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Amperometric immunosensor for nonylphenol determination based on peroxidase indicating reaction

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Abstract

Novel immunosensor for nonylphenol (NP) determination has been developed by immobilization of specific antibodies together with horseradish peroxidase on the surface of carbon screen-printed electrode. The signal of the immunosensor is generated by the involvement of NP accumulated in the peroxidase oxidation of mediator (Methylene Blue, hydroquinone or iodide). This results in the increase of the signal recorded by linear-sweep voltammetry. The sensitivity of the detection depends on the nature of mediator, its concentration and incubation period. Cross-selectivity of the response toward readily oxidized phenolic compounds has been determined. The immunosensor developed makes it possible to detect from 20 $\mu g\,L^{-1}$ to 44 $mg\,L^{-1}$ of NP with detection limit 10 $\mu g\,L^{-1}$ of NP.

Keywords: Immunosensor; Nonylphenol determination; Screen-printed electrodes

1. Introduction

Alkylphenol ethoxylates and essentially nonylphenol (NP) derivatives are widely used as non-ionic surfactants in the formulations of detergents, textiles, paints, petroleum additives, etc. (Renner, 1997). They are present as plasticizers in polycarbonate and epoxy resins, PVC and can contaminate food when released from packaging (Inoue et al., 2001). In the environment, ethoxylated NP isomers biodegrade via shortening the oxyethylene chain and form more lipophilic and hence more toxic compounds (Yoshimura, 1986). The final product of degradation, i.e. NP, is considered as most dangerous because of its enhanced resistance in the environment and its toxicity. The NP used in industry contains about 90% of the 4-NP and other isomers with straight and branched side chain. Symptoms of the NP exposure include eye and skin irritation, headaches, nausea and vomiting (Cox, 1996). Besides, the estrogen disruption effect of the NP has been highlighted in the past decade (Schwaiger et al., 2002; Gomes et al., 2003). It mimics natural estrogens and causes reproductive abnormalities in natural populations (Kledal et al., 2000). Although NP undergoes microbial and enzymatic degradation, the efficiency of these processes in conventional water treatment is insufficient (Sakuyama et al., 2003; Tanaka et al., 2000, 2001). As a result, the NP residues were found in the sediments formed in water treatment (Renner, 1997; Yoshimura, 1986; Cox, 1996; Gomes et al., 2003). For these reasons, it is necessary to develop fast and cost-effective methods for nonylphenol monitoring.

Various biological and chemical approaches have been suggested for the detection and quantification of NP and related compounds. Biological methodologies are based on the in vitro discovery of endocrine disruption effects and often do not imply the identification of a pollutant (Gomes et al., 2003). Thus, a human recombinant estrogen receptor was used in the competitive assay mode to detect down to 7.49 $\mu mol\,L^{-1}$ (1.65 $mg\,L^{-1}$) of 4-NP (Usami et al., 2002). The non-specific detection of endocrine disruptors was accomplished with amperometric tyrosinase sensor. The limits of detection were 1–23 $\mu mol\,L^{-1}$, and that of NP was 10 $\mu mol\,L^{-1}$ (2.2 $mg\,L^{-1}$) (Dempsey et al., 2004).

Chemical approaches to the determination of NP and other alkylphenols involve their extraction coupled with the GC– or HPLC–MS detection. The appropriate limits of detection are of about 10–1000 $ng\,L^{-1}$ (Di Corcia et al., 1994; Crescenzi et al., 1995; Inoue et al., 2002; Penalver et al., 2002). However,

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