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Partial least-squares analysis of time decay data for Eu(III)–tetracycline complexes Simultaneous luminescent determination of tetracycline and oxytetracycline in bovine serum

Gabriela A. Ibañez *

Departamento de Química Analítica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario and Instituto de Química Rosario (IQUIR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Suipacha 531, Rosario S2002LRK, Argentina

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Abstract

A simple and sensitive methodology to simultaneously quantify tetracycline and oxytetracycline in bovine serum samples is described. The method combines the advantages of the lanthanide-sensitized luminescence (i.e., sensitivity and selectivity) with partial least-squares (PLS) analysis, and requires no previous separation steps. Due to the strong overlapping of emission and excitation spectra of the analytes and their europium complexes, the luminescence decay curve (intensity of luminescence vs. time) of analyte–Eu complex was selected to resolve mixtures of tetracycline and oxytetracycline. Partial least-squares uses the luminescence decay as discriminatory parameter and regresses the luminescence versus time onto the concentrations of standards. Using a 16-sample aqueous calibration set, 10 validation samples, 11 spiked serum bovine samples and a blank of serum were studied. The analyte recoveries from serum samples ranged from 87 to 104% for tetracycline and from 94 to 106% for oxytetracycline. The results obtained by the developed method were statistically comparable to those obtained with high performance liquid chromatography.

Keywords: Tetracyclines; Lanthanide-sensitized luminescence; Europium; Partial least-squares; Bovine serum

1. Introduction

Tetracycline (TC) and oxytetracycline (oxy-TC) are antibiotics of the tetracycline group, which possess a wide range of antimicrobial activity against Gram-positive and Gram-negative bacteria. In addition to the employment of tetracyclines (TCs) in humans, there is an ever-increasing use of them for therapeutic veterinary purposes, to maintain the health of animals intended for human consumption, and to enhance the productivity of the farming industry [1,2]. To decrease the resistance in new strains, unnecessary usage of TCs should be minimized. One way of achieving this goal is to decrease their consumption, and this requires monitoring methods for TCs residues in samples such as blood serum, urine, milk, egg and animal tissues [1].

The fluorescent properties of TCs and all of their chelates formed with different metal ions have been extensively studied [2]. On the other hand, it is well established that TCs can be sensitively detected using europium-sensitized luminescence both in solid supports [3,4] and micellar solutions [5–9]. TCs present several coordination sites, being the β-diketone group the main portion which can act as bidentate ligand to form six-membered rings with metal ions such as Eu(III). The stoichiometry of the Eu(III)–TC chelates was reported as 1:1 [6,7]. TCs excited at about 392 nm undergo intersystem crossing to their triplet state, and the associated energy is transferred to the 4*f* level to the europium ion, which yields a characteristic line-type band at about 615 nm. This luminescence signal is proportional to the tetracycline concentration, and this fact has been employed for the direct individual determination of tetracyclines in sev-

* Tel.: +54 341 4372704; fax: +54 341 4372704.
E-mail address: gibanez@fbioyf.unr.edu.ar.

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eral samples (serum, milk, etc.) with a very low detection limit (10 ng mL^{-1}) [5]. The use of a synergistic ligand to form ternary complexes, such as ethylenediaminetetraacetic acid (EDTA) or trioctylphosphine oxide (TOPO) and surfactants such as Triton X-100, cetyltrimethylammonium chloride (CTACl), or dodecylsulfate sodium salt (SDS), allows the sensitivity to increase, due to a luminescence signal enhancement. The synergistic ligand removes water molecules from the coordination sphere of the lanthanide ion, a fact that is deleterious to lanthanide emission. In addition, the micellar environment protects the Eu(III)–TC chelate against non-radiative processes [4,5]. Analytical methods such as high performance liquid chromatography (HPLC) and flow injection analysis (FIA) employing sensitized Eu(III) luminescence as detection system have been reported [10].

Most methods for the determination of TCs use HPLC [11–17], thin layer chromatography [18,19], capillary electrophoresis (CE) [20–22], FIA [23–25] coupled to CE [26], microbiological assay [27–29], immunoassay [30,31], stopped flow mixing [5,32] have also been employed. Other methodologies based on titrimetry [33] and differential-pulse polarography [34] have been described. Spectrofluorometric methods with pre-treatment have been used in the past [35,36] but they involve tedious prior extraction steps. As alternative to these methods, spectrofluorometric techniques combined with multivariate calibration have been proposed [1,2] to individually quantify TCs. Besides separative methods such as chromatography and CE, only few methods have been described for the simultaneous determination of TCs [3,32]. It is noteworthy that methods based on native fluorescence of TCs have not been reported for their simultaneous analysis. An explanation to this fact lies in the strong overlapping between the fluorescence excitation and emission spectra of TC and oxy-TC in aqueous solution, which in principle precludes the application of multivariate calibration of spectroscopic data for the resolution of these mixtures. Even powerful three-way methods based on fluorescence matrices may fail because of the high degree of collinearity between TC and oxy-TC spectra. Cruz Ortiz and co-workers have compared different calibration models (zero, first and second order signal) to individually quantify TCs (TC, chlor-TC or oxy-TC) at a fixed level of the other TCs as interference [2]. On the other hand, the emission spectra of the lanthanide–analyte systems are not useful for analyte resolution, because they correspond to the lanthanide ion. Owing to this fact, we have selected the luminescence decay curve as analytical signal to resolve mixtures of TC and oxy-TC. Recently, a method using photochemically induced fluorescence signals combined with both first- and second-order multivariate calibration have been reported to quantify a mixture of three TCs in surface water samples [37,38].

Multivariate calibration methods are being successfully applied to instrumental data of a variety of sources, mainly spectroscopic, in order to construct predictive models for the determination of mixtures of compounds in several fields [39]. In recent years, multivariate calibration methods have also been applied to the analysis of kinetic [40–42], chromatographic [39,43] and electrochemical data [39]. However, only in few cases these methods have been applied to the analysis of time-resolved data. We can mention the simultaneous determination of human albumin and γ-globulin with 5As-EDTA-Eu(III) complexes employing partial least-squares (PLS) [44]. In a previous work, Alava-Moreno et al. proposed a procedure for analysing mixtures of tetracyclines based on the differences in decay rates by using the Kalman filtering algorithm to process the decay data [3]. It is important to mention that in these previous works the determination of the analytes were not performed in complex matrices. PLS is the most popular regression method for multicomponent analysis due to the performance of its calibration models, the availability of software and the easiness of its implementation [39]. The use of PLS for chemical applications was initiated by Joreskog and Wold [45], and the number of references has increased extensively in recent years [46–48].

PLS is a multivariate regression technique, which has been successfully applied to many analytical systems and shows several important advantages: (1) it employs full spectral data, a feature critical for the resolution of complex multi-analyte mixtures; (2) analytical procedures can be carried out in a short time, usually with no sample clean-up or physical separation; and (3) its calibration models ignore the concentrations of other components except a selected analyte in the studied samples [39]. PLS involves a two-step procedure: (1) calibration, where the relationship between vectorial data such as spectra and reference component concentrations is established from a set of standard samples, and (2) prediction, in which the calibration results are employed to estimate the component concentrations in unknown samples [49]. In the PLS-1 version, all model parameters are optimized for the determination of each analyte at a time. During the model-training step, the calibration data are decomposed by an iterative algorithm, which correlates the data with the calibration concentrations using a so-called 'inverse' model [50]. This provides a set of regression coefficients to be applied to a new sample. Before calibration, however, the optimum number of latent variables should be selected in order to avoid overfitting, by applying the leave-one-out cross-validation method described by Haaland [50] (see Section 3).

In this paper, a simple and sensitive method for the simultaneous determination of TC and oxy-TC in bovine serum samples, combining the advantages of lanthanide-sensitized luminescence and PLS analysis with no previous separation steps, is described. This study is performed on time decay data (intensity of luminescence vs. time) due to the strong overlapping of emission and excitation spectra discussed above. It is important to emphasize that the calibration samples were prepared without serum addition and employing only 16 calibration samples; however the proposed method shows good results even in complex matrices such as bovine serum. With the purpose of validating the developed method, TCs were also quantified by HPLC.

2. Experimental

2.1. Instrumentation and software

For the decay curve measurements, an SLM Aminco Bowman Series 2 luminescence spectrometer, equipped with 7 W Xenon pulse lamp and connected to a PC microcomputer with AB2 software which runs on the OS2 operating system was used.