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Multivariate curve resolution of multidimensional excitation–emission quenching matrices of a Laurentian soil fulvic acid

Joaquim C.G. Esteves da Silva [a,*], Maria J.C.G. Tavares [a], Romá Tauler [b]

[a] *Centro de Investigação em Química, Departamento de Química, Faculdade de Ciências, Universidade do Porto, R. Campo Alegre 687, 4169-007 Porto, Portugal*

[b] *Department of Environmental Chemistry, Institute of Chemical and Environmental Research, CSIC, Jordi Girona 18-26, 08034, Barcelona, Spain*

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Abstract

Fluorescence excitation–emission matrices (EEM) of aqueous solutions of Laurentian soil fulvic acid (LFA) at three concentrations (50, 75 and 100 mg/l) were obtained at two pH values (pH = 4.0 and 6.0) and as function of the Cu(II) ion concentration. The presence of Cu(II) ion provokes quenching of the intrinsic LFA fluorescence due to complex formation. Multivariate curve resolution (MCR-ALS) was used to successfully decompose single EEM into excitation and emission spectra for the detected components. Moreover, multidimensional (up to six dimensions) data matrices were generated by adding EEM collected as function of the LFA and Cu(II) concentrations and pH. MCR-ALS was able to resolve the excitation and emission spectra from these multidimensional data matrices given further information about the spectral variation profiles induced by the experimental factors. Conditional stability constants ($\log K_{\mathrm{LFACu}}$) were calculated from the quenching profiles observed as function of the Cu(II) concentration, as well as, their trends as function of pH and LFA concentration were obtained – average (and standard deviation) of $\log K_{\mathrm{LFACu}} = 4.6 \pm 0.2$. This EEM/MCR-ALS methodology constitutes a new tool for the study of natural organic matter under varying experimental conditions that characterize natural environmental systems.

Keywords: Fulvic acids; Fluorescence excitation–emission matrices (EEM); Quenching profiles; Cu(II) complexation; Multivariate curve resolution (MCR); Stability constants

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1. Introduction

Fulvic acids (FA) are among the most reactive structures of soil organic matter strongly interacting with metal ions (Stevenson, 1992). Consequently, the speciation of metal ions in soil and natural water is dependent on the quantity and quality of FA. Molecular fluorescence spectroscopy is a particularly useful technique for the study of the chemical equilibrium properties of fluorescent FA molecules because it is a highly sensitive analytical technique, allowing measurements to be made at natural environmental concentrations, and measurements are non-destructive (Saar and Weber, 1980; Ryan and Weber, 1982; Cabaniss and Shumam, 1986; Senesi, 1990; Machado et al., 1994; Casassas et al., 1995; Esteves da Silva and Machado, 1995; Pullin and Cabaniss, 1995; Esteves da Silva et al., 1996, 1998a,b,c; Hall et al., 2005). When paramagnetic metal ions, like for example Cu(II), are brought into contact with FA its fluorescence is quenched as consequence of the formation of stable complex species and quantitative information about the complexation phenomena can be obtained (Ryan and Weber, 1982; Machado et al., 1994; Esteves da Silva et al., 1998c).

Due to the intrinsic complexity of the molecular structure of FA, which are roughly defined as naturally synthesized

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[*] Corresponding author. Tel.: +351 226082869; fax: +351 226082959.
E-mail address: jcsilva@fc.up.pt (J.C.G. Esteves da Silva).

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polyelectrolytes, resulting from a multifaceted humification process, the complexation properties are quite difficult to describe. In these types of quite complex chemical systems, the strategy to overcome some ambiguity in the modeling of experimental data is to acquire multidimensional information. The excitation–emission matrix of fluorescence is suitable to the generation of this multidimensional data structures because molecular fluorescence is particularly sensitive to the chemical environment of the fluorophor (Guilbault, 1977).

This paper presents an experimental methodology that allows the generation of multidimensional EEM quenching data, up to six dimensions, to study the complexation equilibrium between a sample of FA and the Cu(II) ion and determination of the corresponding stability constant. However, in this type of systems only conditional stability constants can be obtained which depend on all the experimental factors, mainly the pH, FA concentration and Cu(II) concentration range (Esteves da Silva et al., 1998b,c). Under environmental conditions these factors naturally show seasonable variations. Nevertheless, if experimental data are acquired as function of these three factors multidimensional data are obtained, and only from their global and simultaneous analysis information about the complexation phenomena under analysis can be obtained that is valid for that range of experimental conditions.

Multivariate curve resolution (MCR-ALS) (Tauler et al., 1998, 1999; Smilde et al., 1999; de Juan and Tauler, 2003; López et al., 2003), which is a constrained interactive alternating least squares procedure, is probably the best chemometric technique for the analysis of these multidimensional data matrices because they don't follow a rigorous multilinear model which would allow the use of other more hard-model decomposition techniques, like for example PARAFAC (JiJi et al., 2000). Indeed, the data matrices under analysis in this work hardly follow a multilinear model, mainly because of the following points: exact Cu(II) concentration and pH values reproduction in different experiments is almost impossible to achieve; the spectra collected as function of the concentration may show some distortions due to filter effects, experimental noise and a high spectral colinearity degree is expected.

This paper presents an EEM/MCR-ALS methodology as new tool for the study of natural organic matter under varying experimental conditions that characterize natural environmental systems. This methodology is applied to the study of the interaction of Cu(II) with one sample of a Laurentian soil FA (LFA) under varying LFA concentration, pH and Cu(II) concentration.

2. Experimental

2.1. Reagents and solutions

The sample of LFA was obtained from Fredriks Research Products (Netherlands). 100, 75 and 50 mg/l solutions were prepared in 0.1 M potassium nitrate. A solution of decarbonated potassium hydroxide was used for adjusting the pH to a constant value and, as titrant, a standard solution of Cu(II) nitrate. The following ranges of total Cu(II) concentrations were covered: pH = 4, 0.005–0.3 mM; pH = 6, 0.001–0.1 mM.

2.2. Instruments

Potentiometric titrations with pH measurement were conducted with a PC-controlled system, assembled with a Crison MicropH 2002 pH-meter, a Crison MicroBU 2030 microburette, a glass electrode (Metler Toledo) and a Ag/AgCl reference electrode. The experiments were done under nitrogen at 25.0 ± 0.2 °C. The cell was calibrated with three buffer solutions with ionic strength adjusted to 0.1 M (Vasconcelos and Machado, 1986).

Fluorescence measurements were made with a Perkin–Elmer LS-50 luminescence spectrometer. After addition of a preset volume of the standard Cu(II) solution followed by pH adjustment, a sample of the equilibrated solution was transferred to a standard fluorescence quartz cell for EEM acquisition. Raw EEM was recorded with the following settings: excitation between 220 and 410 nm (every 10 nm); emission between 300 and 600 nm (every 0.5 nm); 15 nm excitation and emission slits widths; and, scan rate 400 nm min^{-1}. For data analysis only a fraction of the raw EEM was used: excitation wavelength between 220 and 360 nm (15 points); and emission wavelength between 400 and 600 nm (401 points).

2.3. Programs and data structures

The calculations associated to the MCR-ALS method were performed using several programs implemented in MATLAB and obtained from http://www.ub.es/gesq/mcr/mcr.htm.

A basic EEM data structure used in this work is composed by 15 rows (15 excitation wavelengths) and 401 columns (401 emission wavelengths), data matrix $\mathbf{D}(15, 401)$, and contains the fluorescence spectra of the fluorophores present in the FA sample. In this study only the fraction of the raw EEM that do not contain scatter bands was selected. Since only fluorescence is observed in this EEM region (*nc* fluorescent components), the bilinear decomposition of **D** can be described by the following equation:

$$\mathbf{D} = \mathbf{S}^{\text{ex}}\mathbf{S}^{\text{emT}} + \mathbf{E} \tag{1}$$

where $\mathbf{S}^{\text{ex}}(15,nc)$ is the matrix of excitation spectra (each one with 15 wavelengths) and $\mathbf{S}^{\text{em}}(401,nc)$ is the matrix of emission spectra of the detected components, and **E** is the residual matrix describing the variance not explained by ($\mathbf{S}^{\text{ex}}\ \mathbf{S}^{\text{emT}}$). A unique decomposition of matrix **D** into $\mathbf{S}^{\text{ex}}$ and $\mathbf{S}^{\text{em}}$ is not assured due to possible rotational ambiguities but, a solution, with physico-chemical meaning can be obtained by application of appropriate constraints (Casassas et al., 1995; Esteves da Silva and Machado, 1995; Esteves da Silva et al., 1996; Esteves da Silva et al., 1998a,b,c;