Sample Handling

This session will consider best practice for:

- Sample storage (sample bottles, storage time)
- Filtration
- Protocols for coloured and turbid samples

Based on recommendations presented at the AGU Chapman Conference on 'Organic Matter Fluorescence', Birmingham, UK. With acknowledgements to Rob Spencer (University of California, Davis) and Paula Coble (University of South Florida)

Overview

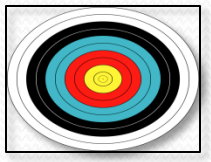
- Introduction
  - QA/QC
  - Accuracy
- Sample collection
  - Contamination sources
  - Equipment cleaning
  - Water samplers
- Sample preservation
  - Filtration
  - Refrigeration / freezing
  - Acidification
- Summary
  - Coloured and turbid samples
  - Conclusions

Introduction

QA/QC – careful sampling and handling prior to analysis are the first steps in producing high quality data.

- Avoid contamination during sampling and handling.

- Separation of particles from the solution. Handling coloured solutions.



Accuracy (i.e. the correctness of the data) – how close is what we measure to the real value ?

- Development of effective and robust laboratory and field SOP's.

- Blanks and replication.

Sample Collection - Contamination sources

Three main areas of contamination:

- 1. Atmospheric – tobacco smoke, cleaning solutions and exhaust fumes.

- 2. Careless handling – working in a dirty environment, not wearing gloves. Polypropylene or nitrile gloves are recommended, latex gloves should be avoided as they can leach absorbing compounds.

- 3. Sampling equipment – insufficient cleaning, leaching of organic substances from new plastics. Many types of plastic are highly fluorescent and should be tested before use. Glass, teflon and 'aged' plastic containers (e.g. polysulphone / polycarbonate) are recommended.

Sample Collection - Equipment cleaning

Cleaning goal is to remove any existing organic material.

- Glass – baked at 450°C for 4-12 hours in a muffle furnace.
- Teflon / plastic containers / caps – rinsed with dilute HCl, rinsed with ultrapure water, rinsed with methanol, soaked in ultrapure water. Dried in drying oven at 60°C.
- Washing procedures should be checked by filling a cleaned bottle with ultrapure water and analyzing for fluorescence and absorbance.

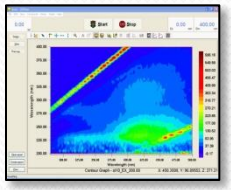
Sample Collection - Water samplers

Avoidance of contamination.

- Niskin bottles or similar samplers should have silicone seals and Teflon-coated springs, as found in those used for trace metal or trace gas analysis.

- The sampler should pass the organic-rich surface micro-layer closed – COC principle e.g. Go-Flo bottles.

Sample Preservation - Filtration



Should you filter?

- Particles can interfere with measurements and can also contain live organisms which produce or metabolize DOM.

What pore size and type of filter ?

- Commonly used ranges of filters are from 0.2 to 1.2 µm and types include nylon, polysulfone, polycarbonate and glass fiber.

Also perfectly fine not to filter. For very turbid samples, particulate matter can scatter light and interfere with the fluorescence signal and filtration may be essential.

Sample Preservation - Filtration

Avoidance of contamination.

- Carbon leaching from the filter or adsorption onto the filter may be significant.
- Commonly used ranges of filters are from 0.2 to 1.2 µm and types include nylon, polysulfone, polycarbonate and glass fiber.
- Filtration pressure should be the lowest necessary to pass the filter in a reasonable time period.
- Clogging reduces the flow rate and may reduce the effective pore size of the filter resulting in increased back pressure over time resulting in DOC from cell lysis.
- All surfaces that come into contact with the sample should be triple rinsed including rinsing the sample bottles with filtrate.

Sample Preservation - Storage

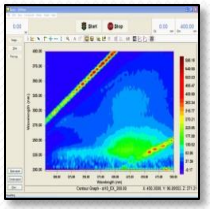
The two most important causes of sample instability are microbial growth and exposure to light.

- Ideally samples should be filtered, stored refrigerated in the dark and analyzed as soon possible after collection.

Considerations when storing samples:

- Storage containers – glass vs. plastics.
- Temperature – room temp. vs. refrigeration vs. freezing.
- Poisoning – acidification vs. preservatives (e.g. $CHCl_3$ and $HgCl_2$.

Sample Preservation – Summary



- Spectrophotometric properties of waters, in particular fluorescence intensities change over time during storage.

- The change during storage under refrigeration is within analytical / sample variability; however, with increasing time these breaks down.

- Analysis is recommended as soon as possible after sampling; data obtained after 5 days may not reflect the natural signal.

- Storage should be under refrigerated conditions in suitably cleaned containers. Storage at room temp. is not recommended.

- Acidification is not recommended as a preservation method as this changes DOM fluorescence.

Sample Preservation - Summary

➢ After defrosting and reanalysis of water samples alterations of fluorescence and absorbance is observed. This varies between samples in an inconsistent manner and can not be predicted from original spectrophotometric characteristics.

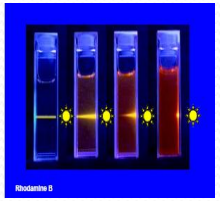
➢ Analysis of defrosted samples must be undertaken with caution.

➢ Little data on preservatives such as $CHCl_3$ and $HgCl_2$ and whether they may be appropriate to use when investigating using spectrophotometric measurements. Current advice: don't

➢ Different filter sizes impact on fluorescence measurements, in particular protein-like fluorescence.

➢ Ideally samples should be filtered, stored refrigerated in the dark and analyzed as soon possible after collection.

Coloured samples – reabsorption effects



- Strongly coloured samples prevent the emitted fluorescence from reacting the detector. The light is reabsorbed by other organic molecules.

- Coloured samples will therefore have lower fluorescence intensity which is emitted at longer wavelengths (lower energy).

- Two solutions to the problem: (1) dilute the sample (2) measure absorption and apply a mathematical correction. Both have their advantages and disadvantages

- Dilution is the practical solution for most applications based situations.