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NEAR REAL-TIME, ON-SITE, QUANTITATIVE ANALYSIS OF PAHS IN THE AQUEOUS ENVIRONMENT USING AN ANTIBODY-BASED BIOSENSOR

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Abstract—Rapid, on-site, quantitative assessments of dissolved polycyclic aromatic hydrocarbons (PAHs) were demonstrated for two field applications. The platform, a KinExA Inline Sensor (Sapidyne Instruments), employed the monoclonal anti-PAH antibody, 7B2.3, which has specificity for 3- to 5-ring PAHs. A spatial study was conducted near a dredging site where contaminated sediments were being removed, and a temporal study was performed during a rainfall event. Most importantly, the generation of near real-time data guided management decisions in the field and determined proper sampling protocols for conventional analyses. The method was able to determine PAH concentrations as low as 0.3 μg/L, within 10 min of sample acquisition, and to assess 80+ samples (not including standards and blanks) in less than 3 d. These results were compared with a laboratory-based gas chromatography–mass spectrometry method in which a wide array of PAHs, including alkylated homologs, were examined. This system shows great promise as a field instrument for the rapid monitoring of PAH pollution. Environ. Toxicol. Chem. 2011;30:1557–1563. © 2011 SETAC

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are natural constituents of petrochemical products or are produced from the incomplete combustion of organic materials. They are usually found in environmental matrices as complex mixtures [1]. The U.S. Environmental Protection Agency (U.S. EPA) has identified 17 PAHs as priority pollutants because of their high toxicity and suspected carcinogenicity [2]. Polycyclic aromatic hydrocarbons enter the environment as a result of natural oil seeps, oil spills, atmospheric deposition, and industrial effluents, among many other means [2]. Moreover, PAHs can be mobilized from land during storm events, transporting them into the aquatic environment, and causing potentially harmful effects on drinking water, recreation, fisheries, and wildlife [3]. Real-time detection would greatly facilitate PAH source detection as well as relative loads accruing during environmental dispersion. Such capabilities can aid in effective mitigation and remediation efforts.

Traditional detection and quantification of low-level PAHs are performed in a laboratory and involve solvent extraction, fractionation, and analysis by high-performance liquid chromatography or gas chromatography (GC) using a variety of detectors [4]. All of these methods are time-consuming, labor-intensive, and costly. Efforts to improve on this technology have become a priority for environmental research and monitoring [5–7]. This is exemplified by the number of newly developed, field-portable technologies such as thin layer chromatography [8], synchronous luminescence spectrofluorometry [9,10], and portable GC-mass spectrometry (MS) [11,12]. Unfortunately, the requirement for solvent extraction and concentration of the samples preclude their widespread use. The development of an inexpensive, easy to use tool would be a significant advancement in this field.

Immunoassays are currently being developed as alternative means for environmental PAH assessments [5–7,13,14]. The advantages of immunoassays are that they can be easy to use, accurate, portable, fast, and cost-efficient. Immunoassays rely on antibody molecules, which exhibit excellent sensitivities, so that low-level detection can be accomplished using aqueous samples without extraction or pretreatment. Although antibodies to biological macromolecules are valued for their exquisite specificity, antibodies to PAHs tend to recognize groups of structurally similar PAHs and related compounds [15]. For this reason, and because PAHs are often found in environmental samples as complex mixtures, immunoassays are best used for determining total PAH concentrations, or a defined subset of PAHs.

The U.S. EPA has adopted an immunoassay test kit (PAH RaPID Assay®; SDIX [16]) for total PAHs in soil (SW-846 Method 4035). More than a decade ago, this kit was evaluated for use with river water samples and was found to be suitable only as a screening method, because it gives 10- to 100-fold higher concentrations than GC-MS assays [17]. Numerous other immunoassay formats are now being developed for the quantitative analysis of PAHs in aqueous environmental samples. Examples include enzyme-linked immunosorbent assays [18,19], amperometric biosensors [20], piezoelectric biosensors [21], surface plasmon resonance biosensors [22,23], and polarized fluorescence biosensors [24].

While most of these immunoassays/biosensors have comparable detection limits (low to sub-ppb [μg/l] range), many have been reported only as a proof of concept. Most studies used natural water samples fortified with a single PAH analyte to test their methods, while only enzyme-linked immunosorbent assay (ELISA)-based methods have used PAH contaminated water [17–19]. Furthermore, in these cases the analysis of contaminated water has fallen short by limiting validation to a subset of unsubstituted PAHs (typically the 16 U.S. EPA priority pollutants) or to a single PAH. Even though portability is often emphasized as an advantage of immunoassays, rarely have the analyses been conducted on site [7].

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Immunoassay speed is dictated by the time required for antibody/antigen (i.e., analyte) equilibrium to occur. Incubation typically requires 30 to 60 min; however, often antibody kinetics are not considered in order to optimize timing. Two surface plasmon resonance immunoassays report a minimum response time of 15 min from sample input to data output; one requires an additional few minutes for sensor regeneration [23] and the other requires 30 min for antibody incubation [22]. A piezoelectric immunosensor for benzo[*a*]pyrene requires 30 min for benzo[*a*]pyrene incubation for competitive displacement of the preadsorbed antibodies and then approximately 5 min for a steady-state response [21]. Although many methods are being developed with the promise of quantitative monitoring of PAHs, none have been exploited for their rapid on-site assessment capabilities.

Immunoassays in the field are susceptible to the effects of extreme temperature on assay performance. The only immunoassays to report ideal operating temperature ranges are those developed for the analysis of PAHs in soil. The RaPID PAH ELISA (now from SDIX) requires the reagents to be within 18 to 27°C [16]. It is speculated by Waters et al. that this same ELISA could adequately operate in the range of 10 to 30°C [25]. The PAH RIS (from Ensys) is reported to perform correctly between ambient temperatures of 4 and 32°C [26].

The primary aim of the present study was to evaluate a new antibody-based biosensor that recognizes PAHs in aqueous environmental samples. A second aim was to exploit the biosensor's ability to generate near real-time, quantitative data to determine which samples should be collected for more extensive laboratory analysis. This is, to our knowledge, the first use of an antibody-based biosensor to guide sampling efforts through near real-time evaluation of environmental contamination.

MATERIALS AND METHODS

Biosensor assay design

Features and principles of the KinExA Inline Sensor (Sapidyne Instruments) have been described elsewhere [27–30]. An attached laptop computer provided instrumental control, graphic signal output, and data storage. The immobilized antigen dibenzothiophene-acrylic acid-bovine serum albumin [31] was coated onto polymethylmethacrylate beads (Sapidyne) following previously described methods [32]. The monoclonal antibody, 7B2.3, specific for three- to five-ring PAHs [31], was coupled to the fluorescent dye, AlexaFluor 647 (Invitrogen), following the manufacturer's protocol. An antibody concentration of 0.38 μg/ml resulted in a voltage change of approximately 1 V.

The speed at which an antibody comes to equilibrium in a sample is determined by the antibody's on and off rates. The antibody employed in the present study, monoclonal antibody 7B2.3 [31], was subjected to kinetics analysis as performed by the K_d and the Kinetics Direct methods employing the KinExA®3000 (KinExA3000 Instrument Manual, Sapidyne). To determine the time required for the antibody to come to equilibrium with a sample, a slight modification to the manufacturer's instructions for the Kinetics Direct method was employed. Essentially, concentrations of antibody and analyte were held constant and the amount of time that they are mixed together (i.e., incubated) was varied and then assessed (voltage recorded).

The automated sample handling program was similar to others that have been described [32]. Briefly, for every sample a new bead pack (≈400 μl) coated with antigen was loaded into the flow cell. Then 400 μl of fluorescently labeled 7B2.3 was pulled into the drawing syringe, followed by 400 μl of deionized water, a standard, or a sample. This mixture was pushed into and drawn out of a mixing syringe to complete mixing. Half of the solution was discarded to waste and the remaining 400 μl was pushed over a previously loaded bead pack in the flow cell. For fluorescence detection, excitation and detection wavelengths were 620 and 670 nm, respectively. The resulting fluorescence signal was based on competitive exclusion by free PAHs in the sample such that the amount of 7B2.3 bound to the antigen-coated beads within the flow cell was inversely proportional to the concentration of three- to five-ring PAHs in the sample. A solution of 50% dimethylsulfoxide (Sigma-Aldrich) in deionized water was incorporated for line rinsing between each sample analysis to prevent cross-contamination of samples by residual PAHs. In total, quantitative assessments were obtained in approximately 3 min, with 7 min for preparation and introduction of the next sample. Up to seven samples can be loaded for analysis with all further steps performed automatically.

Calibration curves for the biosensor

Several PAH formulations were evaluated for biosensor detection (signal response) and eventual use in the generation of a calibration curve. The formulations were SRM 1647b Priority Pollutant PAHs (National Institute of Standards and Technology, Gaithersburg, MD), SRM 2260 Aromatic Hydrocarbons in Toluene (National Institute of Standards and Technology), a solution containing equal concentrations (by mass) of six PAHs (anthracene, 99% J. T. Baker; chrysene, 95% Aldrich; fluoranthrene, 98% Aldrich; fluorene, 98% Aldrich; phenanthrene, ICN Pharmaceuticals; pyrene, 99% Aldrich) and a solution of only phenanthrene. Each solution was diluted in deionized water such that they contained less than 1% of the original solvent. The calibration curves represent the sum of the PAH concentrations for the respective formulations. For each formulation standard, a five-point calibration curve within its linear range was used.

Site descriptions and sample collection

Estuarine monitoring. The Elizabeth River Project, an independent, nonprofit organization, has targeted the Money Point area of the Elizabeth River (Norfolk, VA) as a remediation site. This site has severely PAH-contaminated sediments due to historical creosote releases [33]. Remediation efforts include dredging of contaminated sediments, capping, and wetland restoration. On June 9, 2009, while the dredging operation was under way, the biosensor was employed to monitor PAHs released into the surrounding water column. All samples were collected outside of a sediment curtain that enclosed the dredging activity. Water samples were collected in 30-ml amber glass vials for on-site biosensor analysis and in 4-L amber glass bottles for laboratory-based GC-MS analysis. At this site, the biosensor was operated on board the 6-m vessel, *R/V Oystercatcher*. The biosensor was kept in the front cabin area and powered by a 12-V battery (Energizer E27RC Marine Battery) through a 110-V power inverter (Xantrex Xpower 1750 Plus). Temperatures on board the vessel exceeded 35°C at times, which is capable of causing variable results; therefore, temperature was stabilized by placing ice chips in the reagent block. Biosensor samples were filtered and analyzed on-site, while the samples taken for GC-MS analysis were kept on ice until laboratory analyses could be performed.

Storm water runoff monitoring. Two sites located near the Virginia Institute of Marine Science campus were selected for monitoring during a rain event (April 1 to 16, 2010). The first site was a retention pond located below the Coleman Bridge (CB; lat. 37° 14′ 52″, long. −76° 30′ 9″). The second site was along the Yorktown Creek (YTC) where it passes under the George Washington Memorial Highway/U.S. Route 17 (lat. 37° 13′ 55″, long. −76° 30′ 50″) on the west side. Because the sites were sampled simultaneously and were near the laboratory (i.e., ≈0.5–3 km away), the samples were brought back to the laboratory for analysis. Samples were collected at each site every other day until it began raining, whereupon the samples were collected more frequently (≈hourly). For biosensor analysis, triplicate water samples were collected in 30-ml vials; larger samples (1 L) were collected for GC-MS analysis when biosensor results revealed that PAH concentrations were rapidly changing. Cumulative rainfall readings from rain gauges stationed at each sampling site and the observation times were recorded.

GC-MS analyses of PAHs

Gas chromatography-mass spectrometry analysis was conducted using previously described methods [34] with the following modifications. The estuarine samples were filtered using a 0.45 μm Teflon filter (Millipore) prior to analysis because of high levels of suspended solids, which could potentially clog the biosensor lines. For both studies, 1 L of water sample was transferred to a precleaned separatory funnel, spiked with surrogate standards (1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, perylene-d_{12}, PCB 30, PCB 65, PCB 204, 1,1′-binaphthyl, and perinaphthenone; Ultra Scientific), and extracted three times with 40 ml of dichloromethane (Burdick & Jackson). The volume was reduced under a gentle stream of nitrogen using a TurboVap® evaporator (Zymark) and the internal standard *p*-terphenyl (ChemService) was added. The extracts were analyzed on a Varian 3800 Gas Chromatograph using a Varian CP-8400 Autosampler coupled to a Saturn 2000 GC/MS/MS ion trap MS (Varian) operated in electron ionization mode (70 eV). It was equipped with a split/splitless injector maintained at 320°C. The carrier gas was He and injections were made in splitless mode on a DB5, 60 m × 0.32 mm × 0.25 μm film thickness capillary column from J&W Scientific. The GC temperature program was 75 to 320°C at 4°C/min with an initial hold of 1 min. The MS trap, manifold, and the transfer line temperatures were 220°C, 80°C, and 320°C, respectively. Scans were 100 to 500 *m/z* for 8 to 49 min and then 100 to 650 *m/z* from 49 to 62.25 min; selected ions were used to quantify the targeted analytes. Seven-point calibration curves and sample analyses of individual analytes were performed using the Varian MS Workstation software package, version 6.8 (Varian). The limit of detection was approximately 0.01 μg/l per analyte. Each set of extractions contained a laboratory blank and a replicate environmental sample.

Biosensor analysis of PAHs

The estuarine water samples were filtered (0.45 μm Teflon, Millipore) prior to analysis. The storm water runoff samples were assessed directly, without any manipulation. During the rain event, one to two of the triplicate samples were analyzed in near real-time (within 10–60 min of collection) to guide further sampling. The remaining samples were kept at 4°C and analyzed within 3 d. Standards and blanks were routinely used to ensure the performance of the instrument during each study.

Statistical analysis

The calibration curves, detection limits, and biosensor linear range were determined by log-linear regression analysis. Error estimates of the biosensor method were determined by triplicate analysis of laboratory standards. During laboratory development of the biosensor methodology, the detection limit was determined by performing the EPA method detection limit procedure 40 CFR Part 136, Appendix B. During field studies the limit of detection was defined as a signal-to-noise ratio of 3:1. The PAH concentrations determined by biosensor and GC-MS methods were compared using a Pearson correlation coefficient (r) analysis. Deming (orthogonal) regression analysis was performed to examine the relationship between the biosensor and the GC-MS method. All statistics were performed using Minitab v16 software (Minitab).

RESULTS

Calibration curve for the biosensor method

Kinetic analysis of the antibody resulted in an equilibrium association constant, K_a, of 2.5×10^8 M^{-1} with an on-rate, k_{on}, of 1.52×10^5 M^{-1} s^{-1} and an off-rate, k_{off}, of 6.10×10^{-4} s^{-1}. Equilibrium was also empirically determined to occur within approximately 10 s on the basis of a series of timed reactions. This demonstrated that the biosensor method did not require any additional time for antibody and sample incubation.

The regression curves for the four calibration standards are shown in Figure 1. The concentrations of these solutions ranged from 0.3 to 30 μg/l total PAH. The calibration curves have comparable log-linear best fit lines with minimal variability in each line ($r^2 \geq 0.98$). Based on these results, and for simplicity, the phenanthrene standard curve was selected for use in field applications; therefore, biosensor assessments were reported as phenanthrene equivalents. The detection limit for phenanthrene equivalents, as determined by standard U.S. EPA method, was 0.3 μg/l PAHs with a linear range of 0.3 to 30 μg/l. For field

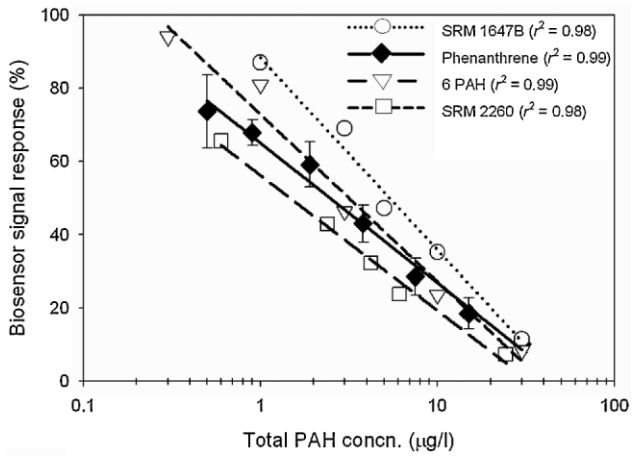

Fig. 1. Calibration curves for solutions containing either a mixture of polycyclic aromatic hydrocarbons (PAHs), SRM 1647b Priority Pollutant PAHs, SRM 2260 Aromatic Hydrocarbons in Toluene, 6 PAH (phenanthrene, anthracene, fluorene, chrysene, pyrene, and fluoranthrene) or phenanthrene. The log-linear lines of best fit are displayed for each solution tested. For the sake of clarity, standard deviation bars are shown only for phenanthrene, as determined by analysis of triplicate standard solutions at each concentration.

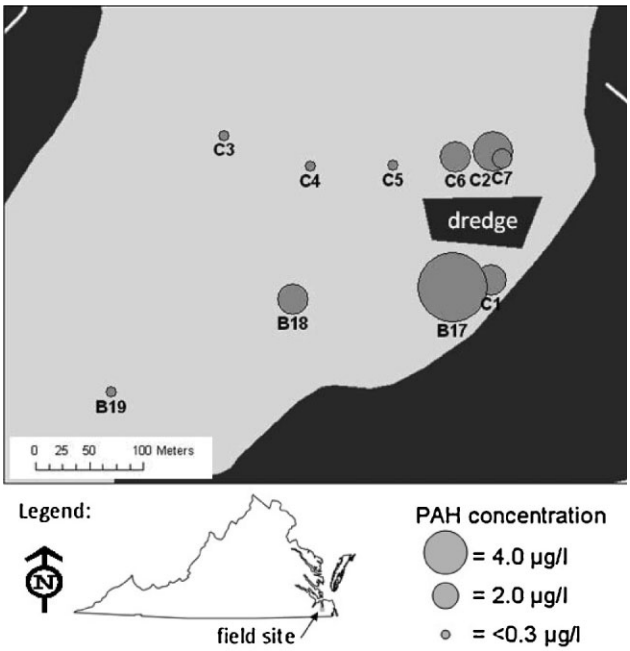

Fig. 2. Real-time monitoring of the remedial dredging project completed at the Money Point site in the Elizabeth River in Norfolk, VA. The polycyclic aromatic hydrocarbon concentrations determined by the biosensor are represented by the gray circles proportional to concentration. The dredge polygon represents the location of the dredging operation. See Figure 3 for concentrations for the sites labeled beginning with a C, as these were validated by gas chromatography-mass spectrometry.

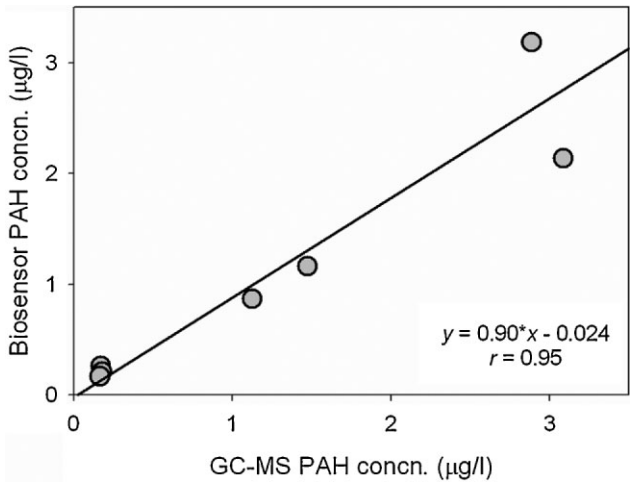

Fig. 3. Polycyclic aromatic hydrocarbon concentrations (µg/l) measured by the biosensor and gas chromatography–mass spectrometry (sum of 11 to 39 analytes) methods during the estuarine monitoring at the Money Point site along the Elizabeth River in Norfolk, VA. A strong correlation was observed between the two methods ($r = 0.95$, $n = 7$). Based on Deming regression analysis, the slope of the line of best fit was 0.90 ± 0.14 (SE).

analyses, the detection limit varied from 0.2 to 1 µg/l. The error in the method, from triplicate analysis of standard solutions, was usually no more than 10% of the signal response, as demonstrated by the standard deviation bars shown for phenanthrene (Fig. 1). The largest error is seen at the highest biosensor response as this is where the PAH concentration is the lowest and is approaching the detection limit.

Estuarine monitoring

A spatial representation of PAH concentrations measured with the biosensor defines a plume ranging from 0.3 to 3.2 µg/l (phenanthrene equivalents) with higher PAH concentrations closer to the dredge site (Fig. 2). Due to the biosensor's rapid and on-site operation, we were able to survey an area of approximately 90,000 m^2 around the operating dredge site, thus defining the extent of the plume relative to background PAH concentrations in the estuary. Polycyclic aromatic hydrocarbon concentrations at seven of the stations, where additional large volume samples were collected, demonstrated a very strong positive correlation between biosensor and GC-MS results ($r = 0.95$, $n = 7$) (Fig. 3). The GC-MS PAH value is the sum of all detected (limit of detection > 0.01 µg/l per analyte) alkylated and nonsubstituted 3- to 5-ring PAHs (11 to 39 compounds identified). As a comparison, the 16 EPA PAHs (excluding naphthalene) comprised 19 to 41% (with one sample containing 88%) of the reported total PAH concentration. The Deming regression slope of the line comparing the biosensor to GC-MS total PAH is 0.90 ± 0.14 (SE).

Storm water runoff monitoring

Polycyclic aromatic hydrocarbon concentrations measured with the biosensor and cumulative rainfall for the CB and YTC sites were documented before, during, and after the rain event (Fig. 4A,B). The rain event began at approximately 1 AM local time. At both sites the initial increase of PAH pollutants was measured approximately 1 to 2 h after the rain started. Although rainfall ceased at approximately 8 AM, sample collection continued until the PAH concentrations declined to background values. From the start of the rain event to the end of the intensive sampling session, 17 h elapsed and 84 samples (14 timepoints, triplicate samples, two sites) were collected for biosensor analysis. Typically, two of the three replicate samples were analyzed within the hour. In total, the CB site received 1 cm of rain, while the YTC site received 0.8 cm. The peak PAH concentration (phenanthrene equivalents) measured was 4.4 µg/l for the CB site and 3.6 µg/l for the YTC site. Samples were collected periodically during the rain event for GC-MS analysis in an attempt to encompass a range of PAH concentrations. The biosensor analyses were used to guide this sampling effort due to its generation of near real-time results. The GC-MS PAH value is the sum of all alkylated and nonsubstituted 3- to 5-ring PAHs (4–9 compounds identified). Good correlation ($r = 0.86$, $n = 7$) between biosensor and GC-MS results was observed (Fig. 5). As a comparison, the sum of the 16 EPA PAHs (excluding naphthalene) comprised 50 to 78% of the reported total PAH concentration. The Deming regression slope of the line comparing the biosensor to GC-MS results is 9.68 ± 2.63.

DISCUSSION

The validation of the immunoassay method for total PAH quantification in environmental samples is difficult, as PAHs commonly occur as complex mixtures. Conventional validation methods (i.e., GC-MS) require the identification and quantification of the individual analytes before summation. However, immunoassays bind structurally similar groups of compounds, resulting in one combined concentration estimate. Therefore, a defined number of these individual analytes detected in the conventional method must be preselected by the analyst to provide a single summed value for which the immunoassay method can be compared. To this end, most methods select a subset of PAHs (typically the 16 EPA priority PAH pollutants) for quantification. This has resulted in the publication of many

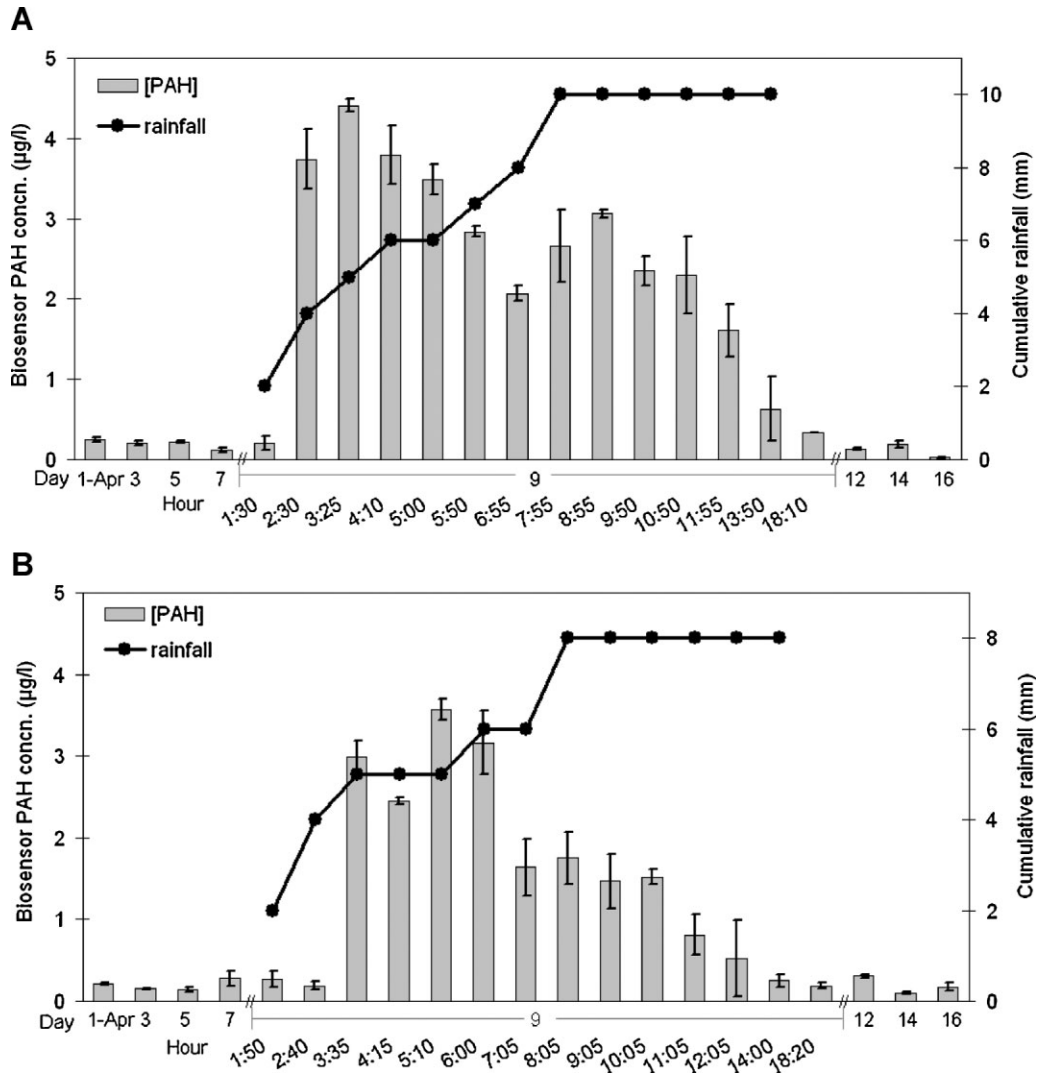

Fig. 4. Near real-time monitoring of polycyclic aromatic hydrocarbon (PAH) concentrations in storm water runoff during a rain event. Concentration profiles of PAHs assessed from samples collected from (**A**) a retention pond located below the Coleman Bridge and (**B**) a culvert into the Yorktown Creek located alongside U.S. Route 17. Error bars represent the standard deviation of three replicate samples. The bars indicate the PAH concentrations and the line shows the cumulative rainfall.

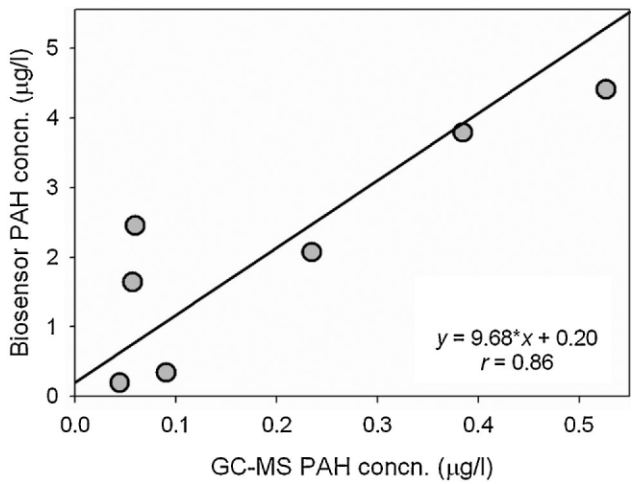

Fig. 5. Polycyclic aromatic hydrocarbon (PAH) concentrations (µg/l) from the storm water runoff study as determined by the biosensor and gas chromatography–mass spectrometry (sum of 4–9 analytes) methods. A strong correlation was noted between the two methods ($r = 0.86$, $n = 7$). Based on Deming regression analysis, the slope of the line of best fit was 9.68 ± 2.63 (SE).

studies in which immunoassays are reported as overestimating the PAH concentrations found in a sample [17–19,35] with the explanation that this is due to the crossreactivity of the antibody with similar analytes in a sample. We propose that much of the increased antibody reactivity could be crossreactivity with alkylated PAHs, as it is well documented that alkylated species may comprise a larger fraction of the PAHs in petrogenic samples [36,37]. Therefore, the total PAH value determined by conventional methods (as defined as an arbitrary set of unsubstituted PAHs) may underestimate actual total PAH concentration in the environment. For this reason, we broadened our definition of total PAHs to include the sum of unsubstituted and alkylated homologs when using the GC-MS method. We found that the higher the number of analytes detected—39 analytes in the estuarine study compared to nine in the storm water study—resulted in a better correlation when comparing the total PAH value in the GC-MS and biosensor method (Figs. 3 and 5; slope of 0.90 and 9.68, respectively).

The second confounding issue with validation of an immunoassay method with a conventional method is the detection limit of the conventional method. When the target analyte is just below the detection limit of the conventional method, it will not be quantified. However, the antibody, due to crossreactivity,

could generate an additive effect and the resultant value will be the summation of all low level analytes. For example, the nearly 10-fold difference seen in the present study could be a result of the limit of detection for individual analytes of 0.01 μg/l by GC-MS. If the sample were to contain, for example, a thousand possible alkylated and unsubstituted PAHs below this limit, a total of 10 μg/l could potentially be undetected. This is also suggested in Figure 3, where the two highest concentration samples vary considerably in the biosensor response but are similar in total PAH concentrations as measured by GC-MS. The highest GC-MS value (3.1 μg/l) is a sum of 39 analytes, with many analytes near the detection limit, while the next highest GC-MS value (2.9 μg/l) is a sum of only 29 analytes and it is likely that many additional compounds were just below the reporting limit. Crossreactivity is considered a limitation of antibody-based analytical methods, but this feature may actually provide a better summation of low level PAHs in complex mixtures not possible with targeted analyte-based conventional analyses. A potential resolution, when directly comparable results are required, is to calibrate the immunoassay result to the source material based on a few confirmatory conventionally assessed samples (GC-MS) [25].

On-site biosensor evaluation of water surrounding a contaminated sediment dredging operation documented a spatial distribution of PAH concentrations in near real-time (Fig. 2). Information about the size and intensity of the plume was provided immediately to engineers monitoring the dredging operation from shore and was able to verify that the PAH plume never exceeded 10 μg/l. If the near real-time results had shown elevated concentrations were a concern, dredging could have been halted and remediative actions put in place. In addition, near real-time data provided information to scientists on board the vessel to guide the collection of large volume water samples for later analysis of specific PAH compounds. This saved time, effort, and money by eliminating the future analysis of samples that might contain PAHs below the detection limit of the GC-MS methods. Overall, the data provided by this near real-time assay allowed for rapid decision-making about sample collection and remediation effectiveness.

The biosensor was employed to document a temporal pulse of PAH concentrations at two locations during a rainfall event and to time the collection of validation samples. In the present study, sample collection for GC-MS validation began 2 h after the rain had started, and continued 4 h after the rain had stopped (Fig. 4), which encompassed the period of all elevated values. Because field sites will differ in topography, run-off volume, and amount of contamination, the timing of the pulse of pollutants and the end of the elevated flux will inevitably vary between sites [38]. Moreover, input loads are a function of concentration of an analyte and flow volume over time. Because concentrations will vary widely over time, higher temporal resolution of measurements can reduce the inaccuracies in estimating input loads of pollutants to the system during a rainfall event [38]. As shown in the present study, measurements made after the rain has stopped (≈4 h later) revealed that elevated concentrations were still present (Fig. 4).

By utilizing the biosensor in both applications, samples indicating a nondetectable PAH concentration (i.e., far from the dredge, before the pulse, and at the end of the flux) were not collected for additional more costly laboratory-based analysis. These advantages can result in considerable savings of time and money for field PAH determinations.

A concern of any conventional on-site field sampling is the need for large sample volumes and the preservation and transportation of samples. In the present demonstrations, sample volumes for biosensor analysis never exceeded 30 ml, although as little as 1 ml could be used for biosensor analysis. Sample holding time is another important consideration to avoid sample degradation. This can be avoided when employing immunoassays, because little need exists for sample pretreatment (i.e., extraction or concentration) [17,18,22,23,35,39], and analysis could be conducted on-site promptly after collection. In the present study, these samples were, at most, filtered. This also minimizes the amount of harmful solvents needed in the field. The only solvent employed was the dimethylsulfoxide used in the biosensor to clean lines between samples; 100 ml of dimethylsulfoxide was more than adequate for 50+ measurements.

Sensitivity, speed, and portability are considered the key advantages of immunoassays over conventional analytical methods for field monitoring of PAHs. In terms of sensitivity, detection limits are often reported in the sub-ppb (μg/l) level [17,18,22,23,35,39], which is consistent with the results of the present study. Of the many immunoassay methods being developed, only ELISA-based methods have been employed for the quantification of PAHs in naturally contaminated water samples [17–19]. We report, in the present study, a new antibody-based biosensor method for the detection of PAHs in contaminated aqueous environmental samples. Moreover, this method required 3 min for a quantitative response (including sample and antibody mixing time) and an additional 7 min for sensor regeneration, which is faster than previously reported antibody-based PAH biosensors [21–23]. Similar to previous studies [16,25,26], we found that maintaining a temperature range of 10 to 35°C ensured proper equipment function and accurate analysis.

Immunoassays have their limitations and optimal operating conditions. Understanding what the antibody recognizes determines the value of the assay and how the results should be interpreted. The primary advantages of this antibody-based biosensor are its sensitivity, speed, and portability, as well as the small volumes required and limited sample manipulations. We have shown that field-based biosensor analyses can be accomplished with good correlation to laboratory-based conventional analytical methods. Overall, the biosensor provided rapid and precise quantification of PAHs that guided a sampling survey and permitted delineation of small changes in concentration. This affords higher resolution than previous technology could easily allow, and has a myriad of uses in environmental monitoring and management.

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