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Determination of photoirradiated tetracyclines in water by high-performance liquid chromatography with chemiluminescence detection based reaction of rhodamine B with cerium (IV)

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Abstract

A simple, selective and sensitive method has been developed for the simultaneous determination of tetracycline, oxytetracycline, chlorotetracycline, demeclocycline, doxycycline and meclocycline based on reversed-phase high-performance liquid chromatography with chemiluminescence detection. The procedure was based on the chemiluminescent enhancement by photoirradiated tetracyclines of the cerium (IV)–rhodamine B system in sulphuric acid medium. The six tetracyclines were separated on an Aquasil-C18 column with a gradient elution using a mixture of acetonitrile and 0.1 mol L^{-1} phosphate buffer as mobile phase, photoderivatized using a photoreactor consisting of a tube reactor coil of PFA and a 8W Xenon lamp. Under the optimized conditions, the method was validated with respect to linearity, precision, limits of detection and quantification and accuracy. The relative standard deviation (RSD) on intra-day precision was below 10% and detection limits ranged between 0.12 and 0.34 μg L^{-1}. The proposed method has been successfully applied to the determination of tetracyclines in surface water samples. A possible mechanism of the chemiluminescence in the system is discussed.

Keywords: Chemiluminescence detection; Photochemical derivatization; HPLC; Cerium (IV); Rhodamine B; Tetracyclines; Water

1. Introduction

In recent years, concern about the presence of pharmaceutical compounds in the environment, their possible adverse effects on humans and ecological systems, and new strains of resistant bacteria, has considerably increased. After they have been administered, a considerable amount of some of these pharmaceuticals is excreted unmetabolized and can remain in the environment [1].

Tetracyclines (TCs) constitute a group of antibiotics, which are naturally obtained by fermentation with some fungi or by semi-synthetic processes, characterized by a broad spectrum of activity against pathogenic microorganisms, acting by inhibiting the formation of proteins within bacteria. These therapeutic compounds are used to control human and animal bacterial infections and have also found applications in preserving harvested fruit and vegetables, exterminating insect pest and supplementing animal feed [2], in such way that they are frequently used in an indiscriminate manner and ends up contaminating the environment.

Possible pathways that allow antibiotics to reach surface waters have been described by Hirch et al. [3]. According to these authors, after intake, antibiotics are subject to metabolic reactions, but a significant amount of the original substances leave the organism unmetabolized via urine or faeces and would therefore enter raw sewage or manure. In addition, the excreted metabolites can even be transformed back to the original active drugs. The polar antibiotics may not be eliminated effectively in sewage treatment plants, as a large part of elimination is achieved by absorption on activated sludge which is partly mediated through hydrophobic interactions and, hence, one can expect to find antibiotic substances in surface waters. Also, an effective pathway into surface water is via runoff, derived from the dispersion of manure on fields as fertilizer. Thus, residues of TCs

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have been found in concentrations ranging from 0.11 $\mu g\,L^{-1}$ in surface water in the United States [4] to 4.2 $\mu g\,L^{-1}$ in surface waters in Germany [5].

Their widespread use and potential adverse effects in the environment have increased interest in their determination. TCs are determined by a number of spectrophotometric [6,7] and fluorimetric [8–11] methods. In addition, several chemiluminescent (CL) methods [12–17] have been exploited for their determination, as well as capillary electrophoretics [18,19] and mass spectrometry methods [1,20,21].

Chemiluninescence is becoming an attractive technique to be used as detection in LC as its good selectivity, high sensitivity, and simple and inexpensive instrumentation, as well as the rapid time of analysis, render it a highly promising tool in the analysis of organic compounds [22]. Several alternative methods have emerged offering the ability to generate light through simple or more complex chemical reactions. Simple methods include direct light emission after oxidation of the target analytes with a suitable oxidant. The most popular agents, are permanganate, periodate, hydrogen peroxide, hypoclorite anions and tetravalent cerium (IV), which preferably oxidize conjugate double bonds [23].

The Ce(IV)-involved CL reactions with sensitizers have been studied and used for the detection of a number of compounds [24–26]. For example, the CL reaction between Ce(IV) and carbaryl in sulphuric acid medium sensitized by rhodamine 6G has been reported for the determination of carbaryl [27]. Rhodamine compounds, as a series of xanthane dyes, have been applied in spectrophometry and fluorescence, and have also been used as CL reagents [28]. Rhodamine B (RhB) was often thought to be a sensitizer for the CL system, but Ma et al. [28] showed that the oxidation of RhB could produce CL and investigated the behaviour of this CL system in an acidic medium. In this way, RhB was used as a CL reagent in the determination of flavonoids [29], L-ascorbic acid [30] and TCs [31] by flow-injection CL detection method.

On the other hand, it has been established that the irradiation of photoreactive analytes leads to the formation of species that can be detected providing very sensitive procedures [32], but no works dealing with photodegradation and CL detection have been published in determining antibiotics. This process, has however been widely used for the determination of pesticides [33–35]. For analytical purposes, photochemical derivatization is extremely useful because of its selectivity and sensitivity and many of these reactions have been adapted as post-column detection systems in HPLC [36–42]. The main advantages of the post-column derivatization are that the analytes are separated in their original form, without the need for a complete derivatization reaction (assuming reproducibility) and the reaction products need no stability for a long period of time [43].

The goal of our study was to check the usefulness of the CL reaction of Ce(IV)–RhB system for the detection of TCs antibiotics. We found that after irradiation with UV light the TCs produced a great enhancement on the CL emission from the RhB oxidation by Ce(IV) in sulphuric medium. This enhancement in the CL emission is proportional to the concentration of the selected compounds, which can be determined by measuring the increase in the CL intensity. Based on these findings, a new HPLC-CL method has been developed for the sensitive determination of TCs, which has been satisfactorily applied in surface water samples.

2. Experimental

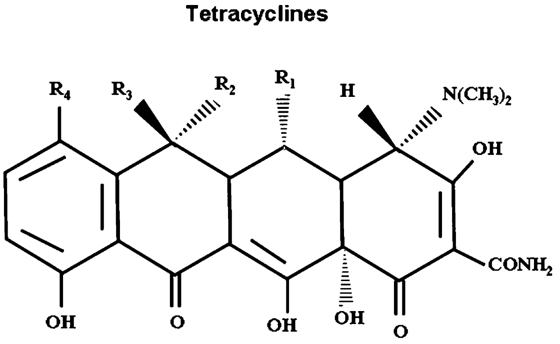
2.1. Chemical and solvents

Analytical standards (pestanal quality) of tetracycline hydrochloride (TTC, 97.3%), oxytetracycline hydrochloride (OTC, 96.2%), chlorotetracycline hydrochloride (CTC, 85.7%) and demeclocycline hydrochloride hemydrate (DMC, 92.8%) were obtained from Riedel-de Haën (Germany); doxycycline hyclate (DC, 98%) was obtained from Fluka (Switzerland) and meclocycline sulfosalicylate salt (MCC, 98%) was obtained from Sigma–Aldrich (Germany). Fig. 1 shows the structural formulas of the six tetracyclines.

Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were obtained from J.T. Baker (Holland). Ortho phosphoric acid (H_3PO_4, 85%), sulphuric acid (H_2SO_4, 96%), hydrochloric acid (HCl, 38%) and ethylenediaminetraacetic acid disodium salt-2hydrate (Na_2-EDTA) were obtained from Panreac (Spain) and potassium dihydrogen phosphate (KH_2PO_4) and rhodamine B *N*,*N*,*N*′*N*′-tetraacetylrhodamine chlorohydrate (RhB) for analysis were obtained from Merck (Germany). Cerium (IV) sulphate tetrahydrate ($Ce(SO_4)_2 \cdot 4H_2O$) was supplied by Riedel-de Haën (Germany).

Ultra pure water, obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA), was used. Mobile phases were filtered through a 0.45 μm cellulose acetate (water) or polytetrafluoroethylene (PTFE) (organic solvents) and degassed with helium prior to and during use.

The solid phase pre-concentration (SPE) of water samples was carried out using Oasis HLB (hydrophilic lipophilic bal-



	R_1	R_2	R_3	R_4
Oxytetracycline (OTC)	OH	CH_3	OH	H
Tetracycline (TC)	H	CH_3	OH	H
Chlortetracycline (CTC)	H	CH_3	OH	Cl
Demeclocycline (DMC)	H	OH	H	Cl
Doxycycline (DC)	OH	CH_3	H	H
Meclocycline (MCC)	OH	-	CH_2	Cl

Fig. 1. Structural formulas of the six tetracycline antibiotics studied.