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Gel-based immunoassay for non-instrumental detection of pyrene in water samples

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Abstract

A new qualitative immunologically based tube test for non-instrumental detection of pyrene (PYR) in water samples was developed. The method combines the pre-concentration of analyte by immunoextraction and its detection by immunoassay using Sepharose 4B-immobilized IgG-fraction of a polyclonal anti-PYR antiserum (immunoaffinity gel) and 1-pyrenebutyric acid-horseradish peroxidase conjugate (PYR-BA-HRP). The immunoaffinity gel was placed in a standard 1-ml SPE column through which a 10-ml aliquot of water sample spiked with 10% acetonitrile was passed. Following, free antibody binding sites were detected by application of PYR-BA-HRP. Four minutes after addition of the chromogenic substrate the results were visually evaluated by occurring or stayed away blue colour development for negative and positive samples, respectively. Total time for assay was about 15 min for six samples. Under optimized conditions a cut-off level for pyrene of 0.04 ng ml^{-1} was found. At this defined concentration, a set of spiked samples ($n = 175$) was analyzed and very low rates of false negatives (1.2%) and false positives (4.6%) determined which fulfils the requirement set by Commission Decision 2002/657/EC for a screening method. No interference by other PAH compounds like naphthalene, fluoranthene, phenanthrene, anthracene, and benzo[*a*]pyrene at a concentration of 20 ng ml^{-1}, i.e., 500-fold excess compared to the defined cut-off level was observed. Different water types like surface water, tap water, bottled water, and melted snow were analyzed for PYR contamination by the proposed method and results confirmed by HPLC-FLD.

Keywords: Enzyme immunoassay; Immunoaffinity column; On-site method; Pyrene; Screening; Non-instrumental test; Visual detection

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds consisting of two or more condensed aromatic rings. PAHs and derivatives are mainly formed during incomplete combustion of organic material arising, in part, from natural combustion such as fires and volcanic eruptions, but for most part from emissions of anthropogenic activities such as industrial manufacturing processes, the incinerating of solid waste, heating, automobile exhaust, but also cooking and tobacco smoke. PAHs have generated considerable interest because of their toxicity and carcinogenicity potential. The most toxic members of this family known to-date are PAH molecules that have four to seven rings. These pollutants have a high persistence in the environment, low biodegradability and high lipophilicity. Natural waters can be heavily polluted with PAHs, which are either dissolved in water or adsorbed on colloids, depending on individual solubility. First of all, washout from the atmosphere by precipitation or runoff from streets and other surfaces are pathways bringing these chemicals into rivers and lakes [1]. In addition, oil and oil products can pollute waters accidentally. PAH compounds can also be found in groundwater, but usually at very low concentrations. As a result of their widespread presence, priority PAHs

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were included in different Directives of the European Union and U.S. EPA. Pyrene constitutes one of the priority PAHs [2].

Generally, GC and HPLC are used for PAHs determination but these assays require pre-concentration of analyte, create large amounts of solvent waste, and are relatively time-consuming and difficult to perform on-site. As an alternative for the detection of PAHs in aqueous matrices non-invasive simpler methods were suggested, e.g., front-face fluorimetry on a solid sorbent and partial-least-square treatment [3]. In contrast, immunoassays are readily adapted to on-site screening. Commonly ELISA was used for PAH screening in different sample types such as surface water, potable water, sea water, sediments, and soil [4–7]. In addition, piezoelectric, electrochemical and optical immunosensors and fluorescent polarisation immunoassay were developed [8–13]. To our knowledge no non-instrumental tests were described for PAHs screening, probably due to problems in providing required sensitivity because of the very low concentration of PAHs in environmental samples. This study aimed at the development of a rapid qualitative non-instrumental immunochemical test which can be performed outside the laboratory for the screening of pyrene in water samples.

2. Experimental

2.1. Materials

Pyrene (PYR), naphthalene (NAPH), anthracene (ANT), phenanthrene (PHE), fluoranthene (FLA), benzo[*a*]pyrene (BAP), 1-pyrenebutyric acid (PYR-BA), Tween 20 and horseradish peroxidase (HRP) were purchased from Sigma (Bornem, Belgium). CNBr-activated Sepharose 4B (Sepharose) was obtained from Amersham Biosciences AB (Uppsala, Sweden). The substrate chromogenic solution used was Colorburst^{TM}Blue TMB/Peroxide (ALerCHEK, Inc., Portland, ME, USA). All other chemicals and solvents were of analytical grade; doubly distilled water was used throughout. Tubes (Bond Elut reservoir, 1 ml) and polyethylene frits (1/4 in. diameter) were supplied by Varian Belgium NV/SA (Sint-Katelijne-Waver, Belgium). Phosphate-buffered saline (PBS) 0.01 M, pH 7.4, was used as assay buffer for the gel-based immunoassay. Proclin 300 (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) was purchased from Supelco (Bellefonte, PA, USA) and was added to PBS as an antimicrobial preservative. PBS with 0.05% Tween 20 (PBS–Tween) was used as wash solution. Diapak C16M columns for solid-phase extraction (SPE) were supplied by BioChimMak (Moscow, Russia). Stock solutions of PAHs (100 μg ml^{-1}) were prepared in acetonitrile (ACN) and diluted in ACN to give standard solutions in the range of 0.01 ng ml^{-1} to 10 μg ml^{-1}.

2.2. Immunoreagents

The polyclonal rabbit antiserum 16.89 was obtained as previously described and IgG-fraction segregated by ammonium sulphate precipitation [14]. The pyrene derivative PYR-BA, which was used for the preparation of the hapten-KLH (keyhole limpet hemocyanine) conjugate (immunogen), was used for synthesis of the enzyme conjugate accordingly [14] (Fig. 1). In brief, 34.5 mg (300 μmol) of *N*-hydroxysuccinimide and 61 mg (300 μmol) of dicyclohexylcarbodiimide were dissolved in 1.5 ml of dimethylformamide. 0.5 ml of this solution was added dropwise under stirring to a solution of PYR-BA (28.8 mg, 100 μmol) in 500 μl of dimethylformamide. The reaction mixture was kept overnight at RT. Next day a small aliquot of activated hapten (5 μl) was added to a solution of 4 mg (0.1 μmol) of HRP in 2 ml of 0.05 M carbonate buffer (pH 9.6). The molar ratio of enzyme and hapten was 1:5. The reaction mixture was kept 1 h at RT followed by overnight incubation at 4 °C. The conjugate PYR-BA-HRP was purified by dialysis in distilled water (3 l) during 4 days with 2 times water change per day. It was stored at a concentration of 0.2 mg ml^{-1} in 50% glycerol at 4 °C.

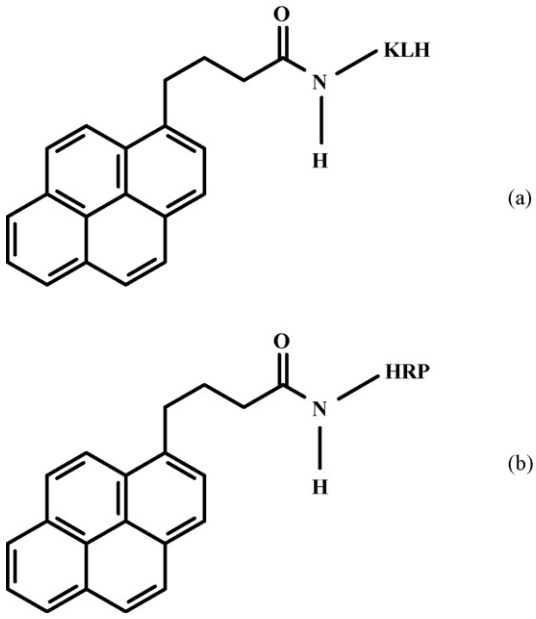

Fig. 1. Chemical structures of the immunogen (a) and conjugate PYR-BA-HRP (b).

2.3. Immunoaffinity gel preparation

The immunoaffinity gel was prepared as a mixture of coupled gel (Sepharose with bound rabbit anti-pyrene antibodies, diluted with PBS 1:3) and blocked gel (Sepharose with glycine blocked active groups, diluted with PBS 1:3). For coupled gel preparation 0.5 g of freeze-dried Sepharose (gave about 1.7 ml final gel volume) was washed on a sintered glass filter using 100 ml of 1 mM HCl. Then, 150 μl of the purified IgG-fraction and 50 μl PBS were added and this suspension was shaken for 2 h at RT. After incubation, the gel was washed with 5 ml of $NaHCO_3$ buffer, 0.1 M, pH 8.3 containing 0.5 M NaCl to remove excess antibody. For blocked gel preparation 2.5 g of Sepharose (gave about 8.5 ml final gel volume) was washed using 500 ml 1 mM HCl. For blocking of active groups five gel volumes of blocking agent (0.2 M glycine, pH 8.0) were added to both gels (cou-