Antibody-based Chemical Sensor Development at VIMS

There is a need for “real-time” analysis of toxic chemicals in the environment

Current technology focuses on physical conditions and inorganic chemicals

Potential uses:
Pollution Monitoring
Public Health
Homeland Defense
Industrial Processes/Outfalls
Interdisciplinary Effort of Immunologists & Environmental Chemists

The laboratories of Dr. Steve Kaattari and Dr. Mike Unger

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We need a method that is REMOTE, RAPID, and RELIABLE.

Environmental samples are extremely complex: 100,000’s of compounds.

The “Black Box”

VIMS Research Focus

Compound specific monoclonal antibodies coupled to electronic detection

Progress?

Electronic output of compatible data.
Antibody-based Chemical Sensor Development at VIMS

Biosensors and Bioelectronics
Published May 2007

Demonstration of the VIMS antibody/Sapidyne Inline as a viable technique to the sensor community

The development of a real-time biosensor for the detection of trace levels of trinitrotoluene (TNT) in aquatic environments

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Abstract

This paper describes the development of a highly sensitive TNT immunosensor consisting of a highly specific monoclonal antibody coupled with a portable fluorescence-based detection system (Sapidyne Inline Biosensor, Sapidyne Inc.). The antibody, which possesses a high affinity for TNT (association constant, K = 9.2) with minimal cross-reactivity with other compounds (as small as 0.2% relative to TNT and 2.3% relative to pentaerythritol tetranitrate), was conjugated to a fluorescein-based detector. This system provides sample consumption within 100 s from sample aspiration and sensitivity greater than 0.015 µg L⁻¹ in standard samples. The sensor was designed to allow a maximum of 100 repeated readings, and has an accuracy near 0.1–0.5% between repeated readings. The design and software allow the sensor to be obtained from up to nine different control stations allowing the user to examine the concentration of the pollutants in the water column. We believe that this immunosensor can be used to rapidly assess trace levels of TNT in environmental water samples.

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Keywords: TNT; Monoclonal antibody; Fluorescence

1. Introduction

The introduction of toxic pollutants into coastal waters can have a dramatic impact on the health of aquatic ecosystems (Collie and Leahy, 1990; Pate et al., 1999). Accidental releases of chemicals into aquatic systems, such as those occurring without explicit use of highly visible and permanent control can be difficult to immediately monitor and contain the spread of the spill. However, most chemicals have a biological impact on aquatic species at concentrations lower than the visually observed (Anderson et al., 1997). Their ability to rapidly detect and quantify trace concentrations of pollutants in aquatic systems has long been the goal of marine managers to protect these valuable ecosystems. The focus of this project was the development and assessment of a biosensor to detect the environmental pollutant trinitrotoluene (TNT) in aquatic systems. Environmental contamination with TNT has occurred particularly in military sites throughout the United States from the manufacturing, storage and handling of munitions. The expected concentration of TNT in situ sites can be as high as 3,000 µg L⁻¹. This contamination occurs through atmospheric deposition or ground water inflows, resulting in the accumulation of TNT in aquatic sediments (Baliga et al., 1999). TNT contamination has been demonstrated to have detrimental effects on both invertebrates (Conder et al., 2006) and fish (CNP, 2005), and has been shown to accumulate in plants and fish (Baliga et al., 2005; Dudley et al., 2005).

Although numerous techniques have been described for the assessment of TNT pollution (Relleau et al., 2000; Green et al., 2000; Hoffman et al., 2003), the standard method employs high performance liquid chromatography (HPLC) coupled with TNT detection (RPA, model 8300, www.rpa.com/ HP- HPLC.html). This technique allows the quantification of TNT and its breakdown products to low ng L⁻¹ levels, however, while the technique is suitable in general, it is expensive, laborious and incapable of real or near-time assessment of low concentrations. More recently, the use of immunochromatographic methods has gained widespread acceptance for monitoring environmental pollutants (for review see Margarita and Ball, 2006), including TNT.

TNT Measurement Method Comparison

\[ y = 1.1164x + 0.9359 \]

\[ R^2 = 0.9849 \]

Antibody (µg/L)

HPLC (µg/L)
Environmental Science and Technology
(in press for 2007)

Demonstrate the utility with field samples
Exposure for our biosensor development to the environmental monitoring community

Antibody-based Chemical Sensor Development at VIMS

Table: TNT in Field Samples

<table>
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The Naval Weapons Station (NWSW) in Virginia, the USA, has been subject to considerable contamination efforts, performed by the US military to clean up the area of environmental, biological, and chemical contaminants. The ongoing remediation efforts require continual monitoring of soil, sediment, surface water, and groundwater for the presence of these contaminants. In this project, public health monitoring was performed by the Naval Weapons Station and Defense Environmental Project (DEP) from 1990 to 1995. TNT was detected in the field in soil and water samples as high as 10 mg/l. Soil with the same composition, similar to the average composition of the monitoring sites, was exposed to high-pressure liquid chromatography with ultraviolet detection (HPLC), mass spectrometry, and antibodies that can detect the presence of TNT in field samples. To demonstrate the potential of our biosensor, field samples were collected from the area of contamination and exposed to the antibodies that can detect the presence of TNT in field samples.
Disclosure Statements filed for antibodies to detect TNT and biphenyl in environmental samples

Next step will be disclosure and publication on an antibody to detect thiophenes (oil)
Antibody-based Chemical Sensor Development at VIMS

Next Steps:

NOAA CICEET funded research
Sept 2007-2009
Bromage, U Mass
Kaattari & Unger, VIMS

Objectives

The overall goal of this proposal is to validate a new field deployable sensor technology for differentiating and quantifying specific petrogenic PAH’s from pyrogenic PAHs in aquatic systems. This will provide water quality managers, as well as environmental monitoring and compliance officer’s access to real-time data, allowing knowledge-based decisions.
Sensor Development & Validation: Basic and Applied Research

Develop New Antibodies

Using real time chemical analyses to answer difficult questions for science and environmental management

Contaminant Sources
- Oil, Creosote
- Groundwater
- Stormwater Runoff

Potential Study Sites
- Elizabeth River, Southern Branch
- Atlantic Wood Industry (AW)
- Paradise Creek (PC)
- Channel Marker 2 (CM)
- Refueling Station (RS)
- Crown Tank Farm (CF)
- Scuffletown Creek (SC)
- Jones Creek (JC)

PAH in Sediment

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14 Years of Restoration

Elizabeth River Project