fe (Iron) Fractionation of Natural Organic Matter on Fluorescence and UV/VIS Spectroscopy

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June 2015

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Abstract

Increase in color in water is becoming a problem due to its physical, chemical and biological implications. The consequences of the increase in color affect the industrial and the domestic purposes. Studies show that one factor affecting the color of water is the increase in dissolved natural organic matter, DNOM. In addition , also iron (Fe) is known for contributing to this effect.
Nevertheless, Fe is present in different forms in the water in lakes and rivers and it affects the color in more than one way. It may scatter light on colloidal forms or absorbs UV radiation when is complexed.
Increase in DNOM also increases the solubility or iron.

In this work different analyses were carried out to elucidate how Fe is present in the solution and how increase in concentration affects the properties of water.
An Fe fractionation method was used to differentiate the species present in solution, finding that pH has great influence on the species distribution for iron, the more acidic the site the more monomeric iron is found and is less complexed with organic compounds. Further analyses with a larger amount of samples should be carried out for better understanding of the mechanisms affecting Fe fractionation.

Acknowledgement

I would like to thank Professor Rolf D. Vogt for giving me the opportunity to work in this project and for providing guidance, advice and feedback during the process.
I also want to express my gratitude to Professor Ståle Haaland for his lead and teaching about the project and science in general. All of it was fundamental during this new and enricher experience.

1. Introduction

1.1 Background

The water used for industrial and domestic purposes usually contains a certain amount of natural organic matter (NOM) (Gjessing, Egeberg et al. 1999). The NOM in the water has different effects, some of which may be negative for the water quality, especially in the drinking water area. This NOM is a complex mixture of heterogeneous organic macromolecules.

Another important concept is DNOM, dissolved natural organic matter, which is operationally defines as the fraction of NOM in solution that is not retained by 0,45 µm membrane filter, which is considered therefor dissolved.

DNOM has various weak acid groups lipophilic sorption abilities, interactions with oxides and other minerals, oxidation and reduction properties and strong metal complexation capacity(Vogt, Akkanen et al. 2004). The last one is the main focus of this project.

During the last 15 years there has been an increase in the color of DNOM in surface waters throughout Europe and North America (Vogt, Akkanen et al. 2004). This increase in water color has far reaching consequences in the ecological and societal area because the increase in color affects the structure and function of the aquatic ecosystem (Kritzberg and Ekstroem 2012). This trend, which is sometimes referred as brownification, has different drivers and there is a consensus that one direct factor of is the increase in concentrations of terrestrial organic matter (OM) in the water since it has shown correlation with water color so is responsible for most of the light absorption in natural waters but not all of it. A study in water brownification showed that iron is responsible for an average of 25% of the water color (Kritzberg and Ekstroem 2012).

Besides the water color, another radiation-related issue has been observed, the attenuation of solar ultraviolet radiation (UVR) in aquatic ecosystems. The ultraviolet radiation is absorbed in the water and this is due phytoplankton, chromophoric dissolved organic matter (CDOM), detritus, water itself and iron as well, specially oxidized Fe increases the absorptive properties of the water. (Maloney, Morris et al. 2005). Besides the absorption of DNOM and Fe alone, they act together increasing the total absorption; Fe oxidizes in water and bind to DOM, which enables this compounds to stay in solution and this the absorption of the lake is increased by this synergy.

UVR absorption is specific to molecular substructures found in dissolver organic matter and CDOM appears to contribute the most for the absorption in lakes, nevertheless, particulate absorption and scattering of light seems to have a contribution to the UVR attenuation. (Maloney, Morris et al. 2005).

UVR has several effects on the water. It causes photochemical degradation of DOM which can convert organic carbon into inorganic carbon, and this is increased when there is iron present due to its oxidation/reduction qualities. In addition, when the iron and the organic compounds dissolved in water interact and form complexes, this increases the UVR absorption in the water (Maloney, Morris et al. 2005).

Iron can affect in different forms the absorption of radiation in lakes, which affects the whole ecosystem, and this can be consequence of the absorption of iron itself in the water, iron and DNOM complexes absorption, iron forming colloidal material that scatter light and iron complexation with other compounds (besides the iron hydrolysis in the water).

Due to this, it is important to consider the different forms in which iron might be present in the water and how all of these would affect the interaction with the radiation.

1.2 Aim of the project

The aim of this project is to study environmental factors which affect the biophysiochemical properties of dissolved natural organic matter in raw water samples from drinking water from the NOMiNOR project[1] and the isolated DNOM samples extracted by a reverse osmosis procedure in the NOM-Typing project (Gjessing, Egeberg et al. 1999). The main focus on this project is on the changes in size distribution of iron fractions and variation of iron concentration on optical properties of the samples using Fluorescence Emission and UV/VIS spectroscopy, and also the variation on other properties such as pH and conductivity. In addition, iron fractionation is carried out to get a better understanding of the interaction of iron and DNOM and how this metal is present in the lakes and how this affects the optical properties.

2. Materials and Methods

2.1 Samples

The analyzed samples were taken from the NOM-Typing project and from the NOMiNOR project.

From NOM-Typinig three Reverse Osmosis (RO) isolates from different locations in Southern Norway were used: Trehørningen (TRE); Birkenes (BIR) and Hellerudmyra (HEO). The preparation of the isolates to their original concentration will be explained later.

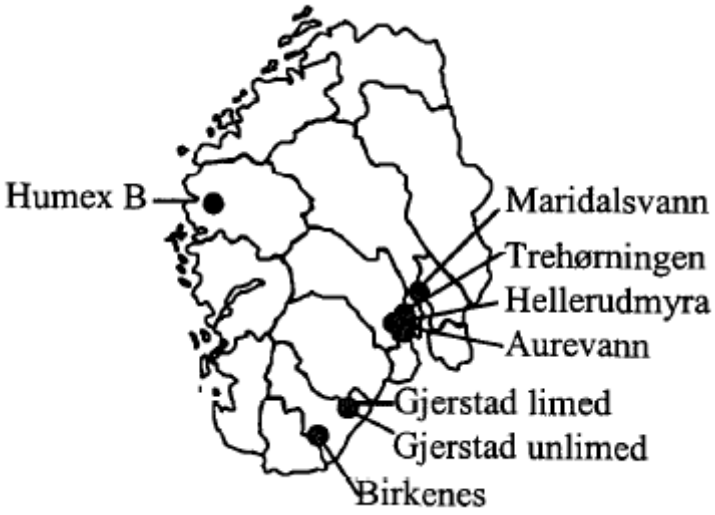

Figure 1: Southern Norway. Location of sampling sites for the NOM-Typing project.

From NOMiNOR five raw water samples were used, all of them were collected during Autumn all over Scandinavia and Scotland. The sampling sites were: Svartediket in Bergenvann (BERGEN) and Langavatnet (IVAR) in Norway; Bolmen of Sydvattenin (SYD) in Sweden; Paijanne (HSY) in Finland, and Burn Crooks Reservoir from Scottish Water (SCW) in Scotland.

1 http://vannforsk.no/nominor/

2.2 UV/VIS absorbency

The term UV-Visible applies to radiation with a wavelength in the range of 200-800 nm. The wavelength range of UV radiation starts at the blue end of visible light (around 400nm) and ends at approximately 200 nm. This radiation has enough energy to excite valence electrons in atoms and molecules; thus UV radiation is involved in electronic excitation. Visible light is considered to be with wavelengths from 800 nm-400 nm and acts in the same as UV light. For this reason spectroscopic instrumentation operates in the range previously mentioned.

The interaction of UV and visible radiation with matter provides a qualitative identification of molecules and polyatomic species. Structural information about molecules can be acquired by observing the UV-Vis Spectrum.
In general, a UV-Vis spectrometer consists of a UV visible light source, two cells through which the light passes, and a detector to measure the amount of light passing through the cells.

In a double-beam UV-Vis spectrophotometer the light is split into to parallel beams, each of which passes through a cell that contains the sample dissolved in a solvent and the other passes through the solvent alone.
The detector measures the intensity of the light transmitted through the solvent alone, in Equation 3 is expressed by I_o. This is compared by intensity of the light transmitted through the sample cell, in Equation 3 is expressed by I. The absorbance A is calculated from the relationship shown in Equation 3.

$$A = log \frac{I_o}{I} \qquad \text{Eq 3.}$$

A common solvent used in UV is Ethanol as it is transparent to UV above 200 nm.

2.3 Excitation Emission Spectrum

When a molecule absorbs radiation of the visible and the UV range, the molecule energy is promoted from the ground vibrational electronic state to an excited level. There is a difference between the ground and the excited state which determines on which wavelength the molecule absorbs the radiation.
Afterwards, when the relaxation of the molecule occurs, this can be accompanied by the emission of radiation, which is fluorescence in most cases. This is a radiative process that consists of the emission of photons with less energy than the absorbed ones that produced the excited state (Andrade-Eiroa, Canle et al. 2013).
An Excitation Emission Spectra contains the fluorescence intensity of a fluorophore at a selected pair of excitation and emission wavelengths. The fluorescence is collected as a function of both the emission and the excitation wavelengths pairs.
Fluorescence can be used to monitor the molecular status of a fluorophore, since fluorescence depends strongly on the chemical environment, the structure of the molecules, the substituents of aromatic moieties and the molecular weight. The

fluorescence intensity provides information of the content of fluorescent components (Andrade-Eiroa, Canle et al. 2013).

2.4 pH

According to Søren Sørenson (introducer of the concept of pH), pH is defined as the negative logarithm of the H_3O^+ ion activity:

$$pH = -log[a_{H^+}] \quad \text{Eq. 1}$$

From Equation 1 it is clear that if the activity of ion H_3O^+ changes tenfold, the value changes by one unite. Thus is possible to measure minimal changes in the pH value of a sample.

A pH-meter has a sensor with a glass membrane, sensitive to hydrogen ions that define the pH value and is sensitive to the reaction between the glass and a sample solution. The outside of this membrane glass forms a gel layer when the membrane is in contact with an aqueous solution. A similar gel layer is formed on the inside of the membrane as the electrode is filled with an electrolyte buffer solution. The H^+ ions in and around the gel layer can diffuse into or out of this layer depending on the pH value and therefore the H^+ ion concentration of the sample solution, which is the difference between the inner and outer charge of the membrane.

A second sensor is needed in order to supply a reference signal or potential for the pH sensor. The difference between both electrodes determines the pH value of the measured solution. To be able to do this reference the electrode is made out of a non-sensitive H^+ ion material and is must be open to the sample to measure. Therefore an opening junction is made in the bottom of the reference electrode through which the inner buffer solution is in contact with the sample. The response of the pH-sensitive electrode is determined by how acidic or alkaline the solution is.

The potential between the two electrodes is a measure of the number of hydrogen ions. Equation 2 shows the linear function of the Hydrogen concentration in solution, which allows quantitative measurements to be made.

$$E = E_o + 2.3\,\frac{RT}{nF} \times\ log[a_{H^+}] \quad \text{Eq. 2}$$

Where E is the measured potential, E_o is the constant potential, n is the ionic charge and F is the Faraday constant (pH Theory Guide, Mettler Toledo).

2.5 Conductivity

When measurements of the conductance of an electrolyte are made, the property is actually measured is the resistance of its dissolution.

To perform the measurement it is placed the sample in a cell conductance and this dissolution behaves like an arm in the circuit of a "Wheatstone bridge". For measuring accurately any electrical resistance is used the Wheatstone bridge where there are two known resistors, one resistor is variable and one resistor is unknown. By adjusting the

variable resistor, the current through a galvanometer reaches zero. Thus, the ratio of the two known resistances is equal to the ratio of the adjusted value of the variable resistance and the value of the unknown resistance. Figure 2 shows how the arrangement of the Wheatstone Bridge and the resistances conforming it.

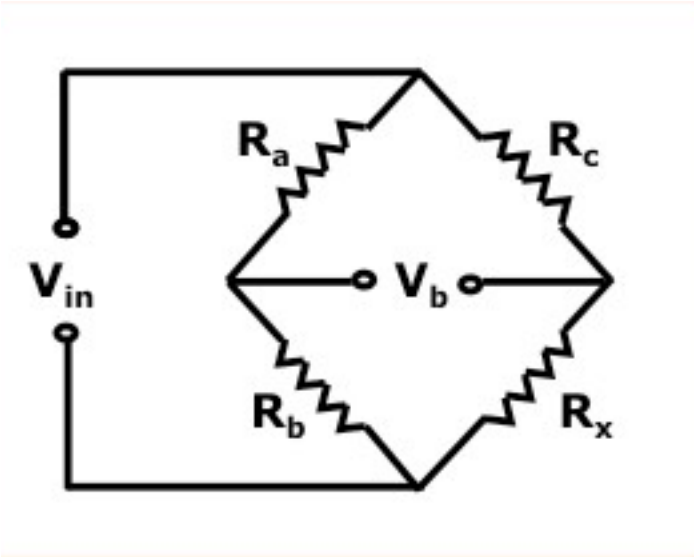

Figure 2. Wheatstone Bridge Circuit

Usually the conductance cells have a glass recipient where two separate inert electrodes measure the resistance of the solution between the two electrodes, because of this, the electrodes are fixed a constant distance. Electrodes used for conductimetry are made our of platinized platinum.

In the case of low conductivity dissolutions it is necessary to reduce the resistance of the cell and is preferable to have the electrodes separated by smaller distances.

On the other side, when very conductive solutions are being studied, it is necessary that the distance between the electrodes is bigger and the surface of the electrodes can be smaller. These changes are applied in order of reducing the residual interference.

2.6 Fe Fractionation

The fractionation of iron is carried out in an analog way of the aluminum fractionation, using the same procedure.

First, the fractionation of the monomeric iron (Fe_a) from the polymeric form is done by complexation of the iron with a ligand, the 8-hydroxyquinoline (8-HQ) at basic pH and extracted the product using Methyl Isobutyl Ketone (MIBK) in the organic phase (Internal UiO doc, Rudberg, 1995 #739).

After this, the organic bound iron (Fe_o) is separated from the inorganic iron (Fe_i), which is mainly labile, and therefor is trapped in an Amberligh IR-120 ion exchange column.

After all the procedure for the fractionation is done, the absorbance of iron in the different phases can be determined photometrically using Fe standards of known concentration and afterward the concentration can be determined using a calibration curve.

The concentration of iron measured after the extraction corresponds to the concentration of total monomeric iron. The concentration determined after the ion exchange and extraction corresponds to the concentration of organic iron. After both

concentrations are determined, it is possible to determine the concentration or inorganic iron using the equation

$$Fe_i = Fe_a - Fe_o \qquad \text{Eq. 3}$$

2.7 Experimental Design

2.7.1 RO isolates preparation

The three selected RO isolates (NOM-Typing) were taken to their original concentration (Gjessing, Egeberg et al. 1999). To achieve this, for the TRE sample 26.60 mg of isolate were dissolved in 1 L of type 1 water to get a 5,90 mgC/L concentration. For the BIR sample, 33,76 mg of isolate were dissolved in 1 L of type 1 water to get a concentration of 5,22 mgC/L. Finally, for the HEO sample, 71,94 mg of isolate were dissolved in 1 L or type 1 water to get a concentration of 24,01 mgC/L. After doing this, the samples were allowed to reach equilibrium. This was done by leaving them stiring, using magnetic stirs, overnight before any filtration.
All the weighting was done by using an analytical balance (OHAUS Discovery).

2.7.2 Raw water samples

Five water samples (approx. 1 L) from different waterworks (NOMiNOR) collected during autumn (described before) were used for the project. No special treatment was necessary prior filtration.

In total, samples from eight different sites were analyzed during the project.

2.7.3 Iron solution

A Fe^{3+} stock solution was prepared in order to be added to the samples and increase the iron concentration in them. The stock solution was prepared from $FeCl_3$ by dissolving 0,06496 g using an analytical balance in 200 mL of type 1 water to get a concentration of $2x10^{-3}$ mol/L.

2.7.4 Filtering and sub-sampling

All eight samples were filtered through cellulose nitrate filter with a pore size of 0,45µm to standardize the measurements and because the focus of the project is on the DNOM, which is by definition not retained in 0,45 µm membrane filter.
After all samples were filtered, an aliquot of 150 mL (aliquot 1) was taken to be analyzed in the future (0,45-1) and another aliquot of approx. 200 mL (aliquot 2) was taken.
From the aliquot 2, approx. 150 mL were taken and filtered through 0,45 µm filter again (0,45-2), 25 mL were taken and filtrated through a cellulose acetate filter with a pore size of 0,2 µm (0,2).
The other 25 mL were stored for filtration through 10.000 Da in future experiments. T
All of the described was done for each of the eight samples.

2.7.5 Iron addition

The remaining 650 mL of each sample were divided into three parts: in the first one 0,5 mL of the iron stock solution were poured into a 200 mL volumetric flask and then it was filled with sample until the mark on the flask (200 mL total) to get a concentration of 5 µM. In another 200 mL volumetric flask 1 mL of Fe stock solution was poured and then filled with sample to the mark to get a total concentration of 10

µM. Finally, in a third 200 mL volumetric flask 2 mL of Fe stock solution were poured and filled to the mark with sample to get a concentration of 20 µM.
This was done for all eight samples. Afterwards they were allowed to stabilize in a slow rotator for a week to reach equilibrium.

2.7.6 Filtering and sub sampling (2)

After one week, each of the three different concentration sub samples were divided into three parts: the first aliquot was 150 mL and it was filtrated through 0,45 µm filter; the second aliquot was 25 mL and it was filtrated through 0,2 µm membrane paper; and the third aliquot, also 25 mL, is to be filtrated through 10 000 Da in future experiments. This was done for each concentration for each one of the original eight samples.

Without considering the 10 000 Da fraction, at the end of all the sub sampling, nine different sub samples were obtained for each one of the original eight samples, and these nine sub samples were analyzed with different procedures.

All of the 10 000 Da were left at the NMBU at Ås for posterior filtration and analysis. In the same way, a sub sample of each fraction was left at NMBU for posterior TOC analysis.

2.7.7 UV/ Vis analysis

All the samples were analyzed using a UV-VIS Spectrophotometer Shimadzu UV-2600 at the NMBU at Ås. Distilled water was used as background for each sample previous the measurement of the UV/VIS absorbency from 700 nm to 250 nm.
To perform the analysis, first 3 mL of distilled water were poured in the cell inside the spectrophotometer to get the background signal. After this, the water was taken out and 3 mL of the sample were poured in the cell using a pipet and the absorbency from 700 nm to 250 nm was measured.
This was done for all samples, with and without iron.

2.7.8 EES analysis

All the eight samples were analyzed using a Cary Eclipse Fluorescence Spectrophotometer instrument from Agilant Technologies. The samples were analyzed from an excitation wavelength of 400 nm to 250 nm and the emission wavelength analyzed was from 400 nm to 700 nm. All the results for the different samples are presented as 3-D graphs.
The measurements were carried out by pouring 3 mL of the sample into the cell which is then placed inside the Fluorescence Spectrophotometer in which emission and excitation were measured in the wavelengths previously mentioned.

2.7.9 Conductivity analysis

A Mettler Toledo conductimeter with an platinum electrode was used to measure the conductivity in all samples at NMBU. The electrode was rinsed between each series to take the interference to the minimum.
For this analysis, a small amount of sample, approx. 10 mL is placed inside a tube so it covers the electrode and then the equipment will show the signal corresponding to the sample.

2.7.10 pH analysis

All eight samples were analyzed for pH at the NMBU as well. An Thermo Scientific pH-meter was used for all samples. The pH meter uses a KCl solution so this analysis was done at the end for each sample to avoid the ion interference from KCl. This experiment is carried out in the same way as the conductivity analysis.

2.7.11 Fe Fractionation

The iron fractionation was carried out using the same procedure as for the aluminum fractionation method (Internal UiO doc, Rudberg, 1995 #739). The difference is that Fe standards were prepared for the analysis to determine the Fe concentration in the samples.
All the reagents were prepared by Gao Pingchuan in advanced.

The Al standards were prepared in the following fashion: first 0,17650 g of $KAl(SO_4)_2 12H_2O$ were dissolved in 5 mL of Milli-Q water, and then 1 mL of H_sSO_4 was added (it was already prepared) and then it was diluted to 100 mL. This was the stock solution.
From the stock solution, 5 mL were piped out and then poured into a 250 mL volumetric flask half filled with ion exchanged water and then diluted with the same water until the mark. This was the working standard solution.
From the working standard, 1 mL, 3 mL, 5 mL and 7 mL were piped out and poured into four different 100 mL flasks and then filled up with water to the mark.

The Fe standards were prepared in the same way that the Al standards: first 0,06211 g of $FeCl_3$ were dissolved in 5 mL of Milli-Q water, and then 1 mL of H_sSO_4 was added (it was already prepared) and then it was diluted to 100 mL. This was the stock solution.
From the stock solution, 5 mL were piped out and then poured into a 250 mL volumetric flask half filled with ion exchanged water and then diluted with the same water until the mark. This was the working standard solution.
From the working standard, 1 mL, 2 mL, 5 mL and 10 mL and 10 mL were piped out and poured into four different 100 mL flasks and then filled up with water to the mark.

For the first part of the fractionation, the extraction, all the samples and the standards were treated in the same way: first, 30 mL of the sample were measured into an extraction funnel and then all the reagents were added in a fast and repetitive fashion; 0,25 mL of phenol red, 1 mL of 8-hydroxyquinoline, approx. 0,25 mL of 25% aqueous NH_3, 2,5 mL of extraction buffer an 10 mL of MIBK.
After the addition of all the reagents, the funnel was shaken for 20 sec., enough time for the monomeric iron to react with the 8-HQ, and then set to rest to allow phases to reach equilibrium and separate. The lower phase went to waste and the organic layer was collected. Afterwards the extracts were stored overnight in a cold, dark space until photometric measurement.

Only the NOM-Typing samples (RO isolates) were fractionated with the ion exchange procedure due to a shortage in volume of the NOMiNOR samples. The TRE, BIR and HEO samples were treated in the following way.
For this part, an ion exchange column was prepared by taking approx. 20 g of ion exchanger Amberlite IR-120 (MERCK), which is a strongly acidic cation exchanger in

H^+ form. The Amberlite was rinsed 3 times with 2 M NaCl, 2 times with ion exchanged water and 2 times with $4x10^{-4}$ M NaCl (eluent). All of this was done according to the Al-fractionation procedure.
After the column was ready, using peristaltic pump speed of approx. 40 mL/min, first 60 mL of eluent were pumped through the column for conditioning, then 60 mL of the sample for waste and then 35 mL of the sample were collected. The last was repeated for every sample. After the ion exchange was performed, the 35 mL collected were extracted with the same method as described for the first part or the fractionation and then stored overnight in order to allow them to reach equilibrium.

After the fractionation was complete, photometric measurement was carried out using UV Shimadzu Spectrophotometer UV-1800 measuring the absorbance of the samples and standards at 395 nm and 600 nm.

3 Results and Discussion

To make the reading of all the data easier, the nomenclature used will be: Sampling site abbreviation-Iron content-Filter Size. (If necessary it woill be specified the number of times the sample was filtrated through the same size. For example, BIR10 0,45 means it is the sample is from Birkenes, it has an iron concentration of 10 µM and it was filtrated through 0,45 µm membrane filter.

3.1 UV/VIS

All the results from the UV/VIS absorbance are presented as graphs for each sampling sites. Each graph (λ vs Abs) includes: the absorbency of sample filtrated through 0,45 µm once with no iron added, filtrated through 0,45 µm twice with no iron added, filtrated through 0,2 µm with no iron added, filtrated through 0,45 µm with a 5 µM iron concentration, filtrated through 0,2 µm with a 5 µM iron concentration, filtrated through 0,45 µm with a 10 µM iron concentration, filtrated through 0,2 µm with a 10 µM iron concentration, filtrated through 0,45 µm with a 20 µM iron concentration and filtrated through 0,2 µm with a 20 µM iron concentration.

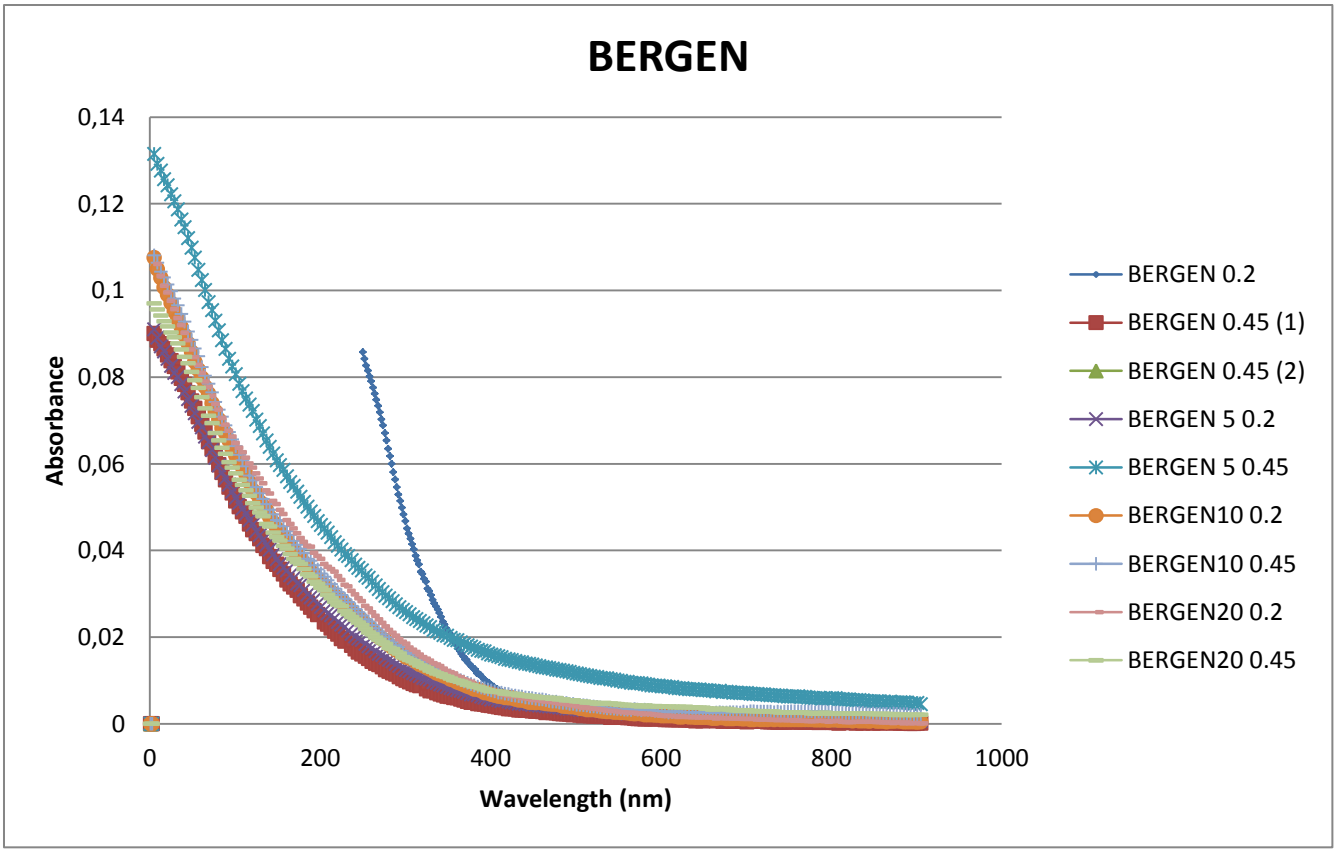

Figure 3: UV Absorbance BERGEN samples.

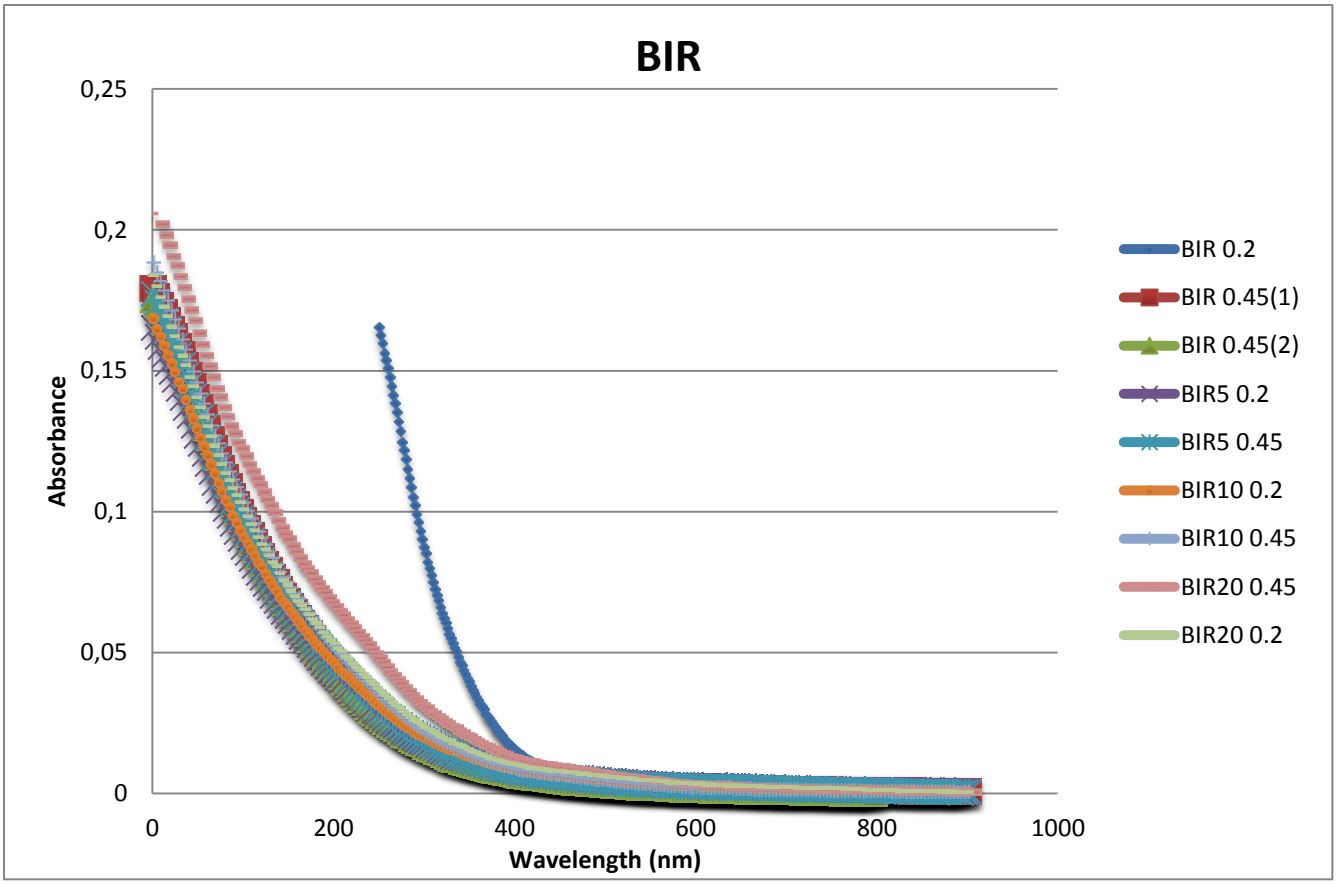

Figure 4: UV Absorbance BIR samples.

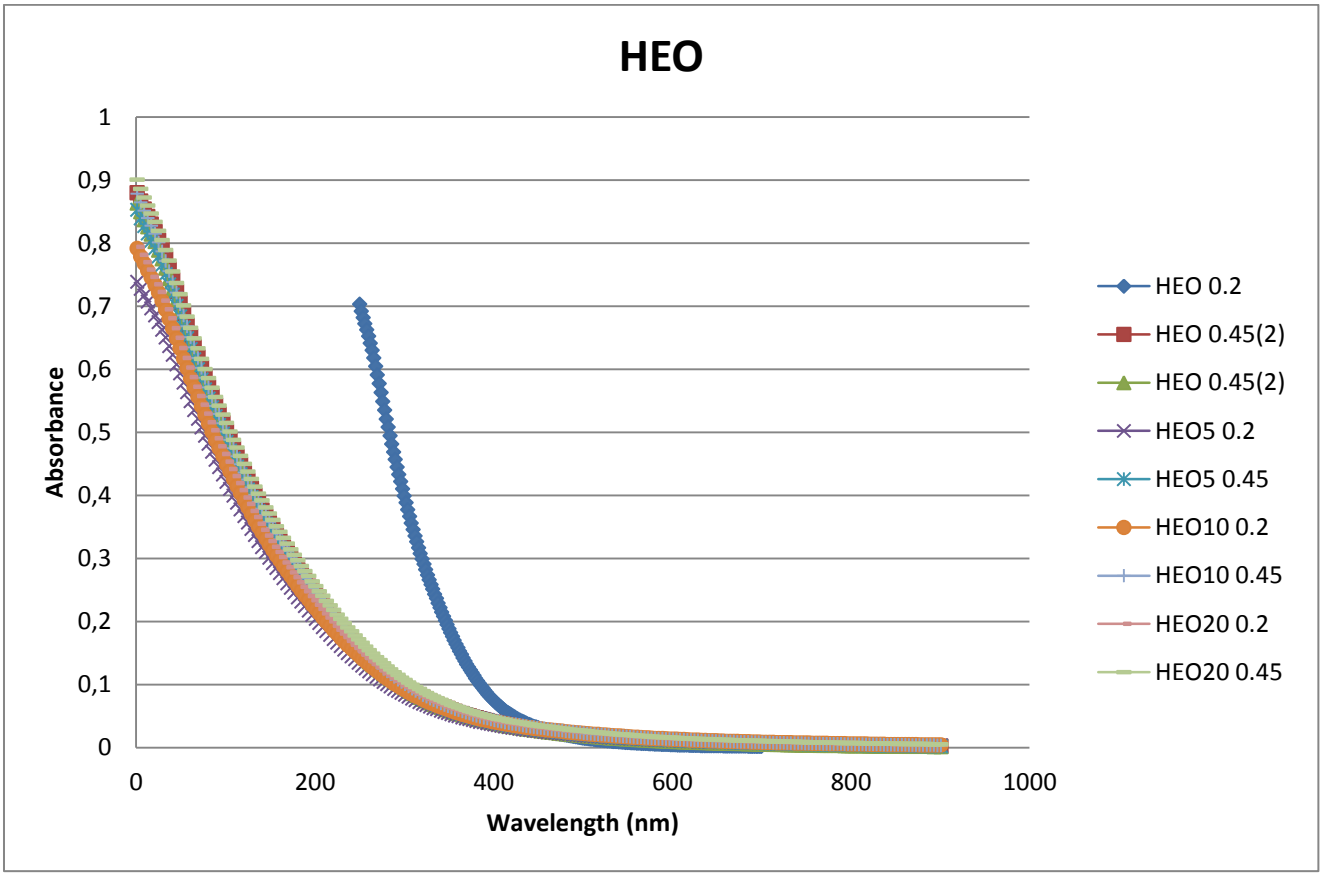

Figure 5: UV Absorbance HEO samples

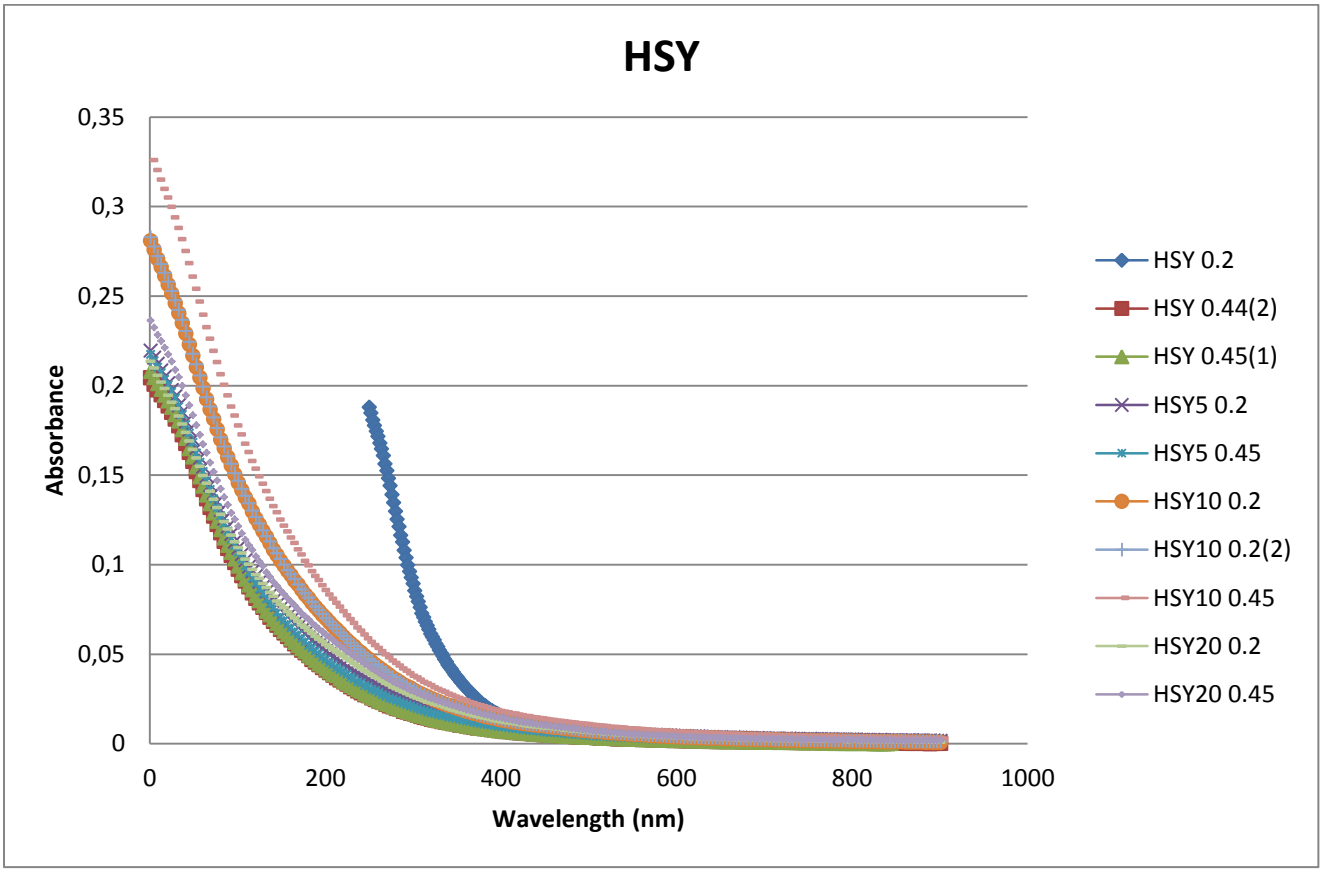

Figure 6: UV Absorbance HSY samples.

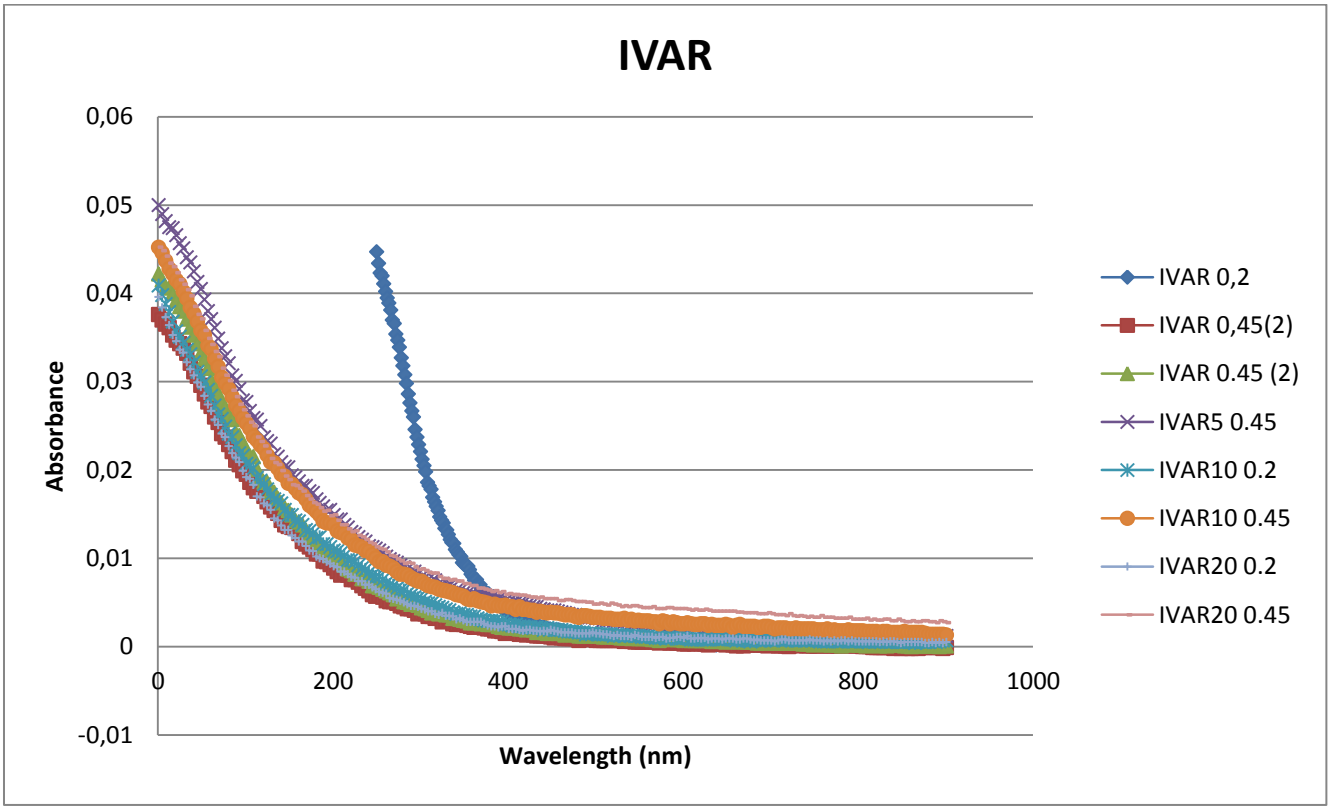

Figure 7: UV Absorbance IVAR samples.

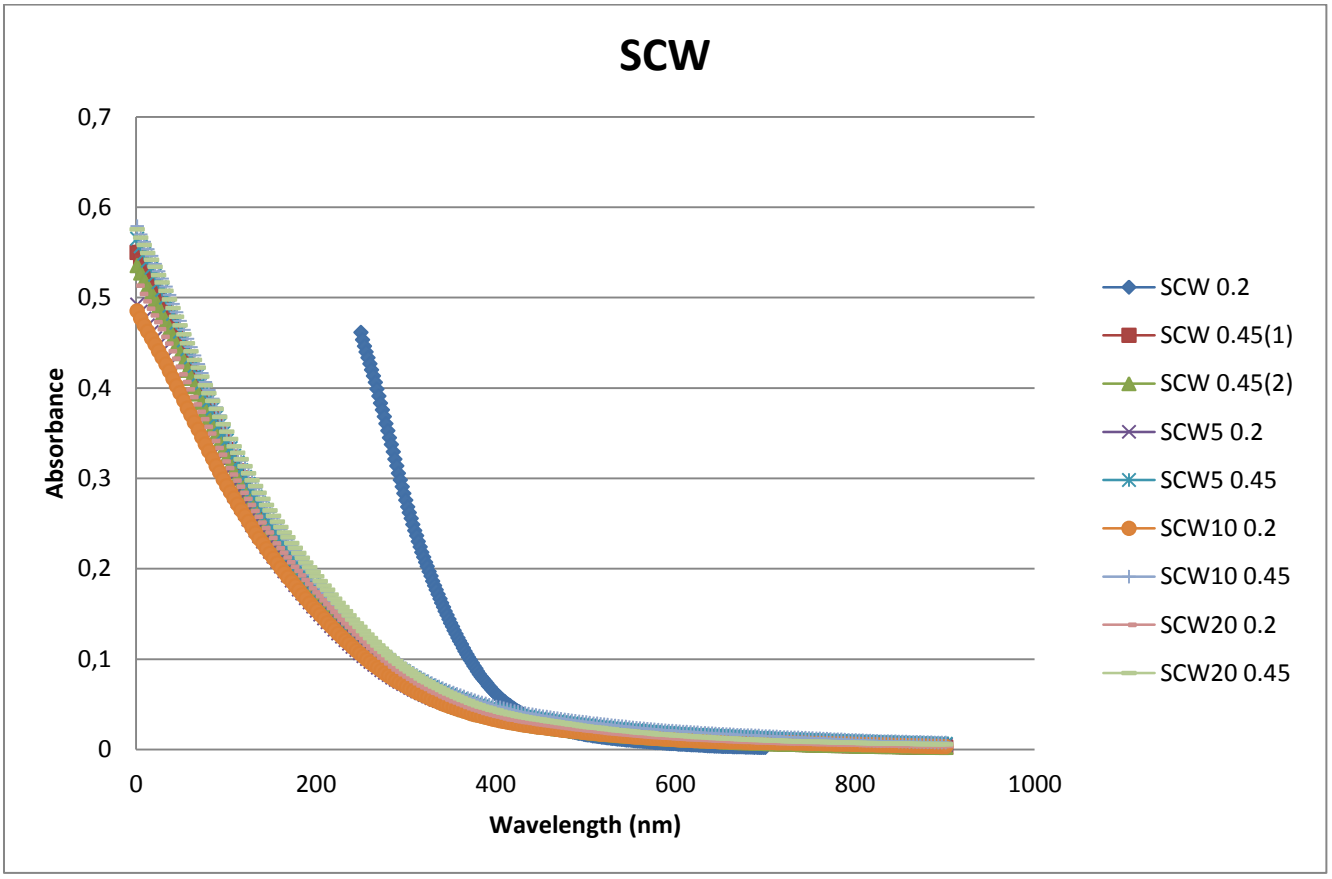

Figure 8: UV Absorbance SCW samples

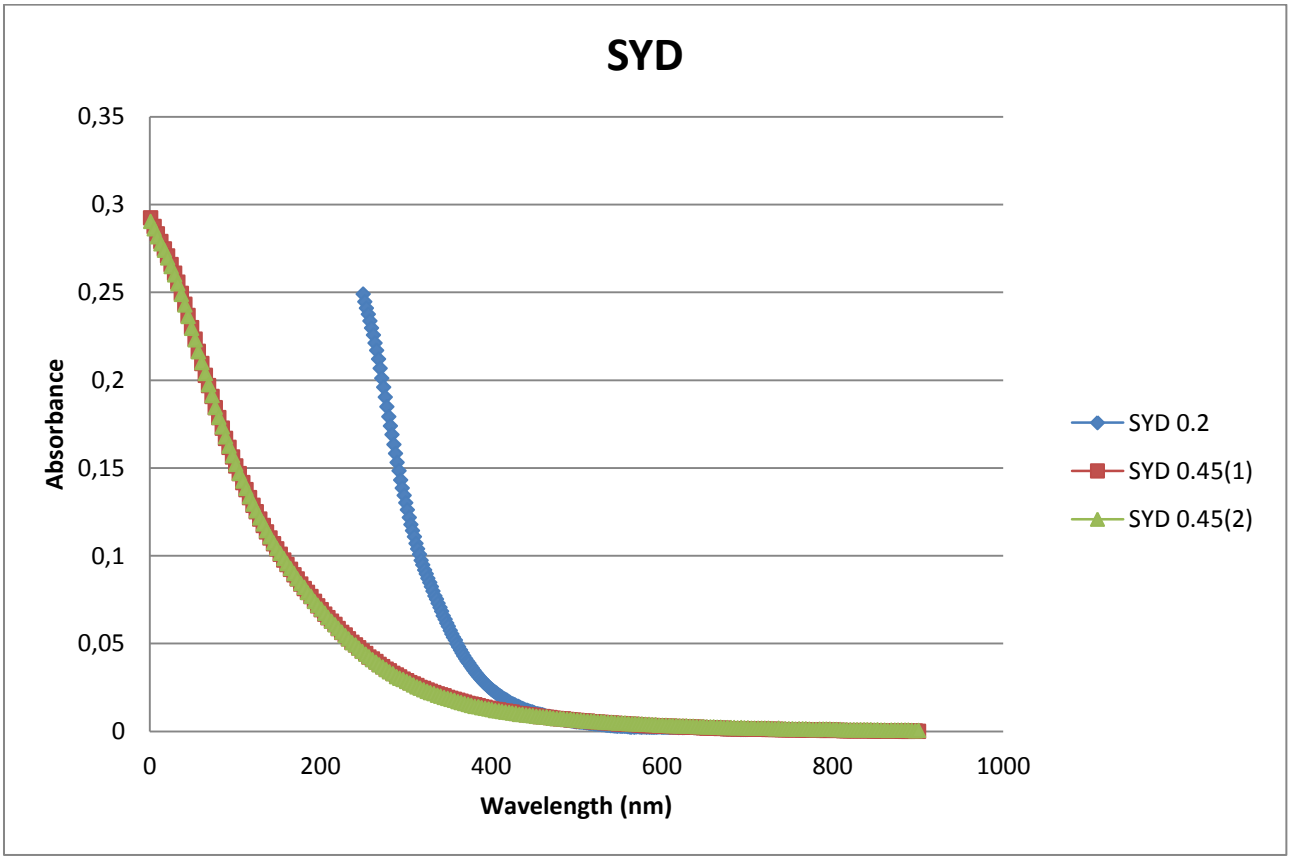

Figure 9: UV Absorbance SYD samples.

On the figures presented is rather difficult to find a tendency. What is clear is that in the samples with no added iron and filtered through 0,2 µm the absorbance is greater around 380 nm, which is not the case for the rest of the samples. That might be due to the retention of larger molecules in the filter, which absorb radiation with higher energy. The presence of iron might cause the fractionation of these larger molecules and therefor they are able to go through the filter (Dinesh and Yang 2014), which would explain the absorbance for that region in the samples with added iron even though they are filtrated through 0,2 µm as well.
The results presented do not give a lot of information related to the differences between the iron concentration which suggest that the UV absorbance is mostly governed by the DNOM in solution rather than other molecules.

3.2 EES

EEs measurements were carried out for all of the 72 samples. The results are for the sample filtrated through 0,45 µm once with no iron added, filtrated through 0,45 µm twice with no iron added, filtrated through 0,2 µm with no iron added, filtrated through 0,45 µm with a 5 µM iron concentration, filtrated through 0,2 µm with a 5 µM iron concentration, filtrated through 0,45 µm with a 10 µM iron concentration, filtrated through 0,2 µm with a 10 µM iron concentration, filtrated through 0,45 µm with a 20 µM iron concentration and filtrated through 0,2 µm with a 20 µM iron concentration from the eight different sites. The results are presented as 3D graphs for fluorescence.

Here only the results from the HEO samples are includes due to those are the ones in which the differences can be appreciated more easily. All the rest can be found at

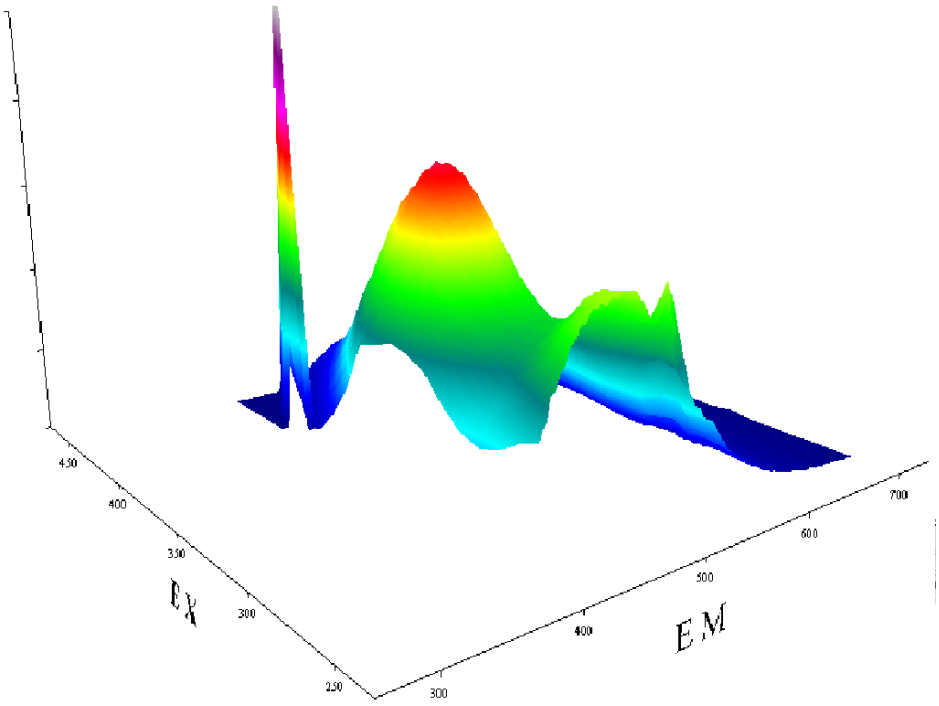

Figure 10: HEO sample filtrated through 0,2 µm filter.

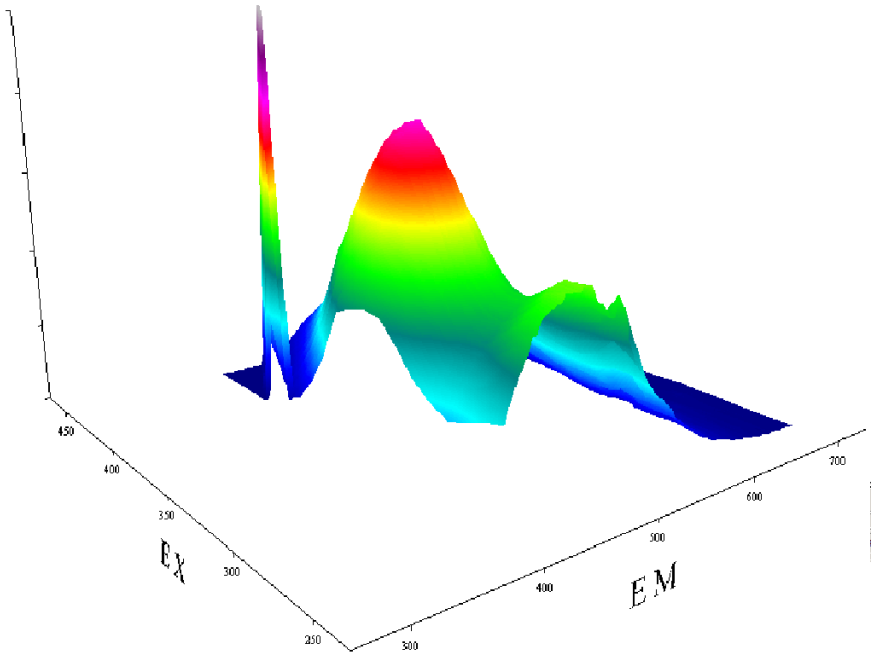

Figure 11: HEO sample filtrated through 0,45 µm filter once.

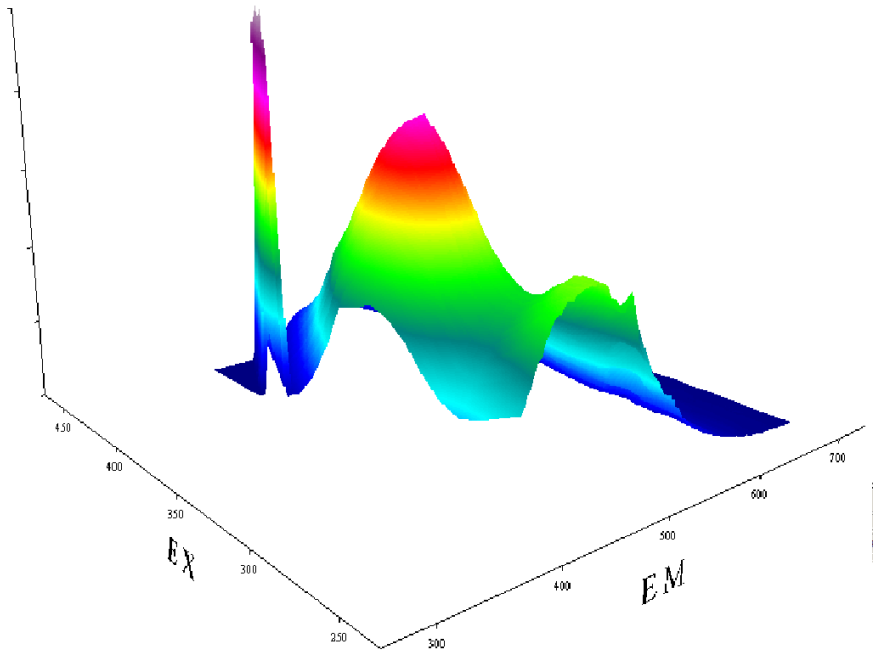

Figure 12: HEO sample filtrated through 0,45 µm filter twice.

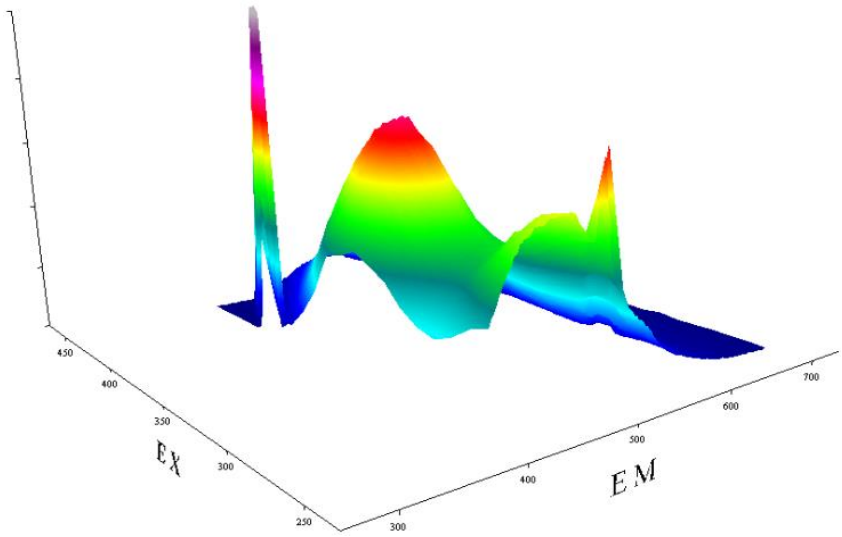

Figure 13: HEO sample with 5 µM added Fe filtrated through 0,2 µm filter.

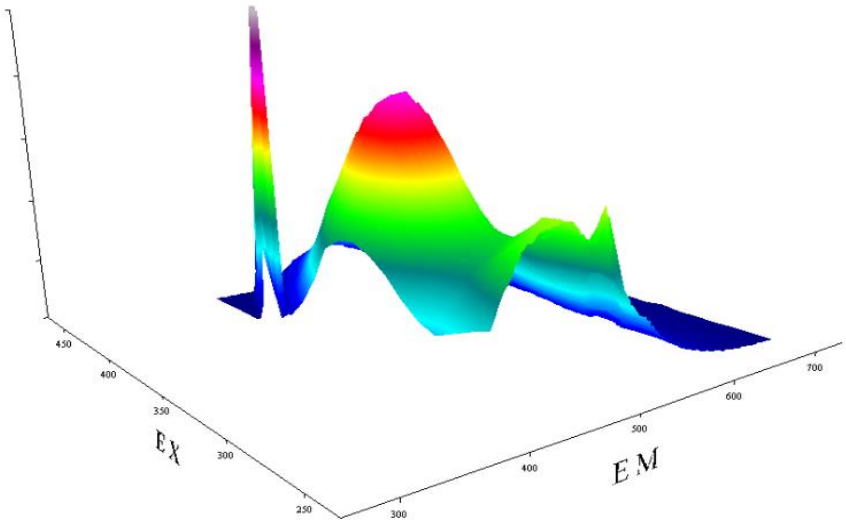

Figure 14: HEO sample with 5 µM added Fe filtrated through 0,45 µm filter.

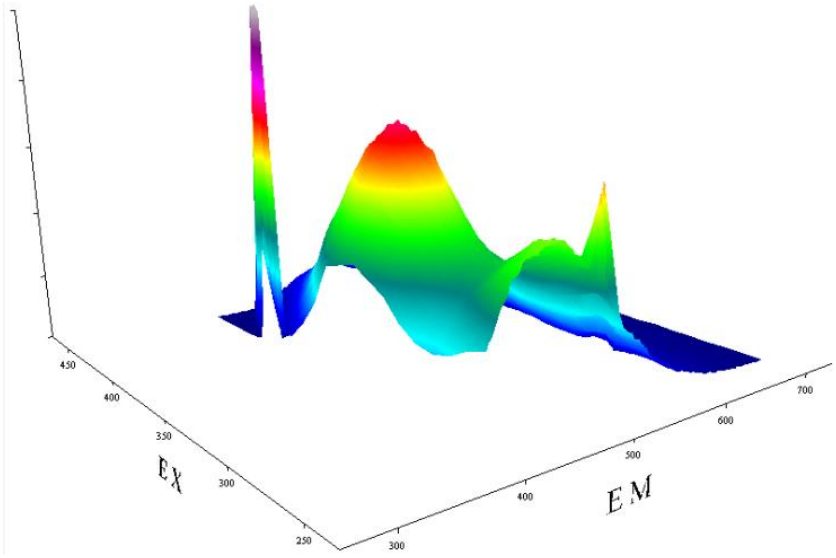

Figure 15: HEO sample with 10 µM added Fe filtrated through 0,2 µm filter.

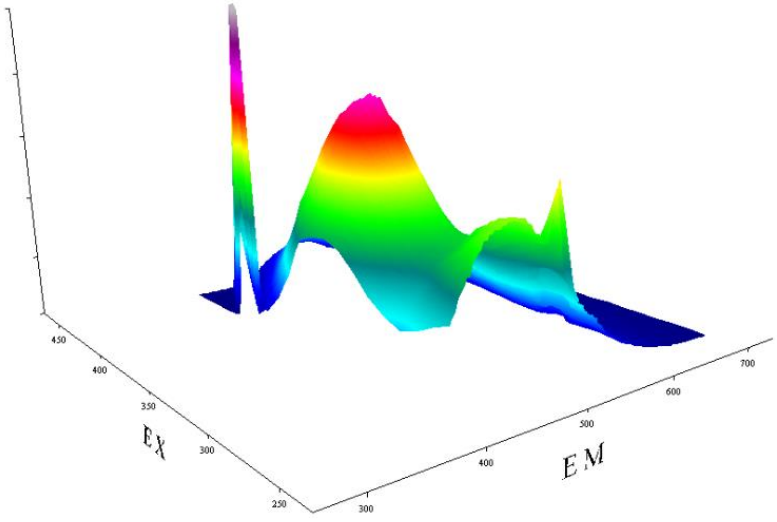

Figure 16: HEO sample with 10 µM added Fe filtrated through 0,45 µm filter.

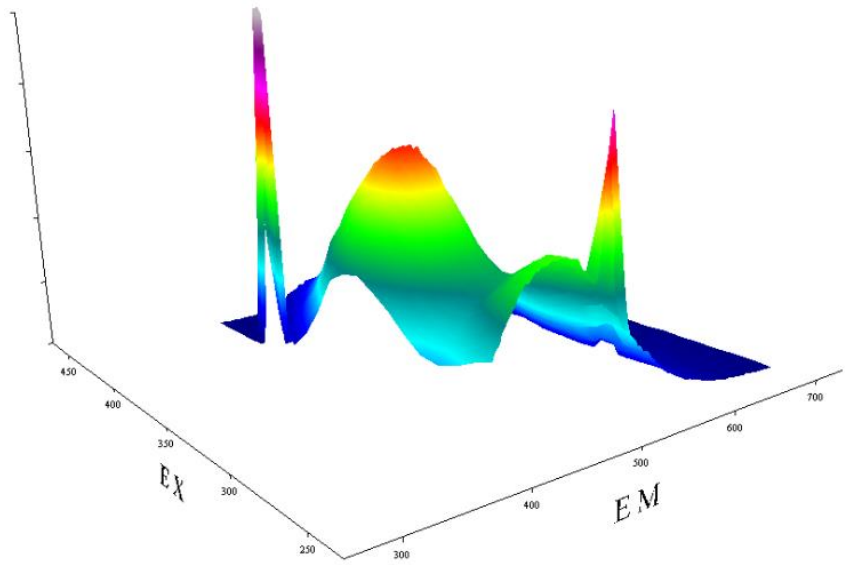

Figure 17: HEO sample with 20 µM added Fe filtrated through 0,2 µm filter.

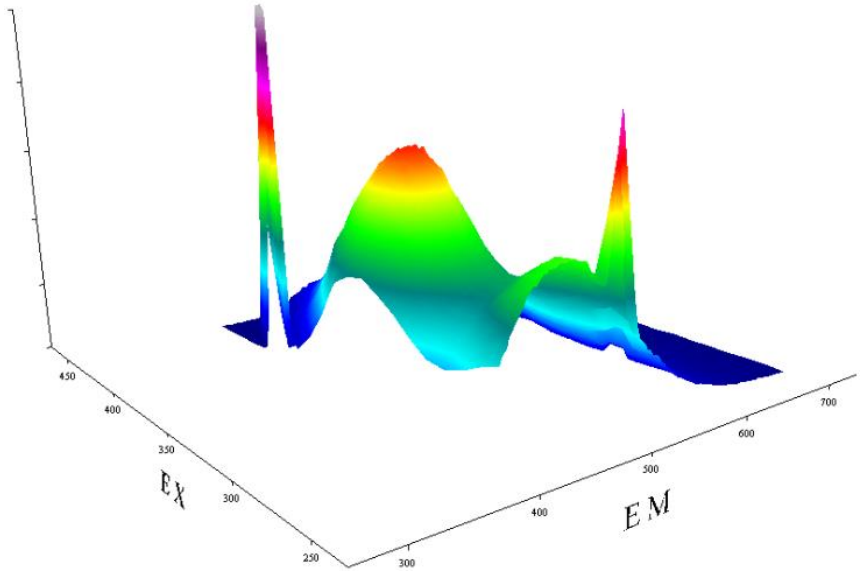

Figure 18: HEO sample with 20 µM added Fe filtrated through 0,45 µm filter.

From the last figures we can notice that as the iron concentration increases in the solution, the fluorescence in the range from 400 nm to 550 nm (EM) is somehow less than with lower concentrations. This can also be observed when the size of the pore decreases. On the other hand, is observed that as the iron concentration increases the intensity of the peaks around 400 nm and 650 nm (EM) increases. This suggests a link between those two sections of the spectra, which might be interpreted as an inversely proportional relation. As the iron concentration increases, it reacts with the DNOM causing the signal in the middle section (Frances, EM, 2015) which causes its concentration to go lower and increases the concentration of the product, which emits radiation with less energy. This might be due to the coordination bond between the iron and the DNOM. Fe^{3+} has a semi full d layer and depending on the atom on which it coordinates the d orbitals crystalline electric field splitting might occur, changing the symmetry, which would allow a greater amount of transitions between electrons, thus the radiation emitted afterwards will have lower energy.

3.3 Conductivity and pH

Here are presented the conductivity and pH values of each of the 72 analyzed samples in eight different tables, one for each sampling site. The results are for the sample filtrated through 0,45 µm once with no iron added, filtrated through 0,45 µm twice with no iron added, filtrated through 0,2 µm with no iron added, filtrated through 0,45 µm with a 5 µM iron concentration, filtrated through 0,2 µm with a 5 µM iron concentration, filtrated through 0,45 µm with a 10 µM iron concentration, filtrated through 0,2 µm with a 10 µM iron concentration, filtrated through 0,45 µm with a 20 µM iron concentration and filtrated through 0,2 µm with a 20 µM iron concentration.

Sample	Conductivity (µS/cm)	pH
BERGEN 0,45 (1)	44,5	6,4
BERGEN 0,45(2)	44,2	6,4
BERGEN 0,2	42,6	6,6
BERGEN5 0,2	40,3	5,5
BERGEN5 0,45	43,5	5,5
BERGEN10 0,2	39,2	5,4
BERGEN10 0,45	43,8	5,6
BERGEN20 0,2	42,7	5,2
BERGEN20 ,045	46,9	5,0

Table 1: Conductivity and pH values for BERGEN samples.

Sample	Conductivity (µS/cm)	pH
BIR 0,45(1)	36,0	4,6
BIR 0,45(2)	35,8	4,7
BIR 0,2	33,1	5,2
BIR5 0,2	31,7	5,1
BIR5 0,45	37,1	4,6
BIR10 0,2	34,3	4,4
BIR10 0,45	40,7	4,2
BIR20 0,2	40,7	4,1
BIR20 0,45	48,3	3,9

Table 2: Conductivity and pH values for BIR samples.

Sample	Conductivity (µS/cm)	pH
HEO 0,45(1)	37,1	4,0
HEO 0,45(2)	37,1	4,1
HEO 0,2	30,0	4,5
HEO5 0,2	33,9	4,4
HEO5 0,45	38,5	4,1
HEO10 0,2	38,3	4,0
HEO10 0,45	42,5	3,8
HEO20 0,2	43,3	3,8
HEO20 0,45	49,5	3,7

Table 3: Conductivity and pH values for HEO samples.

Sample	Conductivity (µS/cm)	pH
HSY 0,45(1)	63,1	6,7
HSY 0,45 (2)	63,2	6,6
HSY 0,2	59,7	6,4
HSY5 0,2	54,8	6,1
HSY5 0,45	61,5	6,3
HSY10 0,2	57,2	6,4
HSY10 0,45	61,3	6,4
HSY20 0,2	56,1	6,4
HSY20 0,45	63,4	6,5

Table 4: Conductivity and pH values for HSY samples.

Sample	Conductivity (µS/cm)	pH
IVAR 0,45(1)	30,4	6,0
IVAR 0,45(2)	29,6	5,9
IVAR 0,2	31,2	6,1
IVAR5 0,2	26,0	5,9
IVAR5 0,45	29,2	5,0
IVAR10 0,2	27,9	4,9
IVAR10 0,45	31,0	4,7
IVAR20 0,2	34,7	4,3
IVAR20 0,45	37,0	4,2

Table 5: Conductivity and pH values for IVAR samples.

Sample	Conductivity (µS/cm)	pH
SCW 0,45(1)	41,7	6,3
SCW 0,45(2)	41,9	6,5
SCW 0,2	38,7	6,6
SCW5 0,2	37,0	6,3
SCW 5 0,45	41,3	6,1
SCW10 0,2	37,4	6,2
SCW10 0,45	41,4	6,1
SCW20 0,2	38,8	6,1
SCW20 0,45	43,5	6,0

Table 6: Conductivity and pH values for SCW samples.

Sample	Conductivity (µS/cm)	pH
SYD 0,45(1)	68,8	6,7
SYD 0,45 (2)	68,6	6,8
SYD 0,2	58,5	6,8
SYD5 0,2	68,3	6,1
SYD5 0,45	67,2	6,3
SYD10 0,2	60,5	6,4
SYD10 0,45	68,1	6,4
SYD20 0,2	61,0	6,4
SYD20 0,45	67,1	6,4

Table 7: Conductivity and pH values for SYD samples.

Sample	Conductivity (µS/cm)	pH
TRE 0,45(1)	21,9	6,4
TRE 0,45(2)	22,5	6,5
TRE 0,2	22,4	6,4
TRE5 0,2	21,8	5,6
TRE5 0,45	22,9	5,6
TRE10 0,2	21,6	5,8
TRE10 0,45	23,4	5,7
TRE20 0,2	23,9	5,7
TRE20 0,45	25,5	5,1

Table 8: Conductivity and pH values for TRE samples.

From the results obtained from the experiments we can notice in four tendencies in general:

- As the concentration or iron increases, also the conductivity increases in the samples. This can be explained by the presence of ions, such as Fe^{3+} and Cl^- in the solution. In addition, the acidic hydrolysis iron generates also may increase the conductivity by increasing the H^+ concentration in the solution.
- As the conductivity of the iron solutions increases, the pH of the sample decreases. This is explained in the last point.
- As the size of the pore decreases, the pH increases. An explanation for this may be that iron binds to the DNOM in the solution and form complexes, but these complexes may also bind to water molecules contributing to the hydrolysis of water. When the pore size is reduced, these rather large molecules, the complexes, might not be able to go through the pore and then the hydrolysis is reduced by this.
- As the size of the pore decreases the conductivity decreases. An explanation for this is also mentioned in the last point, if the hydrolysis is less that might affect the conductivity of the solution.

3.4 Fe Fractionation

The measurement of the absorbance at 600 nm allows to determine the concentration of iron in the different samples. In the NOMiNOR samples only the concentration of the monomeric iron was determined due to only the extraction process was carried out, the ion exchange couldn't be performed due to a lack of sample.
For the NOM-Typing samples both procedures were performed.
Here are shown the results from this. The calibration curves and the raw data can be found in the results carpet shared with the environmental chemistry group.
In all tables, the sample number 1 corresponds to the sample filtrated once through 0,45 µm filter, sample 2 corresponds to the sample filtrated through 0,45 µm twice, sample 3 corresponds to 5µM of added iron filtrated through 0,45 µm, sample 4 corresponds to 10µM of added iron filtrated through 0,45 µm and sample 5 corresponds to 20 µM of added iron filtrated through 0,45 µm.
Some concentrations are shown as negative, which needs to be interpreted in the right way to approach a valid conclusion.

BERGEN	[Fe_a] (µM)
1	-1,150
2	-1,150
3	-0,317
4	-0,817
5	-0,65

Table 9: Fe concentration in BERGEN samples.

HSY	[Fe_a] (µM)
1	-0,983
2	-1,150
3	-1,150
4	0,017
5	-0,483

Table 10: Fe concentration in HSY samples

IVAR	[Fe_a] (µM)
1	26,02*
2	-2,650
3	-1,312
4	2,683
5	14,683

Table 11: Fe concentration in IVAR samples

SCW	[Fe_a] (µM)
1	0,017
2	0,017
3	2,850
4	66,017*
5	1,683

Table 12: Fe concentration in SCW samples

SYD	[Fe_a] (µM)
1	-0,817
2	45,517*
3	-0,817
4	-0,65
5	-0,483

Table 13: Fe concentration in SYD samples

BIR	[Fe_a] (µM)	[Fe_o] (µM)	[Fe_i] (µM)
1	0,017	0,183	-0,1667
2	0,183	-0,150	0,333
3	3,850	1,517	2,333
4	7,350	1,83	6,167
5	15,183	4,017	11,167

Table 14: Fe concentration in BIR samples

HEO	[Fe_a] (µM)	[Fe_o] (µM)	[Fe_i] (µM)
1	10,517	3,683	6,683
2	6,683	3,517	3,167
3	57,850*	4,683	53,167*
4	55,183*	6,850	48,333*
5	24,017	9,017	15,000

Table 15: Fe concentration in HEO samples

TRE	[Fe_a] (µM)	[Fe_o] (µM)	[Fe_i] (µM)
1	-0,817	-0,483	-0,333
2	-0,983	-	
3	0,683	0,517	0,167
4	0,350	0,350	0
5	10,517*	5,017	5,500

Table 16: Fe concentration in TRE samples

The standard deviation of the method is 1%.
The results marked with an asterisk should not be considered as valid results. Some kind of interference was present in those measurements.

From the results shown in the tables above and the pH results is possible to notice that the samples with the lower pH show a higher concentration of monomeric iron, like HEO and BIR. This suggests that the acidic environment is favorable for the monomeric species, thus when the iron concentration is increased in more basic solutions, as SYD, the concentrations are rather low because it doesn't bind with the 8-hydroxyquinoline and the effect of increasing the iron concentration is not as important as in the more acidic solutions. In addition, when the iron concentration is reaching higher values (20µm) the concentration in the monomeric form goes lower, indicating the formation of the polymeric forms.

The organic complexed fraction seems to have in general a correlation with the pH. It appears that the more acidic the solution the less organic complexed the iron (BIR) and when the solution has higher pH values the organic complexed fraction seems to be greater which might be correlated with both the stability of the complexes and the availability on the iron in solution.
Nevertheless, the previous analysis might have deficiencies due to the fact that only a few samples were analyzed with the whole fractionation process which made difficult finding a tendency in the data.

4 Conclusions

In complex processes, such as the ones occurring in nature, it is necessary to take into consideration several factors to understand the behavior of compounds.
The different analyses carried out contribute to the better understanding of the interaction between iron and DNOM.
Fluorescence gives information about how iron affects the composition and behavior of DNOM. On the other hand, pH and conductivity help elucidating how iron affects the chemical environment of water.
The acidity in the solution affects the fractionation of the different iron species in a rather important way. When the solution is more acidic, the iron will be present in greater part as monomeric iron. On the other hand, more alkaline environment will facilitate the formation of organic complexes, in which case the increase in water color will be due to the absorbance of these compounds.
In general, the distribution on iron and how it will affect the color of water is mainly dominated by the composition and properties of the water site that is being studied.
Further analyses with a larger amount of samples should be carried out for better understanding of the mechanisms affecting Fe fractionation

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