# Study of the effect of pH, salinity and DOC on fluorescence of synthetic mixtures of freshwater and marine salts

**Valdemar I. Esteves,* Eduarda B. H. Santos and Armando C. Duarte**

*University of Aveiro, Department of Chemistry, 3810 Aveiro, Portugal*

***Received 29th March 1999, Accepted 28th April 1999***

In order to provide support for the discussion of the fate of organic matter in estuaries, a laboratory simulation was performed by changing freshwater ionic strength, pH and organic matter content. The change in spectroscopic organic matter (DOM) due to increasing salinity (in the range 0 to 5 g $l^{-1}$) is affected by the pH of the samples. The emission fluorescence intensity at the three maxima observed in the fluorescence spectra, is linearly correlated with dissolved organic carbon (DOC) concentration at several salinity values in the same sample. The increase in organic matter concentration caused a shift in the emission peak wavelength at 410 nm for several salinity values. We concluded that it is necessary to take into account the influence of salinity and pH on emission fluorescence of dissolved organic matter if it is to be used as a tracer in estuarine or near shore areas.



## Introduction

The behaviour of riverine dissolved organic carbon (DOC) when entering an estuarine zone has been studied. Some authors[1,2] suggested that fractions of DOC are removed by mechanisms such as flocculation, precipitation, microbial degradation and particulate adsorption while some others[3–5] found that DOC shows an overall conservative behaviour in estuaries.

Fluorescence spectroscopy has been extremely useful as a technique for monitoring dissolved organic matter[3] in freshwater, estuarine or nearshore areas. Both UV-Vis and fluorescence spectroscopy have also been used to quantify DOC in aquatic environments.[6,7] In this paper, we report the results obtained in laboratory experiments of the effects on UV-Vis and fluorescence spectra of changing pH, salinity and DOC concentration of freshwater mixed with prepared salt water.

## Experimental

### Preparation of freeze dried salts from coastal water

Five litres of seawater from a non-polluted beach (Costa Nova, Portugal) were filtered [0.45 μm Gelman (Ann Arbor, MI, USA) membrane filter]. In order to oxidize organic matter to $CO_2$, the seawater was irradiated with UV radiation (1000 W; $\lambda = 254$ nm) over 12 h: six drops of $H_2O_2$ (30% by volume) were added to 200 ml quartz tubes filled with seawater, and the seawater was exposed to UV radiation. After a 12 h exposure, the seawater was freeze dried to obtain the salts used for the preparation of solutions with different salinities.

### Sampling of freshwater

Ten litres of freshwater were sampled in glass containers at Frei-Gil on the Mira channel of the Aveiro lagoon, Portugal. Conductivity, pH and temperature were measured in the field. The sample was filtered (Gelman 0.45 μm) in the laboratory. Dissolved organic carbon (DOC) of this filtered sample was measured using a Dohrmann (Santa Clara, CA, USA) DC-180 Carbon analyser.

### Methodology for studying the effects of the environmental parameters

Adequate amounts of freeze dried sea salts were added to aliquots of filtered freshwater in order to obtain samples with salinity values of 0 (no adjustment), 2, 5, 10, 15, 25 and 35 g $l^{-1}$. The samples were then adjusted to pH 8.0 with NaOH and HCl and left overnight in a darkroom. The following day, samples were filtered through muffled (400 °C, 24 h) GF/F (Whatman, Maidstone, Kent, UK) filters. Filtered samples were characterised by spectral analysis in a UV-Vis spectrophotometer [Shimadzu (Düsseldorf, Germany) Model UV 2101 PC] and in a fluorescence spectrophotometer (JASCO, Tokyo, Japan, Model FP-770).

Emission fluorescence intensities were measured at 410 nm, 440 nm and 460 nm for an excitation wavelength of 360 nm, which corresponded to the three maxima for the freshwater sample. The width of the excitation and emission lines was 5 nm. The wavelength accuracy and repeatability were ±1.5 nm and ±0.3 nm respectively (data from the manufacturer).

For each salinity value a sample of filtered water was subsampled to obtain pH values of 7.0, 8.0 (no pH correction) and 8.5 with NaOH and HCl. The samples obtained were characterized by fluorescence spectroscopy.

In order to study the effect of dilution, for each salinity value, the samples at pH 8.0 were diluted with deionized water prepared at the same salinity as the samples and again characterized by fluorescence spectroscopy.

The fluorescence spectra were corrected for wavelength dependent effect using a dynode-feedback control system.[8]

The same procedure was performed with a series of blanks [Millipore (Milford, MA, USA) Milli-Q 50].

## Results and discussion

### The effect of salinity on the UV-Vis spectra

The variations in absorbance with salinity of a freshwater sample are shown in Fig. 1 where the UV-Vis absorbance of filtered samples is represented as a function of salinity for two

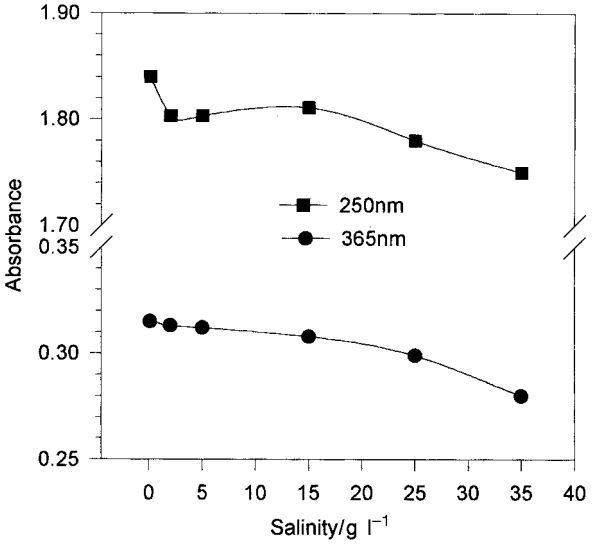


**Fig. 1** Effects of salinity on freshwater absorbance (250 and 350 nm).

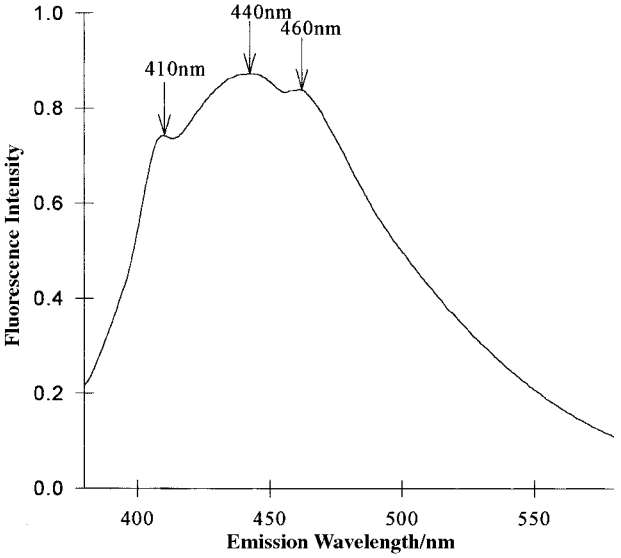


**Fig. 2** Emission fluorescence spectra of a freshwater sample for an excitation wavelength of 360 nm.

wavelengths. A general trend is observed for the wavelengths studied: the UV-Vis absorbance decreases as the salinity increases.

The DOC value of the samples was 3.5 mg l$^{-1}$ of C, before increasing salinity. As the salinity was increased there was a visible increase (not measured) of particulate matter content retained in the GF/F filters associated with the decrease in UV-Vis absorbance of filtered samples. This suggests flocculation of dissolved organic matter (DOM).

The extent of decrease in the UV-Vis absorbance due to the salinity is independent of wavelength: for instance, for wavelengths of 365 nm and 250 nm the UV-Vis absorbance decrease was 11% and 5%, respectively, calculated for a salinity value of 35 g l$^{-1}$. Assuming a strong correlation between DOC and UV-Vis absorbance at 250 nm,[7,9] the results confirm the occurrence of flocculation of DOM during mixing of freshwater and seawater. According to Sholkovitz[1] 3% to 11% of the river DOM is flocculated when in contact with seawater, which is in concordance with our results.

### The effect of salinity and pH on emission fluorescence spectra

A typical emission fluorescence spectrum of river water is shown in Fig. 2; three peaks are easily identified at wavelengths of 410, 440, and 460 nm. The change in salinity and pH of freshwater did not cause peak wavelength displacement in fluorescence emission spectra. However, an increase in DOC content for several salinity values caused a small but consistent shift in the first peak wavelength (attributed to Raman scattering or superimposed with the Raman peak) to higher wavelengths as shown in Fig. 3. Correlating the DOC concentration with the wavelength of the maximum of the first peak of 32 solutions in a range of salinities between 0 and 35 g l$^{-1}$ gave the equation $y=(4.07\times10^2)+0.75x$, and $r^2=0.926$, where $y$ is the peak wavelength and $x$ is DOC concentration (mg l$^{-1}$ of C). The 410 and 440 nm peaks must not be used to evaluate the organic matter content of solutions with high concentrations, to prevent overlap of the two peaks.

The other two peaks did not produce any displacement with changes in organic matter content.

Although fluorescence intensity was affected by salinity, the largest changes in fluorescence intensity were observed when the organic matter content of the samples was varied.

The increase in natural fluorescence intensity observed by Willey[10] during the mixture of freshwater and saltwater in estuaries, was reproduced in this laboratory in terms of salinity, adding adequate amounts of freeze dried sea salt to freshwater

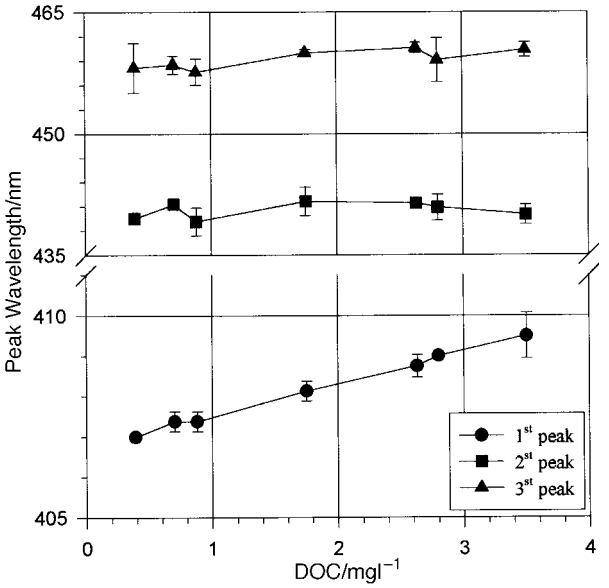


**Fig. 3** Emission peak wavelength ($\lambda_{exc}=360$ nm), as a function of DOC concentration. Each point is the data average for salinities 0, 5, 15, and 35 g l$^{-1}$. Confidence interval has a 95% confidence level.

in order to obtain samples with different salinities and the same amount of organic matter. As shown in Fig. 4, the increase in fluorescence intensity due to the increase in salinity is clear in the range of 2 to 5 g l$^{-1}$ and nearly constant for salinities greater than 5 g l$^{-1}$. Willey[10] attributed this fluorescence increase to the presence in seawater of magnesium which complexes with fluorescent fulvic and humic acids, enhancing fluorescence by crosslinking the structure between internal oxygens. This crosslinking affects fluorescence by changing the positions and energy level of π electrons. Furthermore, removal of the quenching effect of metals such as copper or iron by magnesium could enhance fluorescence.

The effect of salinity on emission fluorescence intensity becomes more accentuated when the pH is higher. For pH = 7.0 the increase in emission fluorescence intensity due to the increase in salinity from 0 to 35 g l$^{-1}$ is 3% ($\lambda_{em}=440$ nm) and 9% when the pH is 8.5 ($\lambda_{em}=440$ nm). The results obtained show that the increase in fluorescence due to the change of

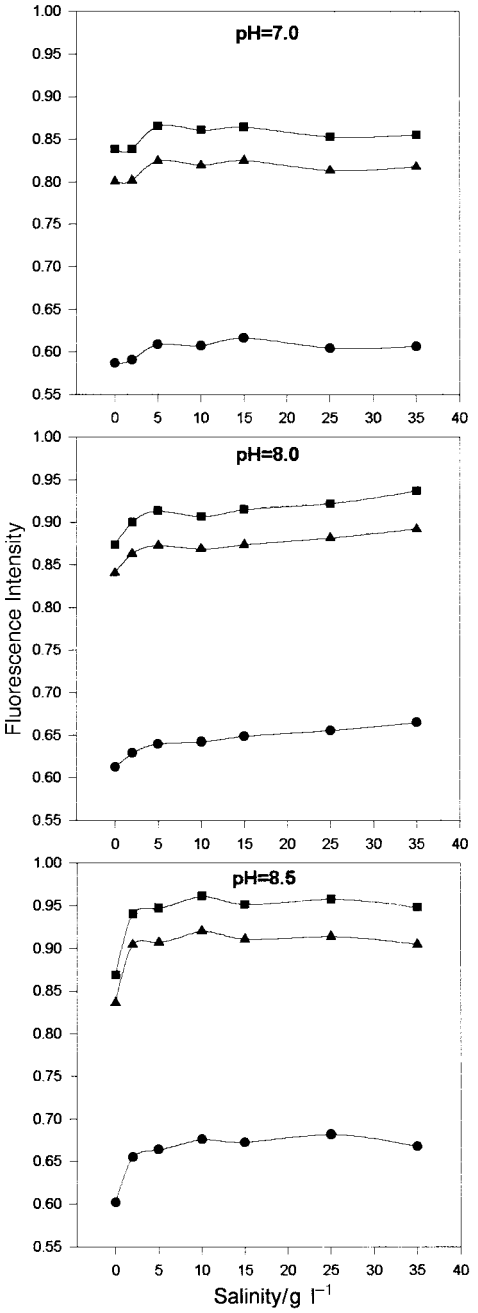


**Fig. 4** Variation of emission fluorescence intensity (at pH 7.0, 8.0 and 8.5) with salinity ($\lambda_{em}$ = ●, 410 nm; ■, 440 nm; and ▲, 460 nm; $\lambda_{exc}$ = 360 nm). The Raman peak of Milli-Q water was subtracted from the fluorescence at 410 nm.

pH from 7.0 to 8.5 is remarkable for salinities greater than 2 where this increase is around 10%.

Several authors[3,5,11] have not observed the increase in fluorescence intensity during the mixture of freshwater and seawater, while others[12] have noted a net influence of the salinity in the range 0 to 5, which can be explained by different pH changes.

Emission spectra of 21 samples with salinities ranging from 0 to 35 g l$^{-1}$ and pH values of 7.0, 8.0 and 8.5 were recorded and no shift was observed in the three wavelengths corresponding to emission maxima. This fact indicates that the increase in fluorescence due to the salinity or change in pH is not a result of a change in the chemical structure of the fluorophores. Therefore, it is possible to compare spectral intensities of samples with different salinities and pH values.

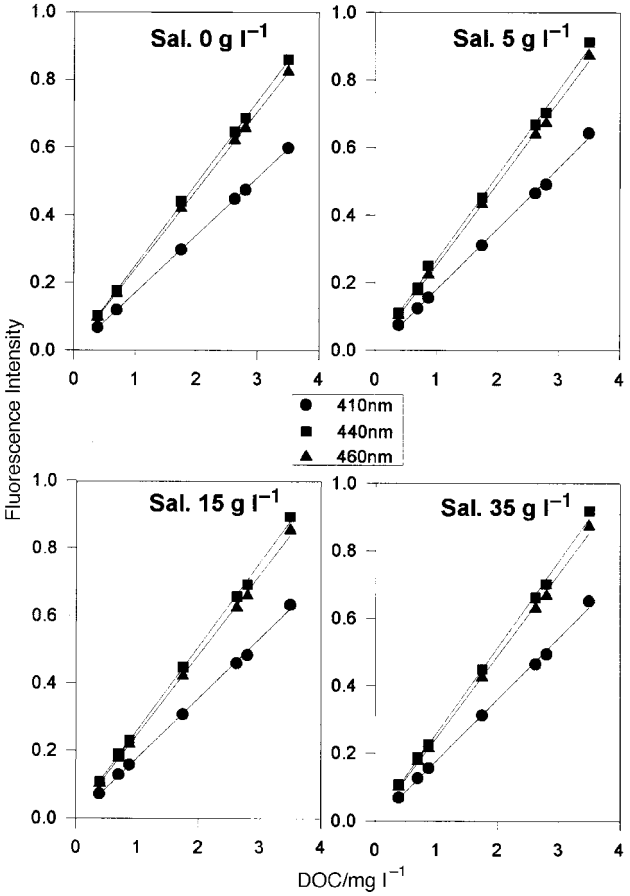


**Fig. 5** Fluorescence intensity as a function of DOC, for salinities (Sal.) of 0, 5, 15 and 35 g l$^{-1}$; pH 8.0.

### The effect of dissolved organic carbon content on the emission fluorescence intensity

A series of samples with salinities of 0, 5, 15 and 35 g l$^{-1}$ was used to study the relationship between DOC content and fluorescence intensity (Fig. 5). For each salinity, seven solutions were prepared with different organic matter content, by diluting each solution with Milli-Q water with the same salinity. The fluorescence intensity was measured at three wavelengths: 410, 440, and 460 nm ($\lambda_{ex}$ = 360 nm). For a given salinity, the fluorescence intensity is linearly correlated with the DOC concentration. The results for all salinity values in this experiment fitted straight lines with correlation coefficients better than 0.997. Any of the three emission wavelengths (410, 440 and 460 nm) could be used to quantify the content of organic matter, but 440 and 460 nm give higher sensitivity (larger slope) than 410 nm.

The good correlation between fluorescence intensity and DOC concentration shows that in the range of 0 to 35 g l$^{-1}$ salinity there are no inner filter effects, hence this is not the reason for the anomalous increase in fluorescence intensity with salinity.

## Conclusions

The mixing of freshwater and seawater, in laboratory studies, removes 5% to 10% of dissolved organic matter as particulate, measured as absorbance decrease. Changes in ionic strength increase fluorescence intensity for salinities ranging between 0 to 5 g l$^{-1}$. The good correlation between fluorescence intensity and DOC concentration shows that, in the range of 0 to 35 g l$^{-1}$ salinity, there are no inner filter effects, hence this is

not the reason for the anomalous increase in fluorescence intensity with salinity.

The increase (%) of emission fluorescence intensity ($\lambda_{exc}$ = 360 nm; $\lambda_{em}$ = 440 nm) due to salinity is affected by pH and is higher for pH = 8.5 (9%) than for pH = 7.0 (3%). Increased concentration of organic matter, for several salinity values, caused a small but consistent shift in the emission peak wavelength at 410 nm while for the other two peaks (440 and 460 nm) no displacement was produced.

To use emission fluorescence of dissolved organic matter as a tracer in estuarine or nearshore areas it is necessary to take into account the influence of salinity and pH on fluorescence intensity.

## References


1 E. R. Sholkovitz, *Geochim. Cosmochim. Acta*, 1976, **40**, 831.
2 E. R. Sholkovitz and D. Copland, *Geochim. Cosmochim. Acta*, 1981, **45**, 181.
3 J. E. Dorsh and T. F. Bidleman, *Estuarine Coastal Shelf Sci.*, 1982, **15**, 701.
4 R. F. C. Mantoura and E. M. S Woodward, *Geochim. Cosmochim. Acta*, 1983, **47**, 1293.
5 P. Berger, R. W. P. M. Laane, A. G. Ilahude, M. Ewald and P. Courtot, *Oceanolog. Acta*, 1984, **7**, 309.
6 P. L. Smart, B. L. Finlayson, W. T. Rylands and C. M Ball, *Water Res.*, 1976, **10**, 805.
7 H. De Haan and T. De Boer, *Water Res.*, 1987, **21**, 731.
8 H. Okahana and M. Arita, *Model FP-770 Spectrofluorometer product description Jasco. Jasco International Co., Ltd.* (4–21, Sennin-cho 2-chome, Hachioji, Tokyo 193, Japan), 1987.
9 R. S. Summers, P. K. Cornel and P. V. Roberts, *Sci. Total Environ.*, 1987, **62**, 27.
10 J. D. Willey, *Marine Chem.*, 1984, **15**, 19.
11 J. D. Willey and L. P. Atkinson, *Estuarine Coastal Shelf Sci.*, 1982, **14**, 49.
12 S. E. Cabaniss and M. S. Shuman, *Mar. Chem.*, 1987, **21**, 37–50.


*Paper 9/02529D*