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High-Throughput Screening of Solid-State Catalysts for Nerve Agent Degradation

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A high-throughput screening (HTS) method was devised to increase the rate of discovery and evaluation of nerve agent degradation catalysts. Using this HTS method, >90 solid state materials, predominantly metal-organic frameworks (MOFs), were analyzed for their ability to hydrolyze the nerve agent simulant methyl paraoxon at two pH values (8 and 10).

Phosphoesters represent one of the most harmful classes of chemical warfare agents (CWAs) in existence.[2] These molecules irreversibly bind to the active site of acetylcholinesterase, a key enzyme involved in the regulation of choline ester-based neurotransmitters. These neurotransmitters are critical to voluntary control of muscle contraction. Exposure to this class of agents in concentrations as low as 0.01 ppm can lead to inhibition of the autonomic nervous system and death by asphyxiation.[3, 4] CWAs remain a clear and present danger to both warfighters and civilian populations.[5] In the past year, two of these agents (sarin, (RS)-propan-2-yl methylphosphonofluoridate and VX, ethyl ({2-[bis(propan-2-yl)amino]ethyl}sulfanyl)(methyl)phosphinate) have received substantial attention due to their use against civilian populations and by terrorist organizations.[6]

Metal-organic frameworks (MOFs) have emerged as promising degradation catalysts against CWAs.[1, 7-9] MOFs are highly porous, crystalline materials constructed from inorganic nodes (secondary building units, SBUs) and multitopic organic ligands forming three-dimensional lattices.[10] MOFs typically exhibit large pores, with high accessible surface areas, and large sorption capacities.[11] Structural diversity and tunability of both the metal nodes and ligand components adds to the attractiveness of MOFs as heterogeneous catalysts.[12, 13]

Zirconium-based MOFs (Zr-MOFs) remain the most successful MOFs to date for CWA degradation, primarily due to the chemical stability and strong Lewis acidity of the Zr(IV) SBUs.[14-16] Recent reports examining the CWA degradation mechanism of Zr-MOFs[1, 7, 17] indicates direct binding of the nerve-agent to the SBU and stabilization of the hydrolysis transition state (Scheme 1). The large body of literature on Zr-MOFs has enabled the study of factors that may affect CWA degradation. Features such as pore size,[18] SBU connectivity, and addition of functional groups on the ligand struts[7, 19] have been examined, leading to steady improvements in catalytic activity. However, efforts to establish broader structure-activity relationships (SARs) over a greater number of solid-state materials has been hindered by time-consuming, low-throughput assays, which limit the number of materials that have been examined.

The vast majority of reports to date employ a screening method that introduces a substrate (e.g., CWA or simulant) into an alkaline solution, typically 0.45 M N-ethylmorpholine at pH = 10.4, in which the MOF or other heterogeneous catalyst has been suspended (Figure 1).[7, 18, 20-23] Product formation is generally monitored over time by ^{31}P-NMR[18] or UV-Visible spectroscopy.[20] The use of strongly alkaline conditions serves to increase the hydrolytic degradation rate of CWA simulants such that they are detectable in a reasonable timeframe (i.e., $t_{1/2}$ of minutes vs. days). These serial assays are both time and material intensive leading to a limited throughput of the materials screened. In most reports, a small set of MOFs are tested, top performers identified, which may be then tested against CWAs.[21, 23-26]
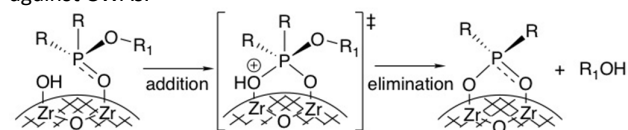
Scheme 1. Proposed mechanism for phosphate hydrolysis by Zr-MOFs. Adapted from Plonka et al.[1]

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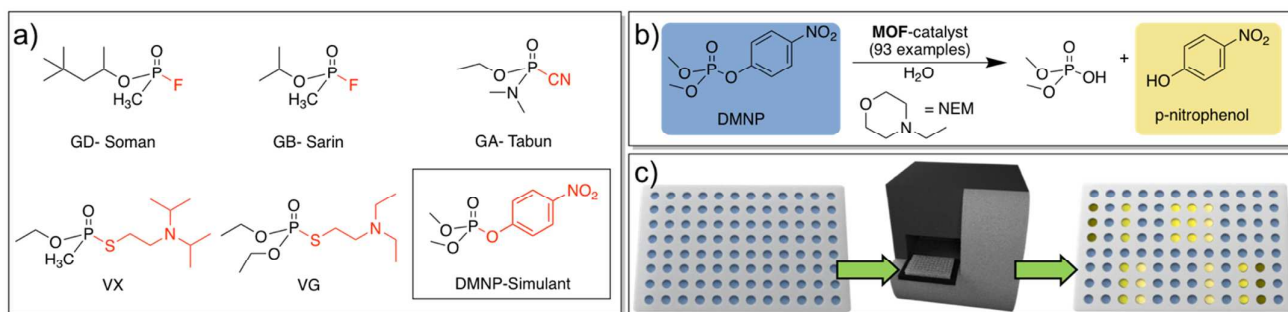
Figure 1. a) A selection of nerve agents with leaving groups highlighted in red. b) Hydrolysis scheme for DMNP. c) High-throughput screening schematic.

In addition to the limited scope of materials assessed, few studies have examined variations in reaction conditions, such as differences in solution pH, concentrations of base, etc. on the rate of catalysis.[19, 26] One report showed that pH had a substantial effect on the catalytic activity of the Zr-based UiO-66 where lowering the pH from 10.4 to 8.8 resulted in an increase in the hydrolysis rate.[26, 27] Factors such as MOF synthesis conditions, particle size, and defect content have been largely overlooked, due in part to the limited throughput of current screening methods.[28] In addition, there are many MOFs with SBUs containing metals other than Zr that may be suitable catalysts, but have not yet been tested.

To address this limitation, we report a fast and reproducible high-throughput screen (HTS) that allows for parallel evaluation of CWA degradants in a fraction of the time required for evaluation using the conventionally reported protocol. HTS is commonly used in the fields of biochemistry and drug discovery, but has not been widely reported for use in the catalytic studies of MOFs or other solid-state materials. In two recent reports, the development of a HTS screen for identification of non-catalytic solid state materials was described.[29, 30] The HTS method described here is time and materials efficient, enabling rapid analysis of a greater variety of materials and assay conditions.

The catalytic degradation assay was scaled to run in a 96-well plate in an unmodified, commercial plate reader (Figure 1). Development of a HTS assay was based on similar colorimetric assays routinely employed in drug discovery.[31] As configured, this assay can analyze 24 materials simultaneously in triplicate in under one hour. Modifications were made to the previously reported assay conditions for dimethyl 4-nitrophenylphosphate (DMNP, Figure 1) simulant hydrolysis, to ensure compatibility and reproducibility in individual assay plate wells. The total volume for each analysis was reduced to 100 µL, and dilution of both simulant and MOF was required to avoid signal saturation. The substrate DMNP has limited solubility in water so it was necessary to employ methanol as a solubilizing agent (see ESI).

Effective dilution of MOF particles to an optimal concentration posed a technical challenge. A high concentration of MOF particles can result in scattering of the UV-Vis beam during analysis, while low MOF concentrations are difficult to accurately dispense. This challenge is not common in biochemical assays, where all of the assay components are soluble. The accuracy of these CWA assays relies on well-dispersed suspensions that are diluted and dispensed into each well. Using the materials ZIF-8, UiO-66, and NU-1000 (which have low, medium, and high activity for CWA degradation, respectively), we demonstrated that controlled amounts of these MOFs could be dispensed into 96-well plates with excellent reproducibility (Figures S1-S3). Consistency in the dispersion of our samples was achieved with rigorous centrifugation and vortexing procedures (see ESI).

Experiments to validate the HTS assay were performed on a small number of MOF catalysts with *N*-ethylmorpholine buffer at both pH 10.0 and 8.0. The results of the HTS are in good agreement with literature reports of the same materials using a conventional assay (Table S1). In addition, the Z-factor of our HTS assay was determined. The Z-factor is a statistical metric for separation of sample and background signal that is a widely accepted parameter of assay quality (see Equation S1).[32] Assays with a Z-factor >0 are considered valid, while assays with a Z-factor >0.5 are considered excellent (with 1 being ideal). For example, UiO-66 and NU-1000 gave calculated Z-factors of >0.45 and >0.50, respectively (Table S2). Indeed, evaluation of several MOFs showed generally outstanding Z-factors >0.5 at both pH 8 and 10 (Table S2). This indicates that our assay is excellent, giving highly reliable data.

Having validated our HTS design, a library of MOFs and related materials were evaluated. Initial linear rates (k, (mM/sec) were calculated assuming zero-order kinetics using the change in absorbance from *p*-nitrophenol generated from the degradation of DMNP (Equation S2, Figure S4). 93 MOFs, metal oxides, carbons, and zeolites were chosen based on their reported water stability and potential catalytic activity. Among this library of compounds were two sets of distinct UiO-type Zr-MOFs: one set that were prepared using different synthetic procedures, and another set that incorporated modified ligands. This library also contained Al, Cu, Zn, Ti, Co, Hf, and Fe based MOFs not previously studied for CWA degradation, as well as one polyMOF.[33]

Figure 2 shows the top 15 MOFs ranked in descending order of activity at pH = 8. The ranking of these materials emphasizes the lack of correlation between activity at pH = 8.0 and 10.0, as catalytic activity at one pH are not predictive of activity at the other. The results of the HTS are consistent with literature reports regarding the effectiveness of MOF materials for organophosphate hydrolysis. Z-factor and regression analysis of these top performers show that reliable statistical

data can be gathered using HTS. None of the zeolite or metal oxide materials show significant activity compared to MOFs.

The HTS assay reveals more subtle SAR among related Zr-MOFs that have largely been treated as a common group. The ability to run a large number of samples in parallel allows for small differences in similar materials to be closely examined. Use of chemical modulators during MOF synthesis, the identity of the modulator, and defect density were all found to have a significant effect on catalytic activity and were easily identified with the HTS assay (Figure 2).
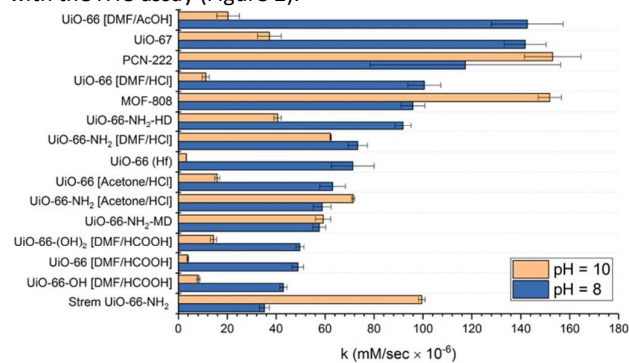

Figure 2. Top 15 catalytic materials ranked based on DMNP degradation at pH = 8 (blue; activity at pH = 10 shown in gold). Activity is based on average slope of three experiments. The solvent and modulator used in the synthesis of the MOFs is shown in brackets for some materials (detailed synthetic conditions are provided in Table S3).

From within the larger library of materials screened, two sets of compounds were chosen to more closely examine the trends that could be revealed using this HTS method. The first set of compounds consists of UiO-66 prepared using four different synthetic conditions (i.e., variations in solvent and modulator). These materials show significantly enhanced catalytic activity at pH 8 vs. pH 10, across all synthetic preparations for UiO-66. For each UiO-66 preparation a >4-fold increase in activity was observed at pH 8 (Figure 3, Table S4), consistent with literature reports on UiO-66.[27]
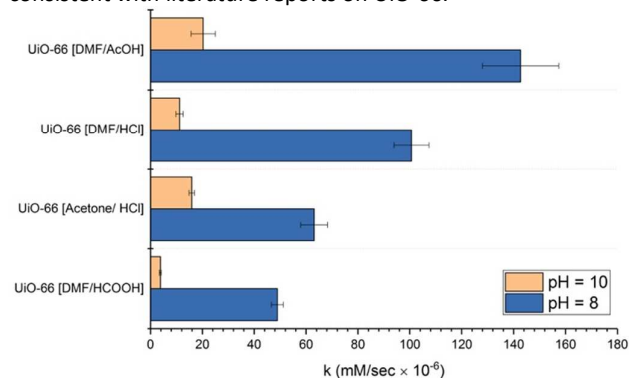

Figure 3. DMNP degradation rates of UiO-66 prepared using different synthetic conditions. The solvent and modulator used in the synthesis of the MOFs is shown in brackets.

The UiO-66 materials used here all have the same structure as determined by powder X-ray diffraction (PXRD, Figures S5-S8), but the activity of the materials varies substantially as a function of their synthetic preparation conditions. Simply by changing from a formic acid (HCOOH) modulator to acetic acid

(AcOH), the activity of UiO-66 increases from an initial rate of 4.9×10^{-5} to 1.43×10^{-4} mM/sec. The particle size of these four UiO-66 preparations is similar as determined by SEM, thus variations in activity cannot be attributed to differences in particle size (Figures S9-12).[28] The differences in catalytic activity may be due to the number of lattice defects generated by the different materials synthesis conditions. Overall, this data shows that both the reaction pH and the material preparation are important parameters to consider in evaluation, optimization, and selection of CWA degradation catalysts.

The high activity of Zr-MOFs has largely been attributed to the inherent Lewis acidity of the Zr metal center.[22] In this screen, we tested the effect of Lewis acidity in a series of four MOFs that used different Group IV metals (Ti, Zr, Hf). Three tetravalent, terephthalate MOFs with similar topologies to UiO-66(Zr), MIL-125-NH$_2$, PCN-415, and UiO-66(Hf) were examined. MIL-125-NH$_2$ is a Ti(IV)-based MOF that is stable in aqueous environments. PCN-415 is a mixed metal MOF (Ti/Zr) that is constructed from a $Ti_6Zr_2O_{12}$ SBU.[34] Finally, UiO-66(Hf) is a direct analog of UiO-66 that has generally displayed few differences from UiO-66(Zr) in other catalytic reactions. Among these Group IV MOFs, UiO-66(Zr) had the highest catalytic rate (Figure 4). Surprisingly, the MOF with the most Lewis acidic metal in the series, MIL-125-NH$_2$, had the lowest activity. Based on this data, it is clear that Lewis acidity alone does not dictate the catalytic degradation rate of the phosphoester bond and other factors potentially serve a more influential role. This suggests that MOFs based on other Lewis acidic metals (e.g., Al or Zn) might show enhanced catalysis in an optimized MOF environment.
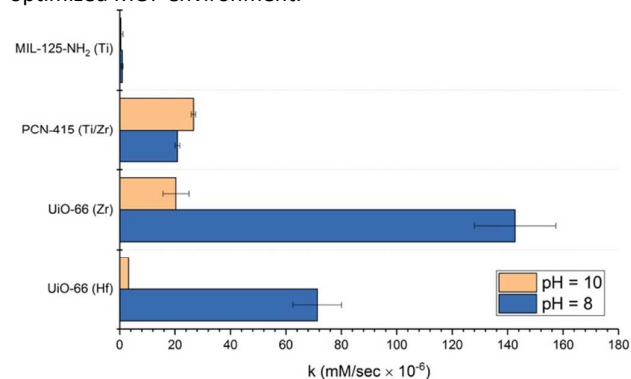

Figure 4. DMNP degradation rates of Group IV metal MOFs with similar structures.

In addition to Lewis acidity, activity of Zr-MOFs has also been correlated to availability of open metal sites on MOF SBUs. A recent study by Peterson et al. examined the relationship between defect density and catalytic activity where defects of UiO-66-NH$_2$ are intentionally varied, characterized and effects measured in both simulant and agent hydrolysis.[35] This result indicates that availability of neighboring open metal sites have a greater effect on catalysis. Data from the HTS supports this hypothesis based on the results with UiO-66 synthesized under different conditions (Figure 3) and from defect regulated UiO-66-NH$_2$ materials (Figure S13, Table S5).

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The HTS method reported herein provides several advantages over traditional DMNP screening methods for solid-state degradation/neutralization catalysts. First, the amount of catalyst, reagents, and solvent required for screening is substantially reduced. Second, the speed of the assay allows for examination of many different materials in a rapid timeframe. Hits can be quickly identified and the best candidates can be selected for more detailed mechanistic studies and optimization. Finally, we can determine the effect of assay conditions by comparing activity under a variety of reaction conditions. By optimizing reaction conditions, we can also identify and optimize catalysts that are active under conditions more relevant to field conditions. In the future it will be important to correlate results from this screen with activity in agents. Ultimately, these findings demonstrate that HTS can be applied to the screening of MOFs and other solid-state catalysts for CWA degradation, as well as other catalytic reactions.

Conflicts of interest

There are no conflicts to declare.

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