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Molecularly imprinted polymer sensors for pesticide and insecticide detection in water

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Antibodies, peptides, and enzymes are often used as molecular recognition elements in chemical and biological sensors. However, their lack of stability and signal transduction mechanisms limits their use as sensing devices. Recent advances in the field of molecularly imprinted polymers (MIPs) have created synthetic materials that can mimic the function of biological receptors but with less stability constraints. These polymers can provide high sensitivity and selectivity while maintaining excellent thermal and mechanical stability. To further enhance the advantages of the traditional imprinted polymer approach, an additional fluorescent component has been introduced into these polymers. Such a component provides enhanced chemical affinity as well as a method for signal transduction. In this type of imprinted polymer, binding of the target analyte invokes a specific spectral signature from the reporter molecule. Previous work has provided molecularly imprinted polymers that are selective for the hydrolysis products of organophosphorus species such as the nerve agents sarin and soman. (A. L. Jenkins, O. M. Uy and G. M. Murray, *Anal. Chem.*, 1999, **71**, 373). In this paper the direct imprinting of non-hydrolyzed organophosphates including pesticides and insecticides is described. Detection limits for these newly developed MIP sensors are less than 10 parts per trillion (ppt) with long linear dynamic ranges (ppt to ppm) and response times of less than 15 min.

Natural waters are contaminated with various pesticides and insecticides because of their wide spread use in commercial and residential applications. These chemicals directly applied to the ground are rapidly transported into the groundwater. In 1988, over 46 pesticides were determined in the ground waters of the United States.[1] Despite today's technology, detection of environmental pollutants like pesticides, insecticides, and herbicides at the levels specified by the Environmental Protection Agency (EPA) remains a challenge. Many of the detection methods currently in use for the determination of these species in water are gas chromatography-atomic emission detection[2,3] (GC-AED), gas chromatography-mass spectrometry[4] (GC-MS), and high performance liquid chromatography with either mass spectrometric or diode array detection[5–7] (LC-MS or LC-DAD).[8–10] These techniques generally have high sensitivity, but are complex and costly, require skilled technicians and large laboratory based instrumentation. Additionally, most of these techniques require sophisticated and time consuming extraction procedures such as liquid solid extraction (LSE) or solid phase micro extraction (SPME).[11–13]

The new focus for detection of these species relies on basic biochemical principles such as enzyme inhibition, selective immunoassays, and blocking of photosynthetic activity.[14] These biosensors are highly sensitive and selective and can be made into very small devices. Enzymes were the first biological receptors to be used as biosensors. Many of these devices are fiber optic based using bound enzymes such as acetylcholinesterase, butyrylcholinesterase, or alkaline phosphatase with detection by reflectance of an indicator material, chemiluminescence, or by total internal reflection of fluorescent radiation (TIRF) which generally results from the inhibiting capacity of the enzyme activity.[15–18] The drawbacks to these devices are that the enzymes are often difficult to purify, are unstable, and often the sensitivity and selectivity depend on the source of the materials. Immunoassay techniques are also utilized for the detection of pesticides. In general, these methods are indirect techniques, relying on antibodies to bind the antigen and requiring labels such as fluorophores or radioactive isotopes. The drawbacks to these technologies are the availability of the antibodies, stability, and irreversible binding which prevents re-use.[19–21]

Recent advances in the field of molecularly imprinted polymers (MIPs) have created synthetic materials that can mimic the function of biological receptors but with less stability constraints. A selective sensor for atrazine using a flexible imprinted polymer has been developed. In this application, detection was based on a change in conductivity when the atrazine was bound. This sensor was sensitive to 5nM, it was extremely stable and suffered only slightly from changes in ionic strength.[22] Imprinted polymer sensors that are selective for the hydrolysis products of the nerve agents sarin and soman have also been developed.[23] To date, only limited imprinted polymer work has been done for direct recognition and sensing of non-hydrolyzed organophosphates.[24] In this paper, new molecularly imprinted polymers that are specific for the detection of pesticides and insecticides are described.

The sensors are based on imprinted polymers that have been directly polymerized onto a fiber optic probe. A luminescent lanthanide (europium) is incorporated into the polymer to act as a signal transducer. The use of lanthanide ions as spectroscopic probes of structure and content is an established technique. The narrow excitation and emission peaks of lanthanide spectra (typically in the order of 0.01–1 nm full width at half maximum), provide for highly sensitive and selective analyses.[25,26] This type of sensor has been shown to provide limits of detection of parts per trillion (ppt), or lower.[27] Detection of the analyte is based upon the changes that occur in the spectrum when the pesticide or insecticide is coordinated to Eu^{3+}. The

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combination of molecular imprinting and luminescence detection provides multiple criteria of selectivity to virtually eliminate the possibility of false positive readings.

Experimental section

Reagents

Unless otherwise indicated, materials were obtained from commercial suppliers and used without further purification. Analytical reagent grade chemicals were used along with deionized water to prepare solutions. Europium (III) oxide, styrene, and azobisisobutyronitrile (AIBN) were obtained from Aldrich (Aldrich, Milwaukee, WI, USA). Neat analytical standards of the pesticides and insecticides were obtained from Supelco (Supelco Chromatography Products, Bellefonte, PA, USA). Malathion, thionazin, and dibutyl chlorendate were obtained as neat liquid standards from Radian (Radian International, Austin, TX, USA).

Instrumentation

Luminescence was excited using a model 60X-argon ion laser (MWK Industries, Corona, CA, USA). A 488 nm holographic filter (Kaiser Optical Systems, Ann Arbor, MI, USA) turned to pass the 465.8nm line, was used to exclude all other laser lines. Spectra were collected using an f/4, 0.5 m DKSP240 monochromator with a direct fiber coupler (CVI Laser Corp., Albuquerque, NM 87123, USA) equipped with a Model ST-6 CCD (Santa Barbara Instruments Group, Santa Barbara, CA, USA) using Kestrel Spec Software (K&M Co., Torrance, CA, USA). Spectra were also obtained with an Ocean Optics S2000 Miniature Fiber Optic Spectrometer (Ocean Optics, Dunedin, FL 34698, USA) equipped with a 1200 line holographic grating, permanently installed 100 micron slits and a 440 nm cutoff filter. Spectra were plotted and calculations performed using Igor Pro Software (WaveMetrics Inc., Lake Oswego, OR, USA).

Complex preparation

The pesticides and insecticides were divided into 4 different groups depending on their functionality,[28] aliphatic organothiophosphates, pyridine organothiophosphates, organophosphates, pyrimidine organothiophosphates, and candidates from each group chosen. Complexes were synthesized, using a stoichiometric ratio of one mol europium, to one mol of pesticide/insecticide and 3 mol of the vinyl benzoate–ligating molecule. (The number of ligating species was chosen to accommodate the 9 coordinate Eu^{3+}). Eu $(NO_3)_3$ was prepared by dissolving the oxide in water with just enough nitric acid to produce a clear solution. The calculated amount of each pesticide/insecticide was diluted/dissolved in a 50 : 50 water–methanol mixture to which the vinyl benzoate monomer was subsequently added. The resulting solution was added to the Eu $(NO_3)_3$ and the pH adjusted to between 9 and 10 for complexation. The resulting solutions were stirred on low heat for approximately 4 h, then covered with a watch glass and left to crystallize overnight. The crystals were filtered, dried and the spectra interpreted to determine the symmetry changes associated with analyte inclusion. A blank complex containing only europium and vinyl benzoate was also prepared as described above.

Pesticide sensor preparation

The fiber optic sensors consisted of a 400 micron optical fiber (Thor Labs, Newton, NJ, USA) with the polymeric sensing element chemically bound on the distal end. The fibers were prepared by terminating one end with an SMA connector using the procedures outlined in the 'Thor Labs Guide to Connectorization and Polishing of Optical Fibers'. The sensing portions of the fibers were prepared by removing the cladding, heating the stripped end in an air–acetylene flame and manually pulling the fibers into tapers (tapered fibers are much more efficient at coupling the evanescent field to the polymer).

Polymers were prepared by dissolving 3 mol% complex compound in 95 mol% styrene with approximately 0.1 mol% azobisisobutyronitrile (AIBN) added as an initiator, and 2 mol% divinyl benzene (DVB) added as a crosslinking agent. Traditionally, higher levels of crosslinking are used, however previous research demonstrated that in real time sensing applications lower amounts of crosslinking allow better accessibility to the site.[23] The structure of the site is stabilized by the addition of the lanthanide that may provide directional bonding in the cavity.

The resulting solutions were placed in glass vials, purged with nitrogen, and sealed using parafilm and screw on tops. The polymers were sonicated at 60 °C for 2–4 h until they became viscous. (Sonication is believed to help maintain homogeneity in the polymer.)[25] The partially polymerized material was then directly dip coated onto the unmodified fibers, (prior research demonstrated that silanization of the fiber is unnecessary), and cured under a small UV lamp for 2 h.[23] Once cured, the polymers were swelled in water with gradually increasing amounts of methanol to remove unreacted monomer and expand the polymer pores to produce accessible sites and facilitate the removal of the imprinting ion.[29] The imprinting ion was removed by washing with 1.0 M nitric acid to facilitate the removal of the analyte and leave in its place a weakly coordinated nitrate. An unimprinted sensor was also constructed by polymerizing 3 mol% of the blank complex in styrene.

Analysis

Measurements for the calibration data, pH study, response time and interference testing within each class were performed using the same fiber to demonstrate the reversibility of the sensors. The fibers evaluated were coated with 200 μm of polymer to allow longer linear dynamic ranges at higher concentrations. Analytical figures of merit were obtained with serial dilutions of the standards in 0.01M NaOH maintaining the pH at 10.5. Luminescence was excited using 1mW of the 465.8 nm line of the argon laser and the active end of the sensor placed in one of the sample dilutions. The performance of the fiber optic sensors was evaluated using the 0.5 m monochromator with 200 micron slits, and an exposure time of 25 s. Spectra were collected at each concentration at different equilibration times. The sensors were rinsed with deionized water between each sample. Standards were analyzed in order of both increasing and decreasing concentration in order to demonstrate the reversibility of the sensor. Calibration curves based on an exposure time of 15 min were obtained and linear regressions performed.

The response time of the sensors and the pH dependence was evaluated using a method similar to the one described above. A series of standards with pH values ranging from 6 to 11 were prepared from the stock standard through the addition of 1.0 M sodium hydroxide. The sensor was placed in a cuvette with each solution and spectra collected at a variety of exposure times. Response was evaluated through a comparison of peak area at each time and pH.

Pesticide and insecticide standards were tested as possible interferences for the sensors. Standard 100 ppm solutions were prepared by the dissolution and/or dilution of the samples in deionized water when possible. The pesticides/insecticides with limited solubility in water were prepared using a 50 : 50 water–methanol mixture. The pH of each of the solutions was adjusted

to 10.5 using 1M sodium hydroxide. Spectra from the fibers for each analyte were taken at regular intervals for 30 min, and then compared with the response from the imprinted analyte. The sensor was rinsed with deionized water between each analysis. The response of the blank sensor to a series of pesticides and insecticides was evaluated using standard solutions at pH 10.5.

Results and discussion

Excitation

Three argon ion excitation wavelengths (465.8, 514 and 488 nm) were used to excite the luminescence of the polymers. The spectra of the sensors excited with the 465.8 nm laser line displayed increased luminescence intensity and greater spectral resolution of the analyte peaks from the parent europium. This increase indicates that excitation using the 465.8 nm line results in a near resonant excitation transition from the ground $^7F_0$ level to the $^5D_2$ level of the europium. As a result, 465.8 nm was kept as the excitation wavelength for the sensor.

pH dependence

Each of the sensors was evaluated to determine the effect of pH on the response time. The study was performed on solutions with pH values ranging from 6 to 11 with measurements taken over a period of 30 min. The sensors tested all show a positive response to the presence of analyte after 3 min for pH values from 6 to 11, and a positive response after 1 min for the solution with a pH of 10.5. Low values of pH (below 6) used to clean the sensors were not evaluated. In all cases, as pH increased, the response time of the sensor decreased as indicated by an increase in luminescence. At high pH values (above 12) the risk of forming the europium hydroxide increases, thus pH 10.5 was chosen for all analyses.

Analytical figures of merit

In all the sensors, the response to increasing concentrations of analyte exhibits an increase in the luminescence intensity of the spectra. (Fig. 1) This increase in luminescence is indicative of rebinding the analyte into the coordination sphere of the lanthanide and the exclusion of water. The resulting peak areas in the 609 to 621 nm spectral region were calculated using Igor Pro Software, and plotted as a function of concentration. Peak areas have been shown to provide a longer, more linear calibration curve than direct peak height, since the bandwidths as well as the peak heights of the lanthanides increase as a function of concentration.[27] Linear regression was performed and the limits of detection calculated. The analytical figures of merit for each of the sensors are given in Table 1. Some residual bands remain visible even when the sensors are cleaned as a result of permanently trapped species deep in the polymer, and should be subtracted out with the background for application purposes. (The trapped particles are estimated to be less than 0.03% based on the amount of complex in the polymer 3%, and the total luminescence of the unwashed polymer.) Variations in the residual peak, the background, or other slight differences between sensors appear to have little effect on the overall calibration curve, linear dynamic range and limit of detection.

Organophosphate sensor

Organophosphate pesticides and insecticides are the closest species chemically to the nerve agent hydrolysis products previously imprinted, and as such were selected as candidates for MIP sensors. Glyphosate was chosen as the target molecule since it was smaller than some of the other candidates, (imprinting the smaller pesticide should help exclude other larger pesticides in the same class). The polymer imprinted for glyphosate provided a 9 ppt limit of detection with a linear dynamic range from 9 ppt to 100 ppm. The 80% response time using a 200 μm coated sensor was 12 min at pH 10.5. Response curves for the sensors are shown in Fig. 2.

Two other organophosphates in the same class phosphamidon and dichlorvos, were also evaluated against the sensor. Fig. 3 shows the chemical structures of all the imprinted molecules

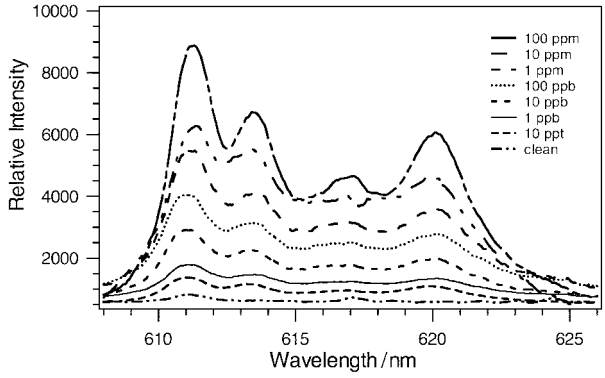

Fig. 1 Spectral response of the glyphosate sensor to concentration, excited at 465.8 nm.

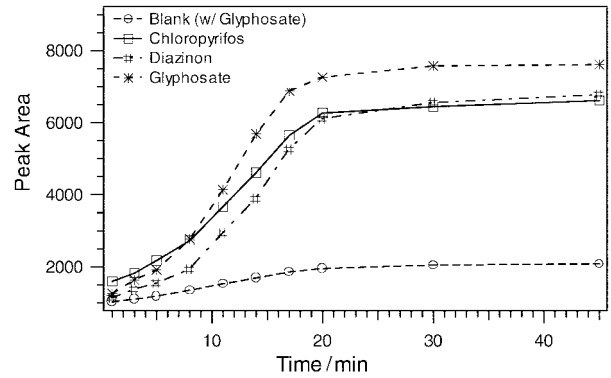

Fig. 2 Response curves for the imprinted and blank polymer sensors. Curves were generated using 10 ppm solutions at pH 10.5.

Table 1 Analytical figures of merit for the sensors

	Blank	Glyphosate	Chloropyrifos Methyl	Diazinon
Limit of detection	≈250 ppb for all	9 ppt	5 ppt	7 ppt
Linear dynamic range	10 ppm to 250 ppb	100 ppm to 9 ppt	100 ppm to 5 ppt	100 ppm to 7 ppt
Correlation coefficient	—	0.9995	0.9996	0.9984
Slope	—	1.377 counts per ppt	1.528 counts per ppt	1.696 counts per ppt
80% Response time	20–30 min	12 min	14 min	15 min

(displayed in boxes) and some of the interferences evaluated against the sensors. The spectral responses to these structurally similar compounds are almost identical but greatly reduced in luminescence intensity. This suggests that the larger pesticides have a harder time entering the imprinted cavity. Despite the similarities in the spectral signatures, the glyphosate spectrum can be resolved from the other two by the characteristic peaks at 613.5 nm and 615.5 nm. For example, in the glyphosate spectrum, the 613.5 nm band is larger than the 615.5 nm band, which is directly opposite from the responses of the other organophosphates. Selected pesticides/insecticides from other classes as well as a sodium phosphate buffer solution were also tested against the sensor at 100 ppm. All of the materials screened displayed very weak luminescence and they all exhibited markedly different spectral signatures, thus they do not interfere with the detection of the imprinted analyte.

Organothiophosphate sensors

The next class of pesticides/insecticides chosen for imprinting was the pyridine organothiophosphates. These were not only chosen for their phosphate functionality but also because it was believed that pyridine could further enhance the luminescence of the lanthanide and allow sensitive detection. The polymer imprinted for methyl chloropyrifos, a small member of this class not only discriminates against other classes of pesticides but also can distinguish against other members within its own class such as ethyl chloropyrifos which differs only by the substitution of a methyl group for an ethyl group. Fig. 4 shows the spectra of 1 ppm methyl chloropyrifos, 10 ppm of the ethyl chloropyrifos, and 100 ppm of selected pesticides and insecticides. Although the methyl and ethyl chloropyrifos are structurally very similar, the methyl-substituted compound exhibits much greater luminescence intensity. Additionally, the 611.5 nm peak is also distinctly shifted to 610.5 nm when the methyl group is substituted by an ethyl in the chloropyrifos.

Other classes of pesticides evaluated as interferences against this sensor had very weak luminescent responses and different spectral signatures. Pyrimidine organothiophosphates such as diazinon and pirimphos ethyl, that only differ from the pyridine organothiophosphates by an additional nitrogen in the ring, had more interaction with the polymer as indicated by an increase in luminescence. However, the spectral signatures resulting from coordination with these pesticides were missing the bands at 611 nm, 618.5 nm, and 620 nm. The limit of detection for this sensor is 5 ppt with a linear dynamic range from 5 ppt to 100 ppm. The 80% response time at pH 10.5 for a sensor with a 200 micron polymer coating was 14 min.

Diazinon a pyrimidine organothiophosphate pesticide was also imprinted as a result of the binding response that it had with the pyridine organothiophosphate sensor. The diazinon-imprinted sensor has a limit of detection of 7 ppt with a linear dynamic range from 7 ppt to 100 ppm and 80% response time of 15 min. Detection is based on intense peaks at 610 nm, 616.5 nm, and a small peak at 623.5 nm. Pirimphos ethyl another pyrimidine containing pesticide was also screened against the sensor. The spectral signatures of the two pesticides were very similar, but the binding of the larger pirimphos ethyl provided a luminescence intensity of less than half that of the diazinon. Additionally, the intensity ratios of the two main peaks at 610 nm and 616.5 nm were reversed in the spectra. Other classes of pesticides were also evaluated against the sensor at 100 ppm. Most of them bound weakly and did not produce peaks in the same regions (Fig. 5).

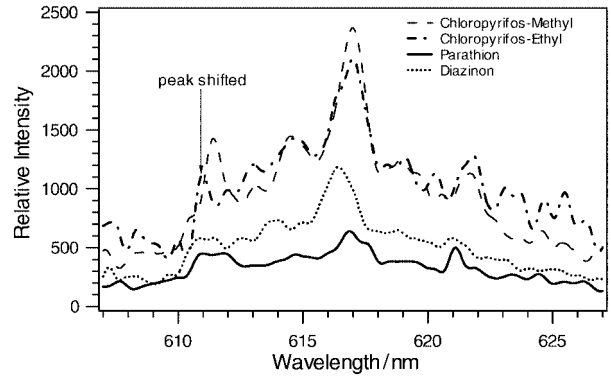

Fig. 4 Spectral response of the methyl chloropyrifos sensor to 100 ppm solutions of structurally similar species, compared to 1 ppm chloropyrifos methyl, excited at 465.8 nm.

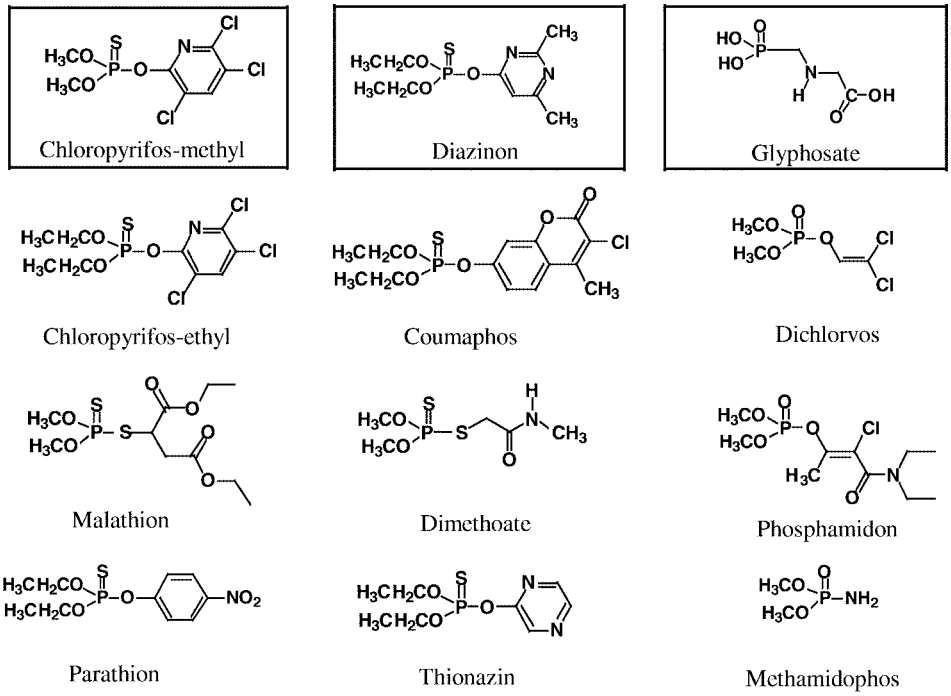

Fig. 3 Chemical structures of the imprinted molecules (enclosed in boxes) and selected chemicals screened as possible interferents.

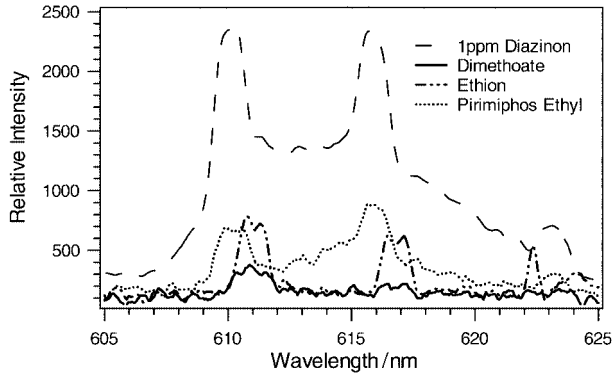

Fig. 5 Response of the diazinon sensor to 100 ppm pesticide and insecticide solutions and to 1ppm diazinon excited at 465.8 nm.

Unimprinted polymer sensor

The unimprinted polymer (blank) was tested against selected pesticides/insecticides from each category imprinted as well as several others. The pesticides and insecticides screened against the sensor were dimethoate, ethion, chloropyrifos, diazinon, glyphosate, and parathion at pH 10.5. All of the compounds evaluated exhibited a positive luminescent response with the sensor at concentrations above 250 ppb, suggesting that they are interacting with the polymer surface and complexing the europium, but not as efficiently or as selectively as with the imprinted polymers. The 80% response time for the sensor ranged from 20 to 30 min, with a positive response in 5 to 10 min. The linear dynamic range of the sensor is from the limit of detection $\approx$250 ppb to 10 ppm in most cases. The decrease in the range at the higher concentrations indicates that without imprinting, the analytes can not completely diffuse into the interior of the polymer and can only interact with the surface binding sites.

Conclusions

Molecularly imprinted polymers for specific sequestering of non-hydrolyzed organophosphate containing pesticides and insecticides have been developed. These polymers can be coated onto optical fibers and used as optical sensors for the detection of these species in aqueous environments. Preliminary results using a small fiber optic spectrometer to decrease the size of the device are promising. Experiments for further miniaturization using blue light emitting diodes as excitation sources are underway. These sensors can be bundled to create an array that can detect multiple analytes in water simultaneously. The superior stability, sensitivity, selectivity, and reversibility of such materials have provided a real time sensing application for molecularly imprinted polymers.

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