



Development of Molecularly Imprinted Polymer Sensors for Chemical Warfare Agents

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To protect personnel from hazardous materials, substances must be unambiguously detected in real time. This goal is being pursued with the application of a wide range of technologies. Most chemical sensors must strike a compromise between sensitivity and selectivity to meet the real-time goal. The sensor platform described in this article is designed to eliminate any such compromise. Thus far, we have successfully synthesized the complexes that will form a foundation for molecularly imprinted polymers (MIPs). The demonstration allows us to pursue incorporation of our MIP into the waveguide sensor system. The platform can also be adapted to many other hazardous substances, such as nerve gases or pesticides, by the preparation and substitution of other MIPs.

INTRODUCTION

Molecularly imprinted polymers (MIPs) are advantageous for sensors because they selectively bind the targeted compound while ignoring all others. Therefore an MIP-based sensor should reduce the number of false positives that often plague other types of sensors (for example, pesticides often cause false alarms with nerve gas sensors).

Molecular imprinting is a process for making selective binding sites in synthetic polymers.¹ The process may be approached by designing the recognition site or by simply choosing monomers that may favorably interact with the imprinting molecule based on information found in various compilations in chemical literature. The design of the binding site requires chemical insights. These insights are derived from studies of molecular recognition and self-assembly and include considerations

of molecular geometry, size, and shape as well as molecule-to-ligand thermodynamic affinity.

The successful application of molecular imprinting to chemical sensing requires the aforementioned design approach.² The process involves building a complex of an imprint molecule and complementary polymerizable ligands (Fig. 1). At least one of the molecular complements must exhibit a discernible physical change associated with binding. This change in property can be any measurable quantity, but a change in luminescence (by a shift in wavelength, intensity, or lifetime of luminescent emission) is the most sensitive and selective analytical technique. By copolymerizing the complexes with a matrix monomer and an empirically determined level of cross-linking monomer, the imprint complex becomes bound in a polymeric network. The network must then

be mechanically and chemically processed to liberate the imprinting species and create the binding site.

For the purpose of sensing, a reporting molecule must be a part of the cavity to indicate when the target (“template”) molecule is bound. The best reporters generate fluorescence, since photoluminescence is highly sensitive and adds greater selectivity through the two wavelengths of light that must be used.

An example of a template molecule is pinacolyl methylphosphonate (PMP), a hydrolysis product of the chemical warfare agent Soman. PMP and similar compounds are complex organic molecules that exhibit a variety of chemical functional groups as substituents. Many of these groups possess an affinity for metal ions in solution and can form complexes. By using these natural affinities, metal-incorporating organic copolymers can be made that have a high thermodynamic affinity for binding molecules. The organic matrix of the polymer improves vapor detection since it can selectively condense organic vapor from air, as is the case in solid-phase micro-extraction methods. The judicious choice of a metal ion with useful spectroscopic properties, in addition to a high thermodynamic binding affinity, results in the formation of highly selective and sensitive sensors.

The desired mechanism to detect nerve agents is to discern their effect on the luminescence of a lanthanide ion, Eu^{3+} (Ref. 3). The optical absorption and emission spectra of the triply charged free lanthanide ions consist of very narrow lines (0.10–0.01 nm) that are easily resolvable, unlike broad emission lines associated with organic fluorophores.

GLOSSARY

- Analyte: the molecule being determined in a chemical analysis
- Chemical insight: the ability to logically sort practical information through the application of experience and basic understanding of chemistry
- Cross-linker: a single molecule that has two or more chemical functional groups that link chains of polymers, making them rigid and insoluble
- Ligand: a molecule that binds to another molecule or ion via some form of electrostatic attraction to form a complex; the ligating atom is the atom of the ligand at the point of contact
- Matrix monomer: the bulk constituent of a polymer that functions as the main structural component
- Monomer: a single molecule with chemical functional group that allows for polymerization
- Reporting molecule: a compound, atom, or ion that shows a discernible physical change upon contact with an analyte

Organic compounds with affinity to form metal ion complexes are called ligands. When ligands are placed around the lanthanide ions, the spectral lines are shifted in position (wavelength), may be split (usually ≈ 1.0 nm), and are often altered in relative intensity (some of them may even be eliminated) owing to electrostatic interactions called the Stark effect. The exact character and degree of these changes are specific to each different ligand environment, the major factors being coordination number, coordination symmetry (geometry), and type(s) of ligand.

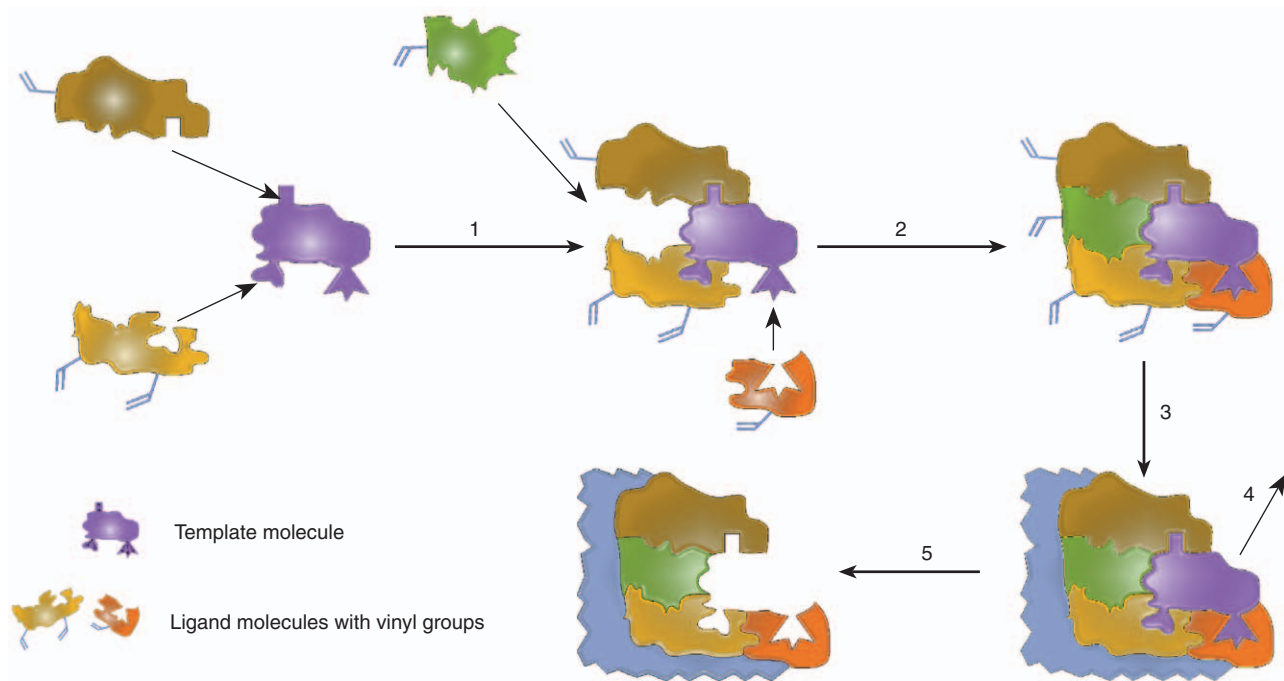


Figure 1. Preparation of molecularly imprinted polymers: 1 and 2, self-assembly of the template and ligand molecules; 3, incorporation of the ligand–template complex into the polymer matrix; 4, removal of the template molecule; and 5, formation of the template cavity.

Even though the ligand field is sufficient to produce these shifts, splits, and intensity alterations, ligand-to-metal electronic coupling is generally not strong enough to result in broadening of the lines. Another consequence of weak coupling and the metal's own electronic transitions is that the radiative lifetimes of lanthanide ions in compounds are quite long, thus simplifying time-resolved measurements that enhance sensitivity. By a judicious choice of coordinating ligand, absorptivity or quantum efficiency can be enhanced and the use of a broadband source such as a light emitting diode (LED) can be used, thereby reducing power requirements and expense considerably. Of the lanthanide ions, Eu^{3+} has the simplest set of splitting possibilities and the most favorable level structure for sensitization. These spectroscopic properties make Eu^{3+} ions attractive for chemical sensing.⁴

The exchange of one ligand for another results in spectral changes that are useful for sensing. In the case of a vapor sensor, PMP need only displace a solvent molecule (typically water) to bind to the Eu^{3+} ion. This process is facilitated by the imprinted cavity geometry and the affinity of organic polymers to absorb organic molecules.

To develop a binding site with appropriate characteristics for sensing, candidate complexes must be screened. Our selection process is now based on finding a complex that has an increase in luminescence with complexation and that will absorb light from an inexpensive and low power source. The complexing species also must bind Eu^{3+} tightly enough to keep it from being removed from the polymer when the imprinting species is removed.

To satisfy these criteria we began screening compounds called β -diketones. Once the pair of complexes, with and without PMP, were shown to exhibit the desirable spectroscopic properties, it was necessary to functionalize the complexing ligands with vinyl groups. This process can be the rate-limiting step in making imprinted polymer sensors; however, the ligands must be polymerizable for the imprinted site to be bound to the polymer.⁵

CHEMICAL DEVELOPMENTS

PMP. Soman is a nerve gas that was developed by Germany before World War II as a pesticide but was found to be lethal to people as well. Fortunately, Soman is readily hydrolyzed and deactivated by aqueous agents to PMP (Fig. 2). Understandably, since Soman is very toxic, PMP has been chosen as the analyte of interest because its presence directly indicates the presence of Soman.

Ligand synthesis. The key to the success of ligand synthesis is to make a proper binding site for PMP. Our initial polymerizable ligand, 4-vinylbenzoyl acetone, was synthesized using a modification of a published method.⁶

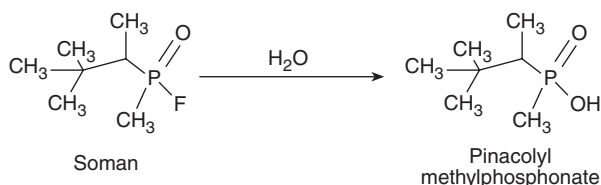


Figure 2. The hydrolysis of Soman to pinacolyl methylphosphonate (PMP).

A more versatile method for making this ligand and a number of vinyl-substituted dibenzoylmethanes was to condense bromine-substituted aromatic compounds with desired ketones (Fig. 3). Ultimately, bromine-substituted β -diketones were formed and converted to vinyl-substituted β -diketones by "Heck coupling," a means for forming carbon-carbon bonds from olefinic compounds and aromatic halides.⁷ The new ligating monomers were used to make imprinting complexes.

The imprinting complexes were synthesized by dissolving three equivalents of β -diketone in acetone (Fig. 4). A slight excess of base was added to generate the β -diketonate anion, to which europium trichloride was added. The resulting tris(β -diketonate) europium was purified and dissolved in acetone to which a basic solution of the template molecule, PMP, in acetone was added. The sodium tris(β -diketonate) europium PMP (the imprint complex) was isolated by its precipitation from solution.

β -Diketone complexes. β -diketone complexes of lanthanides have a long history of use due to their stability and utility as optical sensitizers, two properties useful in sensor applications. When the project began, the choice of excitation sources was limited to LEDs operating at blue wavelengths of 430 nm and higher. To sensitize a Eu^{3+} complex to such a long wavelength, a ligand with extensive conjugation would be needed. Thus, the initial choice for a complexing ligand was dibenzoylmethane. The addition of one or two vinyl substituents would increase the conjugation and give us the best chance of using blue LED excitation. The alternate choice was benzoylacetone, a ligand with less conjugation but one that is less sterically hindered, allowing easier PMP addition.

With the recent availability of 370-nm LEDs, both systems were explored. Figure 5 gives the spectra of the old imprinting complex with vinylbenzoate compared to the new complex using vinyl dibenzoylmethane. Not only does an absorbance appear at the LED wavelength, but the intensity of luminescence increases roughly 10-fold as measured by the areas of the emission bands.

Polymer synthesis. Polymers are typically prepared using 0.1–2.0 mol% imprinting complex with 2 mol% cross-linking agent divinylbenzene, 1 mol% initiator 2,2'-azobisisobutyronitrile, and the matrix monomer. The resulting solutions are placed in glass vials, purged with nitrogen, and sealed using screw-on tops. Polymers

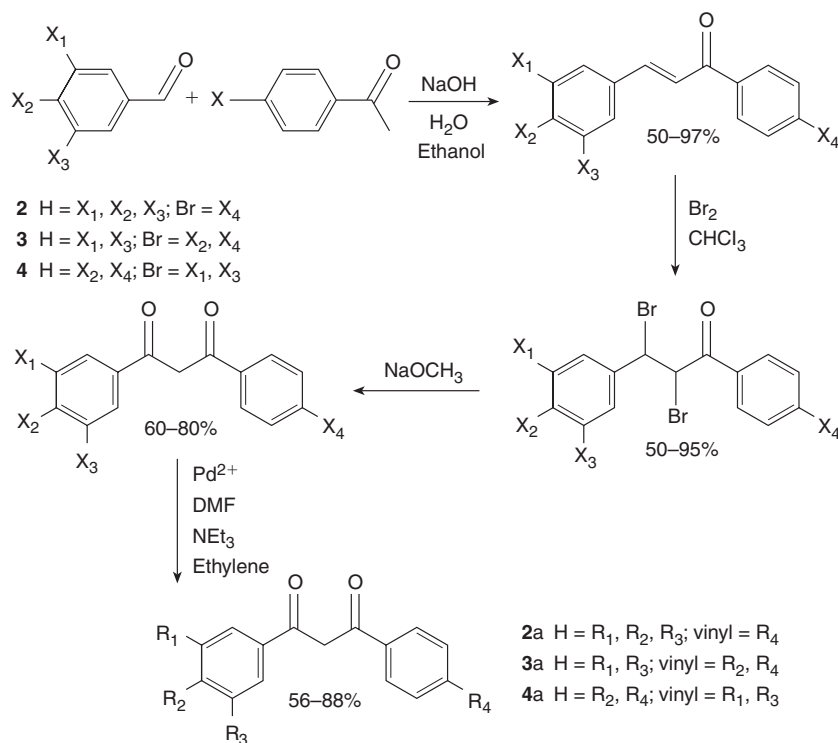


Figure 3. Synthesis methods for vinyl-substituted dibenzoylmethane derivatives using "Heck coupling." The X's and R's represent generic substituents distributed among four positions on the molecule. A hydrogen atom is represented by H, a bromine atom is represented as Br, and a vinyl group is stated as such. Exchanging X's for R's through the synthetic scheme represents a distinct change in the chemical nature of generic substituents.

are typically sonicated for 1–2 h at 60°C. After sonication, the viscous solution (partially polymerized) is coated on optical substrates and polymerization is completed by ultraviolet irradiation. The resulting polymer-coated substrates are swelled in an appropriate solvent, which removes the imprinting molecules. Polymer-coated fibers are employed in the liquid light guides described below.

OPTICAL DETECTION HARDWARE

Advances in spectroscopic-based techniques have resulted in the continuous improvement in sensitivity for measuring a variety of organic and inorganic species. A recent advance involves long-pathlength detection cells for absorbance and luminescence measurements using liquid-filled optical waveguides. Early attempts at producing liquid core waveguides primarily focused on increasing the refractive index (RI) of the liquid contained inside a tube or by physically creating a reflective surface

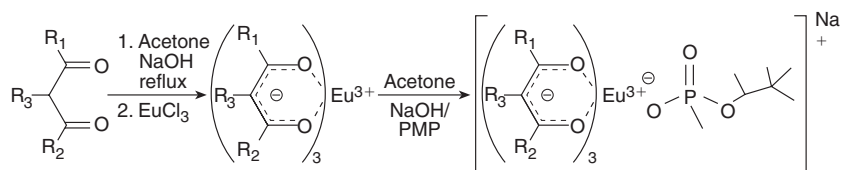


Figure 4. Synthesis of imprinting complexes (R₁ = 4-vinylphenyl or 3,5-divinylphenyl, R₂ = methyl or phenyl, and R₃ = H, allyl, or 4-vinylbenzyl).

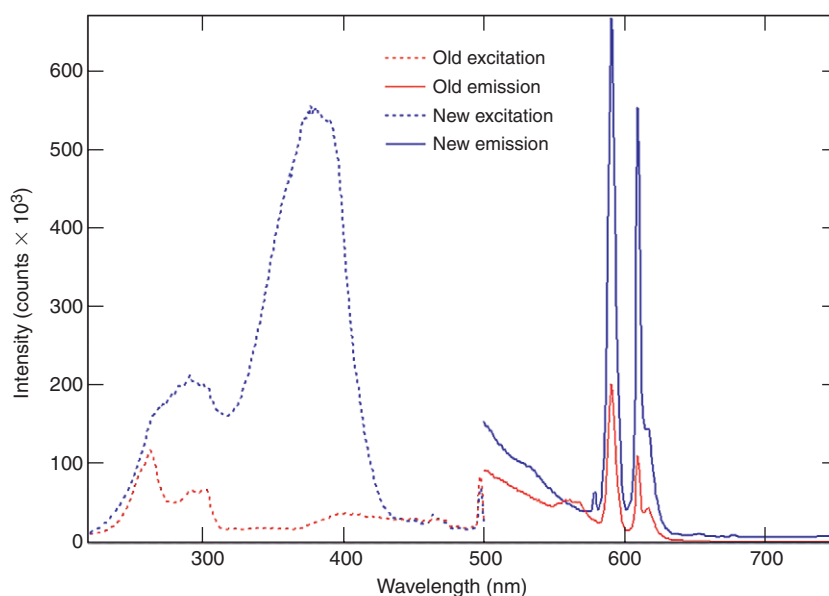


Figure 5. Spectra of old and new imprinting complexes.

inside or outside a tube such that light waves could be reflected along the inside of the tube. Both methods have their obvious limitations. Cells created with higher RI liquids (e.g., carbon disulfide, saturated sucrose solutions) have limited applicability for most analytical purposes. Reflective surfaces can be reactive or can cause light to be absorbed, and subsequently lost, in the tubing walls, resulting in low signal-to-noise values.

The development of a new class of amorphous fluoropolymers has opened the door to a variety of innovative analytical techniques based on optical detection. Certain polymeric formulations have resulted in an amorphous fluoropolymer with an RI of 1.29, which is slightly less than that of water (RI = 1.33). This low RI allows a great degree of versatility for luminescent reactions within a tube detection cell. Further, this fluoropolymer is optically transparent from 200 to 2000 nm, allowing detection cells to be axially or transversely illuminated.⁸ Structural properties allow an increase in permeability for gases and vapors through the fluoropolymer, often being 3 orders of magnitude higher than Teflon.⁹ While fluoropolymer tubing offers the basis for a permeable optical detection cell with increased sensitivity, it lacks the selectivity necessary for today's complex airborne environments.

A sensor prototype has been developed for the detection of PMP and is awaiting final production with active MIP. The waveguide consists of fluoropolymer tubing filled with dilute basic solution. The analyte of interest (AOI) will hydrolyze under the basic conditions within the waveguide to allow selective and reversible binding to the MIP. The AOI-MIP complex will be excited by an inexpensive light source and will produce a narrow luminescence band in the 610-nm region of the lanthanide spectrum. The fluoropolymer waveguide propagates emitted light to a photomultiplier for detection. Reversible binding of the hydrolysis product should allow the sensor to be washed, ultimately resetting the sensor for continued deployment. Because of the inherent sensitivity and fast response time of the liquid core waveguide and the selectivity of the MIP, this sensor has the potential to respond within seconds to a chemical release at or below the time-weighted average/airborne exposure limit, which is in the low parts-per-trillion range for the nerve agents in question. Evaluations of the response

time and detection limits of this innovative sensor are continuing.³

CONCLUSIONS

A detection system has been presented that has the ability to recognize PMP, opening the door to the development of additional MIPs for the detection of a wide range of chemical weapons and explosives. MIPs will be incorporated into a large cross-section detection system for the detection of nerve gases. This fiber-optic-based system can be woven into fabric or wallpaper. The wallpaper can be placed in high traffic areas of buildings (e.g., entranceways) to detect threats being transported through those areas. Ultimately, this system will lead to an array detector that can be interfaced with the building's security system.

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