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Analysis of bisphenol A in natural waters by means of an optical immunosensor

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Abstract

This work describes a very simple, fast and sensitive method based on the use of the optical immunosensor "RIver ANAlyser" (RIANA) to the determination of bisphenol A in a waters. RIANA is based on a rapid solid-phase indirect inhibition immunoassay that takes place at an optical transducer chip chemically modified with an analyte derivative. Fluorescence produced by labelled antibodies bound to the transducer is detected by photodiodes and can be correlated with the analyte concentration. The sensor surface can be regenerated thus allowing the performance of several measurements (around 300) with the same transducer. Each test cycle, including one regeneration step, is accomplished in 15 min. The detection limit achieved in the direct determination of bisphenol A in water with this system was 0.014 μg/L. Satisfactory repeatability, with relative standard deviations (RSD) ranging between 1.48% and 6.93% were obtained. The immunosensor method developed was applied to the monitoring of bisphenol A in various types of water collected in a waterworks (from the river water source to the finished drinking water) and validated against the results obtained in the same approach by a more traditional method, based on solid-phase extraction followed by liquid chromatography–mass spectrometry. Results obtained by both techniques were in general good agreement (considering the typical overestimation bias of immunoassays), and served to prove the satisfactory removal efficiency of the overall purification process applied in the waterworks and, in particular, of the sand filtration step.

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1. Introduction

Bisphenol A (4,4′-isopropylidenediphenol) is a typical product of the industrial society produced in large quantities worldwide, 90% or more being used as a monomer for the production of polycarbonate, epoxy resins, and unsaturated polyester-styrene resins. The final products are used as coatings on cans, as powder paints, as additives in thermal paper, in dental fillings, and as antioxidants in plastics (Staples et al., 1998). It has also been used as an inert ingredient in pesticides, as a fungicide, antioxidant, as flame retardant, rubber chemical and polyvinyl chloride stabilizer. Human exposure to bisphenol A eventually released from these materials is of increasing concern due to its endocrine-disrupting potential. In fact, BPA was one of the first chemicals discovered to mimic estrogens. In 1936, bisphenol A was found to stimulate growth of the rodent uterus, an indication of estrogenic action (Dodds and Lawson, 1936). The estrogenic effect of bisphenol A has been shown both in in vitro and in vivo experiments (Rehmann et al., 1999; Christiansen et al., 2000; Mehmood et al., 2000; Suzuki and Hattori 2003). This substance can enter the environment through

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leaching from final products and during manufacturing processes (Fromme et al., 2002). Data shows that the current release of bisphenol A into streams and rivers occurs mainly via discharges of sewage treatment plants (STPs), which have led to elevated levels in both, surface waters and sediments (Bolz et al., 2001). Bisphenol A may decompose microbially in water, with a half-life of between 2.5 and 4 days (Staples et al., 1998). However, persistence and bioaccumulation of bisphenol A has also been reported. Schonfelder et al. measured bisphenol A in the blood of pregnant women, in umbilical blood at birth and in placental tissue, and all samples examined were found to contain bisphenol A at levels within the range shown to alter development (Schonfelder et al., 2002). the determination of bisphenol A, although weakly estrogenic —approximately 10000 fold less potent than 17βestradiol (Rubin et al., 2001; Gaido et al., 1997)—, is very important due to its extensive use and environmental ubiquity. Of particular concern are the levels found for this compound in river waters, quite high already in some of them (up to 0.4 μg/L) and expected to only increase as a consequence of their widespread application (Bolz et al., 2001; Shin et al., 2001; Fromme et al., 2002; Heemken et al., 2001).

The methods most frequently used for the determination of bisphenol A in environmental water are gas chromatography (GC) (Kawaguchi et al., 2004; Liu et al., 2004) and liquid chromatography (LC) (Shin et al., 2001; Watabe et al., 2004), both coupled to mass spectrometry (MS). These methods are highly sensitive and specific, but require time-consuming pre-treatment steps and thus do not allow rapid processing of multiple samples. In recent years, different bioassays have been developed as a cheap and quick alternative to traditional chromatographic methods for high-throughput analysis or real-time monitoring. Some of these bioassays have been applied to the determination of the potential biological impact of bisphenol A through methods based on the measurement of effects, such as toxicity (Kim et al., 2002) or estrogenicity (Usami et al., 2002). Other type of bioassays for environmental monitoring aim at the detection of bisphenol A based on its specific recognition by a certain biomolecule. Examples of this are the fully automated immunoassay systems that use monoclonal antibodies chemically conjugated to bacterial magnetic particles (BMPs) (Matsunaga et al., 2003) or the competitive immunoassays based on surface plasmon resonance (SPR) (Soh et al., 2003). The application of various enzyme linked immunosorbent assays (ELISA) for the determination of bisphenol A in environmental and biological samples has also been reported (Zhao et al., 2002; De Meulenaer et al., 2002). Lately, immunosensors have appeared as new biological analytical tools complying with the current requirements for the analysis of micropollutants in the environment. Immunosensors incorporate either antigens or derived antigens or antibodies, intimately associated with or integrated within a physico-chemical transducer, which, in contrast to immunoassays, is permanently fixed in the construction of the device. Immunosensors offer a broad number of advantages such as high specificity, low detection limits, rapidity, potential portability, simplicity, low cost and versatility. In this work, an optical immunosensor based on total internal reflection fluorescence has been applied to the determination of bisphenol A in different water samples. This optical immunosensor was designed a few years ago and although its performance for the determination of bisphenol A in water has been recently evaluated (Tschmelak et al., 2004), this is, to the best of our knowledge, the first work reporting its application to the monitoring of bisphenol A in natural and treated waters from a drinking water treatment plant. Validation of results with chromatographic methods was also fulfilled. This article follows previous works of our group in which the RIANA was applied to the determination of the pesticides atrazine, simazine, paraquat, alachlor, 2,4-dichlorphenoxyacetic acid (2,4-D) and isoproturon, and the estrogen estrone (Mallat et al., 1999, 2001a, b, c; Rodriguez-Mozaz et al., 2004).

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Potassium hydroxide, potassium dihydrogen phosphate, sodium chloride, hydrochloric acid, sodium dodecyl sulphate (SDS) and HPLC-grade water, acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Fluorescent Cy5.5-dye and bifunctional reactive N-hydroxysuccinimide (NHS) ester were obtained from Amersham Life Science (Braunschweig, Germany). Di-isopropylcarbodiimide (DIC) and Ovalbumin (OVA) were purchased from Sigma (Deisenhofen, Germany). Bisphenol A standard was obtained from Riedel-de-Haen (Seelze, FRG). Lyophilised polyclonal anti-bisphenol A was kindly supplied by Dr. Ram Abuknesha (King's College London, UK). Labelling of the antibodies with Cy5.5 was performed according to the method described by Southwick et al. (1990).

Phosphate buffered saline (PBS) was prepared in MilliQ water at a concentration of 150 mM sodium chloride and 10 mM mono-potassium phosphate, and adjusted with a potassium hydroxide solution to pH 7.4. A stock standard solution of bisphenol A was prepared in methanol at 1000 mg/L. Two sets of working standard solutions were then prepared at various concentrations by appropriate dilution of the stock solution with both, methanol and PBS for LC–MS and RIANA determination, respectively.

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