

# NANO-BIOMIMETIC MATERIALS FOR THE DETECTION OF CHEMICAL AGENTS IN GASES, AEROSOLS, AND SOLUTIONS

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## ABSTRACT

Recent advances in molecularly imprinted polymers (MIPs) have created a new class of synthetic materials that can mimic the function and selectivity of the bioreceptors with significantly improved stability and real-time sensing capability.<sup>1, 2</sup> As a result; molecularly imprinted polymers can provide high sensitivity and selectivity for molecular recognition while maintaining excellent thermal and mechanical stability. MIPs have been demonstrated to be extremely selective for the agents and related pesticides in water and organic matrices, with detection limits in the low parts per trillion<sup>3, 4</sup> however the application of these materials for air/vapor detection has never been demonstrated. This work combines the sensitivity and selectivity of previously demonstrated MIP sensors with the capture efficiency of denuder technology to create a sensor platform capable of detecting phosphorus based chemical agents, hydrolysis products and pesticides in all weaponized forms.

## 1. INTRODUCTION

Nanomaterials and detector platforms capable of capturing and detecting agents of chemical and biological significance in a variety of environments are desperately needed. Previous traditional approaches have used micro-organisms, enzymes, and antibodies for the molecular recognition of chemical compounds. However, all of these assays require an additional tagging step for signal transduction, making the real-time sensing very difficult. In addition, bioreceptors (i.e. antibodies) technologies suffer shelf-stability problems, are not reversible (one time use) and are not always well suited for small molecule recognition.<sup>3</sup> Non-biologically based detection technologies, such as ion mobility spectrometry (IMS), suffer from sensitivity problems and “false positives”. Still other instrument-based detector technologies either require complicated algorithms to get meaningful data or their size, weight, and logistics prohibit their use in the battlefield.<sup>1, 2</sup> Few if any are capable of doing liquid and vapor/gaseous samples in the same platform in real time without complex sample preparation or extraction.

## 1.1 The Imprinting Approach

The nanobiomimetic materials for this work are produced using molecularly imprinted polymer (MIP) techniques. MIPs have been extensively studied in a variety of academic laboratories.<sup>5-11</sup> Since the polymers can closely mimic antibody-antigen reactions, a majority of these research efforts have focused on separation science related applications such as chiral compound separation and drug screening. However there is a large push to use these materials in state of the art highly selective sensing schemes.<sup>12-17</sup>

In the imprinting technique, the target molecules are first bound into a polymeric matrix using carefully selected monomers and cross-linkers. (Figure 1) The target is then chemically removed, leaving cavities that possess the exact size, shape, and corresponding functional groups capable of rebinding the target. As a result, the molecularly imprinted polymers have high sensitivity and selectivity for molecular recognition while maintaining excellent thermal and mechanical stability first immobilizing the target into a complex containing polymerizable monomers.

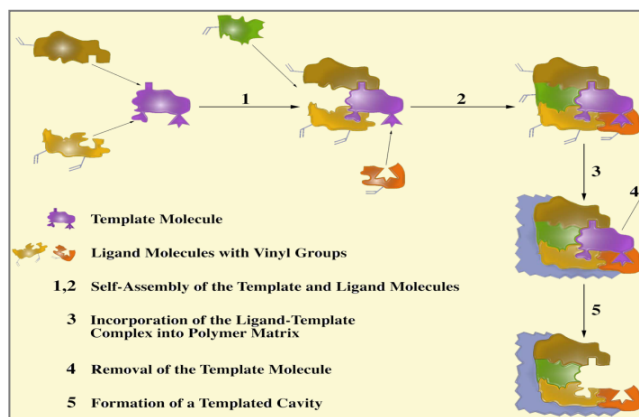


Figure1. The generic imprinting process

In this approach real-time identification of binding can be achieved by incorporating lanthanide

metal ions into the polymer network. Upon binding and coordinating with the target analyte, a unique luminescence signal in the 600-700 nm range arises from the energy transfer of the target to the lanthanide. The recognition mechanism is based on the size, shape, and chemical functionality of the analyte, very similar to that of antibody-antigen binding reactions, but without the need for fluorescence tags to do signal transduction.

### 1.2 Lanthanide Signal Transduction

The use of lanthanide (Eu) ions as spectroscopic structural probes is an established technique.<sup>19-20</sup> The enhancement of luminescence intensity by complexation of tripositive luminescent lanthanide, Ln (III), ions has been explained on the basis of a ligand-to-metal energy transfer mechanism.<sup>21</sup> Energy transfer occurs when a coordinating excited triplet state ligand overlaps a lanthanide excited electronic level. The lanthanide luminescence is pumped by the large cross-section molecular absorbance of the ligand rather than weak lanthanide absorbance. The narrow excitation and emission peaks of lanthanide spectra (typically on the order of 0.01-1 nm full width at half maximum) provide highly sensitive and selective analyses.<sup>22-24</sup>

Lanthanide complexes exhibit long and intense luminescent lifetimes when complexed with appropriate ligands. Proper ligand choice is required to immobilize the lanthanide probe, and provide intensity enhancement needed for detection limits in the parts per trillion (or lower). Europium is typically the lanthanide of choice as it has a “hyper sensitive” transition in the  ${}^7F_2 \leftarrow {}^5D_0$  manifold. This transition is so sensitive to changes in its environment that simple chemical changes (such as the addition of a methyl group) will result in readily distinguishable changes in the splitting patterns.<sup>23-25</sup>

### Denuder Technology

The denuder technology was adapted from a method developed by Steve Hoke and coworkers at the Aberdeen test Center to detect acid gases in fire fighting applications.<sup>26-28</sup> The denuders have been redesigned to be smaller but basically function on the same principles. Denuders are like continuous impingers using gas injection analysis technology (similar to flow injection analysis) to continuously introduce a gas or aerosol sample into a solvent stream where the analyte of interest is taken up into a “trapping” solution. The trapping solution is delivered to the denuder at a rate between 1-4 mL/min using a chemical pump, i.e. a solenoid, syringe, peristaltic, rotary or HPLC pump. Airflow on the order of 1-10 L/min is obtained using a mass flow controller (rotometers or critical orifice) and a vacuum source. This vacuum source located external to the analyzer draws sample air into the denuder where the chemical agent

gases and aerosols are immediately extracted into the liquid phase of the trapping solution to be detected. The solution can then be discarded or re-circulated to continue collection and provide a cumulative detection scheme.

## 2. EXPERIMENTAL

### 2.1 Polymer and Fiber Preparation

Pinacolyl methylphosphonate, (PMP), the hydrolysis product of the nerve agents sarin and soman was chosen as the target since that MIP sensor has been well characterized in prior publications.<sup>1-3</sup> The PMP-MIP was synthesized by preparing complexes of the target using a stoichiometric ratio of 1:1:3 europium, target (PMP) and monomer, vinyl benzoate. (The number of binding species was chosen to accommodate the nine coordinate Eu (III).) Europium nitrate ( $\text{Eu}(\text{NO}_3)_3$ ) was prepared by dissolving europium oxide in water with just enough nitric acid to produce a clear solution. The PMP was diluted and dissolved in a 50/50 water-methanol mixture to which the vinyl monomer was subsequently added. The resulting solution was added to the  $\text{Eu}(\text{NO}_3)_3$  and the pH adjusted with sodium hydroxide to a pH of 9 and 10 for complexation. The resulting solutions were stirred approximately 2 hours, then covered with a watch glass and left to crystallize overnight. The crystals were then removed and the spectra interpreted.

Once the complex was made, polymeric coatings were prepared by dissolving 3 mole percent complex compound in styrene with approximately 0.1 mole percent azobis(isobutyronitrile) (AIBN) added as an initiator, and 2 mole percent divinyl benzene (DVB) added as a cross linking agent. The resulting solution was placed in a glass vial, purged with nitrogen, and sealed using parafilm and screw on tops. The solution was sonicated at approx. 60C until it became viscous.

The fiber optic sensors consisted of a 400micron multimode optical fiber (Thor Labs, Newton, NJ) with the polymeric sensing element chemically bound on the distal end. The fibers were prepared by terminating one end with an SMA connector. The end to be coated was tapered by heating it in an air/acetylene flame and manually pulling the stripped end. (Tapered fibers are much more efficient at coupling the evanescent field to the polymer.) The tapered fiber tips were dipped into the chemically initiated viscous copolymer leaving a uniform layer on the fiber. The polymer finished curing under a small UV lamp, overnight.

Once cured, the polymers were swelled in water with gradually increasing amounts of methanol to remove unreacted monomer and expand the polymer pores which produces accessible sites and facilitates the removal of the

imprinting molecules. The imprinted molecules were removed by washing with 1.0M nitric acid which leaves a weakly coordinated nitrate ion in the cavity. Template removal was verified spectroscopically.

## 2.1 Instrumentation

The detection platform is built on commercially available off the shelf (COTS) technologies. Polymer luminescence was excited using a model 60X-argon ion laser (Melles Griot, Carlsbad, CA). A 488 nm holographic filter (Kaiser Optical Systems, Ann Arbor, Michigan) turned to pass the 465.8 nm line, was used to exclude all other laser lines. Spectra were collected using an f/4, 0.5m monochromator (CVI, Albuquerque, NM) equipped with a Model ST-6 CCD (Santa Barbara Instruments Group, Santa Barbara, CA) using Kestrel Spec Software (K&M Co., Torrance, CA, USA).

Spectra were also collected with a StellarNet Blue Wave miniature fiber optic UV-vis Spectrometer (StellarNet Inc, Tampa, FL). It has an integrated thermo electric cooler (TEC) to stabilize references and allow long detector integration times. The spectral range is 500-850nm with a single element holographic concave grating that produces a flat field image for uniform resolution. The grating is aberration corrected for spectral imaging. The detector has a 25um slit for 1nm resolution, and runs on Spectra Wiz software. A second StellarNet system identical to the one described above but also containing an integrated 15 Watt Deuterium light source with 4000 hour Hamamatsu L2D2 bulb, and 0.5inch diameter filter to allow only the 470-490nm light through was also used.

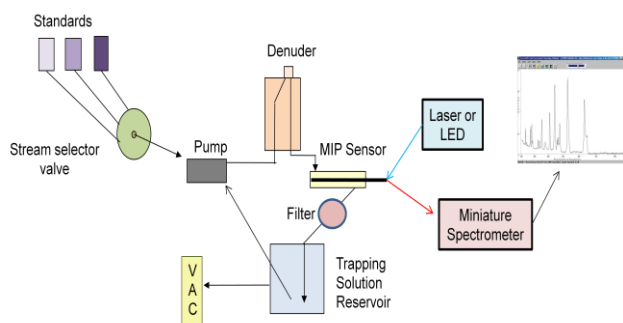


Figure 2. The MIP-Denuder system.

The MIP-Denuder system combined the MIP setup (both the large bench top version and the miniature StellarNet setup) with the denuder. The trapping solution (water adjusted to pH =9.5 with 1M NaOH) was delivered to the denuder at a flow rate ranging from 2 mL/min using an HPLC pump. Airflow on the order of 2 L/min was obtained using a vacuum source that drew sample air with trapping solution into the denuder where the target gases

and aerosols were immediately extracted into the trapping solution. The resulting mixture was then passed over the MIPs coated fiber that senses the captured CWA/NTA. A series of 6 standards were incorporated into the system to provide an internal calibration check. (Figure 2.)

## 3. RESULTS AND DISCUSSION

The first step in this work was to verify that the PMP sensors prepared for this work were performing as well as previously reported.<sup>1</sup> Measurements for the calibration data and response time for all tests were performed using the same fiber to demonstrate the reversibility of the sensors and maintain continuity. For the first test, the large monochromator laser system was used. The MIP fiber was inserted directly into each solution and spectra collected at one minute intervals. The response leveled off after about 11 minutes and that was taken to be our response time. A calibration curve was generated based on the 11minute data. Analytical figures of merit were calculated and the sensor was determined to have a detection limit of about 1ppt (0.7ppt was the reported LOD in the previous work). Detection limit is calculated as 3sigma or 3 times the standard deviation of the blank. Standards were analyzed in the order of both increasing and decreasing concentration in order to demonstrate the reversibility of the sensor. This response was taken as the baseline measurement for the technology and used for comparison with the denuder and miniature systems.

### 3.1 The MIP-Denuder System

Once the sensor and detector were determined to be working properly we began the denuder study. The MIP was placed into "T" connector so the effluent from the denuder would pass over it. The standards were then pumped through the denuder and over the MIP inline. The response from the MIP was collected at one minute. In this phase of the work, maximum response was seen in 9 minutes (so the denuder decreases the response time).

Detection limits for the sensor were calculated based on the 9 minute data. (Figure 3) The detection limit for the standards through the denuder was calculated to be 54ppt. This rise in detection limit was believed to be a result of the fast flow rate over the MIP which possibly decreased the contact time. Several different air and liquid flows were evaluated to see if the detection limit was influenced by the flow. Lower flows did increase the detection limit of the MIP sensor but were at a level that they decreased the efficiency of the denuder. In order to improve the contact time of the solution with the fiber without compromising the denuder, a specially designed glass U shaped tube was developed to collect and hold the solution near the MIP for a slightly longer time. Testing with this tube in the system is underway.

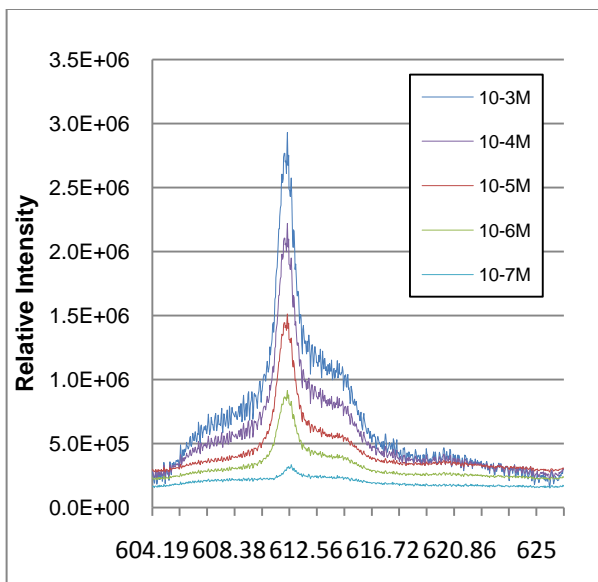


Figure 3. Results from the denuder calibration testing.

### 3.1 The MIP-Denuder Mini Spectrometer System

In the third set of tests we substituted the large monochromator CCD detection system with the StellarNet Blue wave portable system. (The argon laser was still used for excitation.) The system was run with the denuder, MIP, and standards in line. The optimal response was reached within 9 minutes as seen with the denuder in the larger system. (Figure 4) Limits of detection were calculated to be around 75ppt. Considering the size and weight difference between the two systems and taking into account that the smaller system has smaller slits installed (less light/better resolution) this was a promising step towards miniaturization of the system

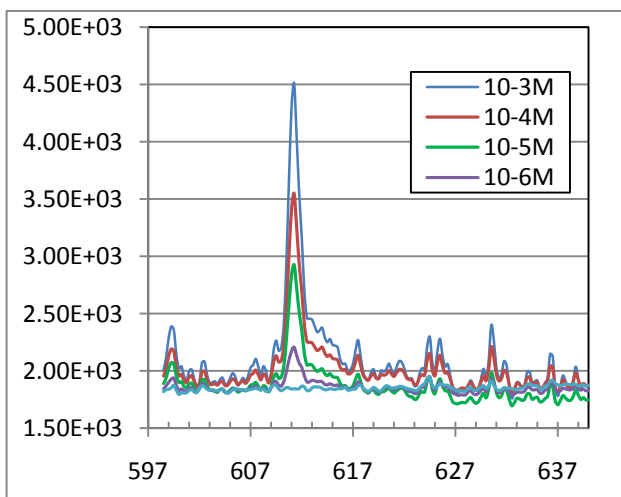


Figure 4. Calibration of the dynamic calibration of PMP with the miniature spectrometer.

### 3.2 Onboard LED System

In the next series of tests we substituted the fiber optic spectrometer with the onboard LED for the large monochromator laser system. Limit of detection at 9 minutes was determined to be 12ppb. (Figure 5) The decrease in sensitivity is primarily attributed to the decrease in the intensity of the light source at the required wavelength 465.8nm. Using the LED directly without the band-pass filter gives more light but was also found to decrease the overall luminescence signal in the area of interest. The wide wavelength range of the LED must excite other pathways of luminescence or cause completion in some way. Adding the filter to limit the light to a 5nm range on either side of the desired wavelength decreases the overall amount of light but facilitates the detection. When technology is able to produce a diode laser in the 466nm range, total miniaturization of this sensor can be realized. For now it is a tradeoff between sensitivity and size.

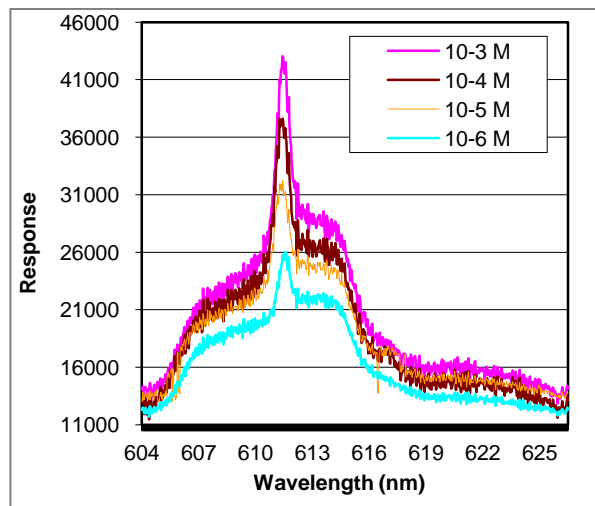


Figure 5. PMP calibration using the StellarNet miniature Spectrometer with internal LED.

### 3.3 Headspace Analysis

In an effort to verify the ability to detect vapors a headspace study was conducted. The intake portion of the denuder system was placed over an empty (but not cleaned) bottle of the PMP. Signals were collected every minute using the large scale system. Positive detection of the PMP vapor was seen within the first minute of analysis and the maximum signal was detected within 7 minutes. This represents the first time that this type of MIP sensor previously used for liquid samples has ever detected a vapor. (Figure 6) The amount of PMP in the headspace was calculated to be 0.9 ppm.

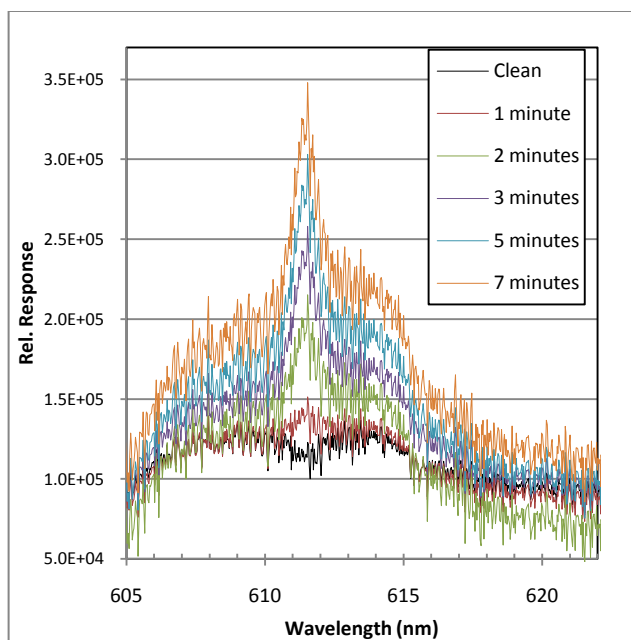


Figure 6. Headspace analysis of empty PMP bottle.

## CONCLUSIONS

This work represents the first time a chemical agent has been able to be detected in any weaponized form (liquid or gas) using a single molecularly imprinted polymer platform. These polymers have been demonstrated to have unprecedented sensitivity for over 30 chemical agents and related compounds. The applicability of this technique to air and vapor will open new opportunities for detection of these species. Further enhancements to the denuder collection system should allow the MIP-denuder system to have the same performance characteristics of the MIP sensors themselves including low ppt detection limits, long linear ranges and virtually no false positives.

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