# Coupling Size exclusion chromatography and 3D Fluorescence for studying DOM in estuarine waters

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## Introduction

In estuarine systems, dissolved organic matter (DOM) has been extensively studied by quantifying total dissolved organic carbon concentration (DOC) or using fluorescence technics. Although these approaches can give clues to the bulk DOM complexity and reactivity, uncertainties remain on the acquisition of quantitative data and relationship with the size distribution is poorly resolved. Size exclusion chromatography combined with UV, carbon and nitrogen detection (LC-OCD-OND-UVD called LC-OCD) could partially make the gap between these different technics by providing information on size distribution, polarity, aromaticity and C/N ratio of DOM without any sample processing[1]. In this work we examined how 3D fluorescence can give further insights to the information provided by LC-OCD on DOM changes in estuarine systems.

## Materials and Methods

Estuarine waters were collected in January 2018 along the Aulne estuary (Bay of Brest, France) on board of R.V. Hesione (INSU-CNRS-UBO). Stations were selected according to their salinity from 0 to 35 with a step of 2 (17 sampling stations). Chromatography analyses were performed by a LC-OCD designed and assembled by DOC-Labor® (Karlsruhe, Germany). The chromatographic sector consisted in two weak cation exchange columns on polymethacrylate gel (250 mm x 20 mm, TSK HW 50S, Toso Japan) wherein the on-line filtered (0.45 µm PES-filter ) mixture is separated into 6 distinct fractions : i) the bypass used to calculate the total DOC and the global absorbance of the sample ; ii) the fraction called biopolymer (BP) typically representative of high molecular weight compounds including polysaccharides and proteins ; iii) the humic fraction (HS); iv) the building blocks fraction (BB), composed by breakdown products of HS ; v) the low molecular weight acids fraction (LMW-acids) and vi) the low molecular weight neutrals fraction (LMW-neutrals) mostly composed of alcohols, aldehydes and ketones. In this work, we isolated as extracts some of these fractions and 3D fluorescence was applied to further study the footprint of DOM. The fluorescence emission spectra were obtained on a Cary Eclipse spectrophotometer with an arc-xenon lamp pulsed at 80 Hz as source excitation.

## Results and Discussion

Concentrations of the LC-OCD fractions within the estuarine system varied between 700 and 4000 ppb-C for DOC, 50 and 120 ppb-C for BP, 400 and 2000 ppb-C for HS, 150 and 400 ppb-C for BB and between 200 and 800 for LMWN. The HS fraction was the main contributor to DOC representing 68 % at low salinities and 52 % in the marine part of the estuary. All the fractions displayed a relative conservative behavior in the estuary with a decrease in concentrations from upstream to downstream. The BP and SH+BB fractions were extracted and analyzed by 3D fluorescence. The fluorescence of BP extracts was characteristic of tryptophan and proteins compounds and the signal intensity of this fraction significantly increased in the marine part of the estuary (salinity > 28). By contrast, the fluorescence of SH+BB extract corresponded to pedogenic fulvic acids and the intensity of the related signal decreased conservatively with salinity.

## Conclusion

The application of 3D fluorescence on fractions separated by LC-OCD allowed a better characterization of the nature and the sources of the biopolymers and the humic compounds in the Aulne estuary. Our study is a first step demonstrating that the coupling of LC-OCD and 3D fluorescence can be used for a finer characterization of DOM in natural waters.

## Bibliography

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