Analytica Chimica Acta 788 (2013) 99–107

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

The inner filter effects and their correction in fluorescence spectra of salt marsh humic matter

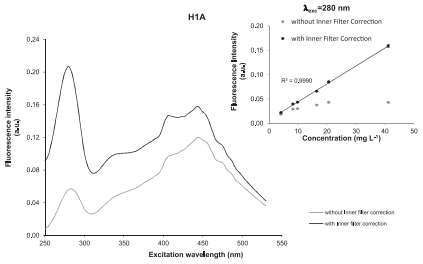
Ana Mendonça, Ana C. Rocha, Armando C. Duarte, Eduarda B.H. Santos*

Centro de Estudos do Ambiente e do Mar (CESAM), Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

HIGHLIGHTS

- Inner filter effects (IFE) in salt marsh humic fluorescence spectra were studied.
- IFE act differently depending on the type of salt marsh humic acids (SMHA).
- The tryptophan (Trp) band of some SMHA is severally obscured by IFE.
- IFE were studied in mixtures of model fluorophores for SMHA.
- IFE in SMHA spectra were mathematically corrected.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form 22 May 2013
Accepted 30 May 2013
Available online 8 June 2013

Keywords:
Inner filter effects
Spectra correction
Fluorescence spectroscopy
Saltmarsh sediment
Humic substances

ABSTRACT

The inner filter effects in synchronous fluorescence spectra ($\Delta\lambda = 60$ nm) of sedimentary humic substances from a salt marsh were studied. Accordingly to their type and the influence of plant colonization, these humic substances have different spectral features and the inner filter effects act in a different manner. The fluorescence spectra of the humic substances from sediments with colonizing plants have a protein like band ($\lambda_{exc} = 280$ nm) which is strongly affected by primary and secondary inner filter effects. These effects were also observed for the bands situated at longer wavelengths, i.e., at $\lambda_{exc} = 350$ nm and $\lambda_{ex} = 454$ nm for the fulvic acids (FA) and humic acids (HA), respectively. However, they are more important for the band at 280 nm, causing spectral distortions which can be clearly seen when the spectra of solutions 40 mg L^{-1} of different samples (Dissolved Organic Carbon – DOC ~ 20 mg L^{-1}) are compared with and without correction of the inner filter effects. The importance of the spectral distortions caused by inner filter effects has been demonstrated in solutions containing a mixture of model compounds which represent the fluorophores detected in the spectra of sedimentary humic samples. The effectiveness of the mathematical correction of the inner filter effects in the spectra of those solutions and of solutions of sedimentary humic substances was studied. It was observed that inner filter effects in the sedimentary humic substances spectra can be mathematically corrected, allowing to obtain a linear relationship between the fluorescence intensity and humic substances concentration and preventing distortions at concentrations as high as 50 mg L^{-1} which otherwise would obscure the protein like band.

1. Introduction

Humic substances (HS) are heterogeneous mixtures of high molecular weight organic compounds, with aromatic and aliphatic moieties, that are rich in oxygen-containing functional groups [1,2]. Accounting for 50–80% and 5–30% of dissolved organic carbon from river and seawater, respectively, they are the major fraction of

* Corresponding author. Tel.: +351 234 370 725; fax: +351 234 370 084.
E-mail address: edsantos@ua.pt (E.B.H. Santos).

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.05.051

the aquatic organic matter [3–6]. In estuarine soils, HS are also an important fraction of the organic matter [7,8]. These substances can regulate the biogeochemical cycles, acting as carbon and nitrogen reservoirs [9–11] and the fate of metal ions, acting as organic ligands [12,13]. Considering that estuarine salt marshes can be an important source of humic matter, the characterization of the sedimentary humic substances (SHS) of these environments raised the interest of several authors to understand their origin and composition [8,10,14].

With a high potential due to its sensitivity and to its non-destructive character, fluorescence spectroscopy has been largely used for the characterization of humic substances [1,15–19]. The HS can be considered as mixtures of fluorophores with different absorption and emission maxima. In consequence, their molecular fluorescence spectra give information on their structure and composition. However, with high sample concentrations, some fluorophores can be obscured by the absorption phenomena called the "Inner Filter Effects" (IFE) [1,20,21]. The IFE consist of the conjugation of two absorption phenomena [20]. The first one, called primary inner filter effect (PIFE), is an attenuation of the excitation beam before reaching the interrogation region, due to the absorption by chromophores in the solution. The second phenomenon, the secondary inner filter effect (SIFE), results from the absorption of the emitted fluorescence radiation, by other chromophores in solution. As a result of these IFE, the shape of the HS fluorescence spectra can be distorted leading to erroneous interpretations.

Although mathematical expressions for the correction of IFE have been published since 1938 [22,23], the first report where this phenomena and its correction are extensively analysed on fluorescence spectra of humic substances was published by Mobed et al. [1]. These authors applied the mathematical correction of PIFE and SIFE proposed by Tucker et al. [20] to excitation-emission matrix (EEM) spectra of humic substances obtained from the International Humic Substances Society (IHSS). By the comparison between corrected and uncorrected EEM spectra, Mobed et al. [1] showed that at high concentrations it is essential to perform the absorbance correction. Some authors have proposed a DOC concentration of 10 mg C L^{-1} as an upper limit for the concentration of humic substances to obtain fluorescence spectra without significant IFE [24], and this concentration has been used for spectrofluorimetric studies of humic substances without correction of IFE [25,26]. All these studies show results for fluorescence intensities with excitation wavelengths higher than 300 nm.

Nevertheless, there are several studies where solutions with high concentrations of HS still are analysed by fluorescence spectroscopy without any correction of inner filter effects [15,19,27,28]. In fact, there are authors who argue that, in some cases, the IFE do not significantly affect the fluorescence spectra [18,29].

For example, Antunes and Esteves da Silva [18], in their work where the multivariate curve resolution alternating least squares was applied to the EEM spectra of some humic samples, observed inner filter effects at concentrations higher than 30 mg L^{-1}, depending on the fluorophore and/or sample. However, according to the authors, these effects do not cause measurable distortions in the fluorescence spectra of the detected fluorophores, whose excitation wavelengths (λ_{exc}) were higher than 300 nm.

The sedimentary humic substances (SHS) from a salt marsh inside Aveiro Lagoon System were previously analysed by synchronous molecular fluorescence spectroscopy ($\Delta\lambda$ = 60 nm) [10]. In that study, a fluorescence band at λ_{exc} = 280 nm (λ_{em} = 340 nm) was detected in some humic samples from sediments colonized by the halophyte *Halimione portulacoides*. The same fluorescence band was also present in the spectra of sediment pore waters collected from the same salt marsh [30]. This band has an excitation wavelength lower than 300 nm, which was not present in humic substances spectra obtained by Antunes and Esteves da Silva [18]. Besides, considering that this material fluoresces at wavelengths where the other common humic fluorophores strongly absorb [31], the IFE may obscure the presence of this band, giving rise to higher spectral distortions in this type of samples.

The fluorescence band at $\lambda_{exc} \sim 280$ nm/$\lambda_{em} \sim 340$ nm, often referred to as a protein-like fluorescence [31,32], has been detected in several studies where the aquatic and terrestrial organic matter were characterized by molecular fluorescence spectroscopy [33–36]. According to these studies, this fluorescence band is related to the presence of tryptophan (Trp) which is an essential amino acid. This amino acid is ubiquitous in both aquatic and terrestrial environments [32,37,38] and is the proteins constituent with the highest fluorescence emission [33].

Considering that the proteinaceous materials are important sources of nitrogen for humic matter composition [9,39,40], the detection and quantification of Trp moieties may contribute to the knowledge of the humic substances role for the sequestration of sedimentary nitrogen in salt marsh environments. Additionally, as nitrogen containing ligands [31,41], this proteinaceous material can play an important role in humic–metal interactions. Considering these facts, in order to know the real contribution of protein fluorescent materials to the bulk of the different sedimentary humic samples, accurate determination of the fluorescence intensity at the Trp excitation wavelength, without IFE, must be made.

In the studies of Mobed et al. [1] about the IFE in the EEM spectra of reference humic substances and their correction by the procedure proposed by Tucker et al. [20], the lower limits of the wavelengths of these EEM spectra were 300 and 390 nm, for the excitation and emission, respectively. Thus, they did not cover the wavelength region of the protein-like band.

In the present study the inner filter effects in the fluorescence spectra of salt marsh sedimentary humic samples were analysed with special emphasis on the protein-like band. Additionally, the efficiency of two methods for the mathematical correction of the fluorescence spectra was analysed and compared in order to support the choice of the easiest one that effectively assures the IFC of sedimentary humic substances spectra.

Since the difference between λ_{em} and λ_{exc} of the tryptophan band is 60 nm (λ_{exc} = 280 nm, λ_{em} = 340 nm) the protein-like band is visible with clearness in spectra of SHS, if the excitation and emission wavelengths are both scanned simultaneously with a constant difference of 60 nm between them. Thus, we used synchronous fluorescence spectra ($\Delta\lambda$ = 60 nm) for the studies of IFE and their correction.

2. Methods

2.1. Salt marsh humic samples

The experiments were conducted with sedimentary humic substances extracted from a salt marsh situated in "Largo do Laranjo" at the Aveiro Lagoon ("Ria de Aveiro"), Portugal. The sedimentary humic acids (HA) used for the experiments were extracted according to the IHSS method for soil samples [42], with some modifications. Their extraction and purification are described elsewhere [10]. These humic substances were extracted from different depths of sediments. The sediments depths chosen for this study were 0–5 cm and 45–50 cm corresponding, respectively, to the upper layer with fresh vegetal biomass and the deepest layer where the vegetal biomass was scarce.

From the same sediments, fulvic acid (FA) samples were extracted simultaneously with the humic acids. However, while the HA have been purified with a dialysis system [10], the FA purification and desalinization was performed by adsorption onto an XAD-8 adsorbent resin column (pH 2) followed by a protonation through a DOWEX 50W-X8 cationic exchange resin column