ABSTRACT

Molecularly imprinted polymers (MIPs) have numerous practical applications, including integration with quartz crystal microbalances to make specific, stable, chemical sensors, but most published research literature does not provide details concerning the specificity or stability of such an imprinted polymer. A polymer made from polyacrylic acid monomers, templated with benzoic acid, was tested for specificity with solutions of benzoic acid, acetic acid, phenol, and terephthalic acid passed through samples of uniform size under vacuum filtration. Additionally, MIP samples were also stored for extended periods of time in varied microclimates and then tested for performance, and consequently, stability. Initial conclusions indicate that a benzoic acid-templated MIP can capture the specific targets of benzoic acid, benzaldehyde, and terephthalic acid, while excluding species of similar size and functionality. Furthermore, benzoic acid-templated MIPs operate best when stored in a dry environment between 9ºC and far below 120ºC with shelf-lives for at least months.

EXPLORING SPECIFICITY AND STABILITY OF A MOLECULARLY IMPRINTED POLYMER

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Introduction & Background

Acoustic wave devices, either bulk acoustic wave (BAW) or surface acoustic wave (SAW), operate on the transduction of mechanical energy to electrical energy, and *vice versa*, within a piezoelectric (PZ) solid state material. Materials that experience the piezoelectric effect, in which an applied alternating electric current is generated or altered by applying some form of mechanical stress, can make very sensitive gravimetric (mass-sensitive) devices. SAW-based devices operate using waves that only penetrate a small distance into a material, whereas BAW-based devices operate with a wave that penetrates the throughout the bulk of the substrate. One such example of a piezoelectric BAW-based device is a quartz crystal microbalance, most commonly called a QCM. Typically about the size of a dime, QCMs are primarily made of quartz, and often have some sort of thin metal electrode on part of its surface, such as gold or silver. When changes in mass (Δm), even at the sub-nanogram level, occur on a QCM surface, variations in resonance frequency (Δf) can efficiently be readily detected. This is in accordance with Sauerbreys' equation, shown in Eqn. 1:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\mu_q \rho_q}}\Delta m \qquad \text{(Eqn. 1)}$$

[where Δf is frequency change, f_0 is the fundamental resonant frequency, Δm is the mass change, A is the active vibrating area, μ_q is the shear modulus of the material, and ρ_q is the density.][1] It is important to note from this equation that the frequency change is linear with mass change.

QCM sensitivity comes from how the crystal is naturally cut. For example, if the quartz has an AT-cut, the result is a temperature independence in gas phases and ideal use for gas sensors.[2] Functionalization of the gold electrode surface allows for the specificity of the QCMs. There are multiple different strategies available to selectively capture target molecules atop a quartz crystal microbalance and induce a frequency change, including selective chemical tethers with receptor sites on their distal ends. These theoretical tethers, however, seem less selective than might be desired. After intensive literature review on the manufacture of molecular imprinted polymers, most commonly called MIPs, the MIP strategy was posited to potentially be more fruitful for sensor research, especially since MIPs are known to be highly selective in regard to detection of biologically relevant molecules.

Much like enzymes with the "lock and key" model, in which an enzyme is the lock and an appropriate substrate is the key,[3] MIPs are "locks" formed around template molecules. Functional monomers, the repeating building blocks of MIPs, are essentially "molded" into specific receptor sites ("keyholes") by imprinting on the target analyte, able to allow only a very select number of target molecules ("keys") to fit their specialized shapes and relevant bonding locations,[4] which often involve hydrogen bonding.[5] Once the MIP is formed, the template molecules must be removed by some chemical means, either with solvent or gentle heating. After that, only template molecules or target molecules (which are very similar to the template molecules in size, shape, and/or chemical functionality) should theoretically able to be "captured" by these MIP receptor sites,[6] informally known as "pockets." An example of the MIP approach for a small molecular target (Propofol) is shown in Figure 0 on the next page.

Figure 0: Propofol (top left) is used as a template for the formation of highly selective and sensitive molecularly imprinted polymers (MIPs).

[Hydrogen atoms have been omitted for clarity in this diagram.]
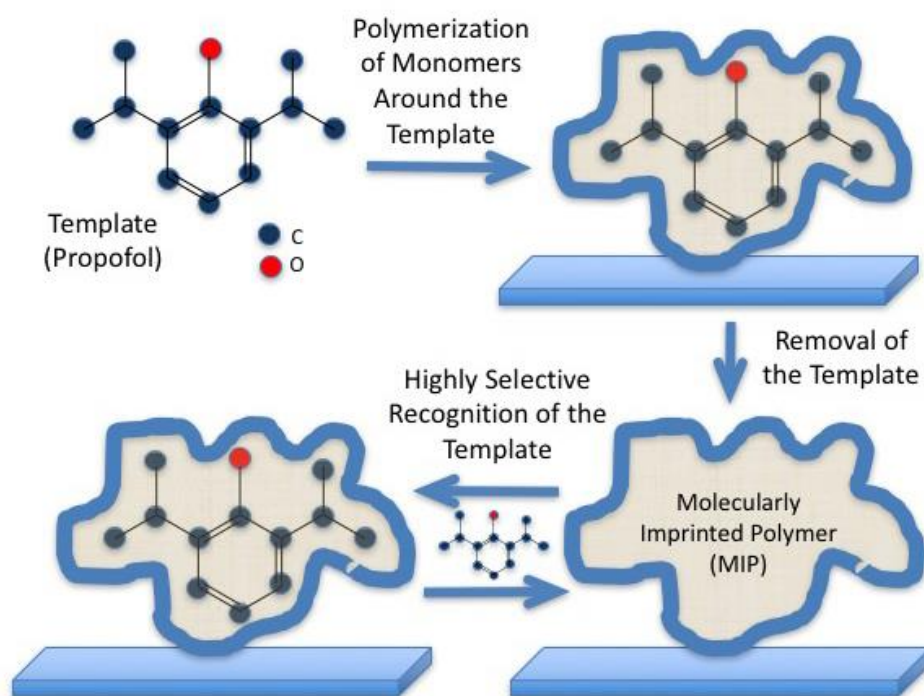
Literature research on MIP-based QCM sensors revealed the countless applications they are being considered for, ranging from forensic science in trace evidence detection[7,8] to biomedical in pathogen detection and monitoring[9,10] to national and international security.[11] In regard to security, our research is particularly interested in the detection of gaseous chemical agents, including paraoxon[12] and related species.[13] For example, sarin gas was used in the relatively recent attacks in Syria[14] and the subway attack in Tokyo. Researchers have since been able to use a non-toxic analog molecule of Sarin called diisopropylfluorophosphate in other

projects focused on rapid and reliable detection,[15] allowing for laboratory work with Sarin MIPs as a reasonable academic goal.

Sarin, diisopropylfluorophosphate, and other relatively small substrates, like simple carboxylic acids, have very few challenges with regard to the lock-and-key model. There is less molecular surface area, and consequently fewer necessary confirmatory bonds and linkages, to be concerned about compared to macro-biomolecules, such as proteins,[16] viruses,[17] and pollen.[18] However, because there are few challenges in that regard, there is a scarce amount of studies to back up both the specificity and stability of MIPs.[19]

In the bulk of the literature regarding MIP production and use, relatively little has been reported about the specificity of these polymers towards smaller molecular substrates, specifically related to their selectivity and longevity. In this study, the decision was made to explore the selectivity and sensitivity of a fabricated MIP for one small molecule, benzoic acid, in relation to different chemicals that could potentially fit and stay in MIP receptor sites, and to evaluate the MIP's stability over time and varied temperatures.

Experimental

Materials:

Acetic Acid, Glac., Reagent ACS – $C_2H_4O_2$; FW: 60.05; aqueous (Fisher Scientific, CAS 64-19-7, Batch # UN2739): potential MIP target

Benzoic acid, 99%, extra pure – $C_7H_6O_2$; FW: 122.12; solid (Acros Organics, CAS 65-85-0, Lot # A0375236): MIP template & potential MIP target

Buffer Solution pH 4.00 +/-0.01 – aqueous (Fisher Science Education, Code S25849A, Lot # 8GA190): pink, acidic buffer solution for titration pH probe calibration

Buffer Solution pH 10.00 +/-0.01 – aqueous (Fisher Science Education, Code S25849, Lot # 7GD951): blue, basic buffer solution for titration pH probe calibration

Celite 545 Filter Aid – CNa_2O_3; FW: 105.99; solid (Fisher Scientific, CAS 68855-54-9): substrate to test with vacuum filtration of solutions

Ethyl alcohol, 200 Proof, Absolute, Anhydrous, ACS/USP Grade – C_2H_5OH; FW: 46.07; liquid (Pharmco-Aaper, CAS 64-17-5, Batch # 11243-23 & Lot # KKH25C): solvent used in MIP preparation & vacuum filtration testing, and titration

Ethyl ether (Diethyl ether), Reagent Grade ACS Anhydrous – $(C_2H_5)_2O$; FW: 74.12; liquid (Pharmco-Aaper, CAS 60-29-7, Lot # C18B21CAS00000EE): solvent used for extracting templates when preparing MIPs and used in vacuum filtration tests of various solutions

Hydrochloric acid, 37%, ACS reagent – HCl; FW: 36.46; aqueous (Sigma-Aldrich, CAS 7647-01-0, Batch #11396JJ): acid used to help polymerization

Phenol, 99+% – C_6H_5OH; FW: 94.11; liquid/solid (Aldrich, CAS 108-95-2, Lot # 2229CJ): potential MIP target

Phenolphthalein – $C_{20}H_{14}O_4$; FW: 318.32; aqueous (Aldrich, CAS 77-09-8): used as indicator in acid-base titration

Potassium hydrogen phthalate, ACS primary standard, 99.95-100.05%, GOLD LABEL – 2-$(HO_2C)C_6H_4CO_2K$; FW: 204.23; solid (Aldrich, CAS 877-24-7, Lot # 121223): used to standardize concentration of basic titrant

Poly(acrylic acid) – $(C_3H_4O_2)_n$; FW: variable; solid (Aldrich, CAS 9003-01-4, Batch # 117K5055 & Lot # 04610EIV): MIP "monomer" & substrate to test with vacuum filtration of solutions

Sodium Hydroxide Certified ACS Pellets – NaOH; FW: 40.00; solid (Fisher Scientific, CAS 1310-73-2, Lot # 093309): dissolved in deionized water to make basic titrant

Materials (cont.):

Terephthalic acid, 98% – $C_8H_6O_4$; FW 166.13; solid (Aldrich, CAS 100-21-0, Batch # 03704LH): potential MIP target

Water, deionized/distilled – H_2O; FW: 18.00; liquid (City of Syracuse Water Department): universal solvent, used to make various solutions and dilutions

Preparation of Benzoic Acid Molecularly Imprinted Polymers (MIP): The following experimental method was adapted from a recent procedure published in *Talanta*.[20] A 2.0 gram sample of polyacrylic acid (PAA) was dissolved in 40 mL of dry ethanol in a round bottom flask. Then, 0.080 g of benzoic acid, partially dissolved in ~0.05 mL of ethanol to ease transfer, was injected *via* syringe into the stirred solution, followed by 0.20 mL of hydrochloric acid. All of this mixture, except the miniscule amount that could not be recovered from the stir bar, was transferred to a Rotovap, with additional 20-40 mL of ethanol. The solvent was removed until what remained was either a film inside the flask that had the appearance of *Saran* wrap, which could be peeled off by a spatula (Figure 1), or a colorless solid. In order to remove the template molecule, benzoic acid, and other remaining volatile molecules, such as water, the polymer was soaked in diethyl ether (abbreviated as ether) at least three times for several hours.
For all MIPs other than MIP #2, the polymer was air dried before use. For MIP #2, it was dried in an oven set at 120 degrees Celsius for 90 minutes.

Testing the Benzoic-Acid (BA) Templated MIP: To test each BA-MIP, a sample between 0.127 g and 0.128 g was placed of the desired medium (MIP, PAA, Celite) into a 200-mL sintered disc frit on top of a filtration Erlenmeyer flask. 10 mL solutions of the chosen target solute were made in ether solvent, or in the case of terephthalic acid, a 10% (v/v) solution of ethanol in water. 3 mL of each were set aside to be considered the "before filtration" solutions. The remaining 7 mL of each were poured through the medium and frit into the flask while the

vacuum is sealed and on its highest setting. The vacuum was generated by an aspirator connected to a standard laboratory sink. Each filtrate remainder was put it into its own vial. All "before" and "after" vials were uncapped, in order to allow solvent to evaporate, leaving behind residual acid substrates. This was done to help make dilution easier and also in order to account for ether's easily observable high evaporation rate, which could affect filtrate collection and make dilution easier.

With the original solvent taken care of, tenfold dilutions of the original concentrations were made with either water or 10% (v/v) ethanol in water. (Ex: Since I started with 0.50 M acid in ether, the final dilution would be ~10 mL 0.050 M acid in solvent.) All titrations with MIPs #1-3 were done with water as the dilution solvents. The final titration with phenol and MIP #4 and all titrations with MIP #5 were done with 10% (v/v) ethanol in water as the solvent. A separate, basic, bulk solution of ~0.10 M sodium hydroxide in water was also prepared, standardized with oven-dried KHP.

Titrations of BA-functionalized MIPs: There were two possible ways to carry out the acid-base titration, both of which used standardized ~0.10 M sodium hydroxide for the base titrant. For MIPs #1-#3, two drops of phenolphthalein indicator were added to the acidic solution, and the base was released dropwise from a biuret into the vial of acid, with constant mixing. For MIPs #4 onward, a clean, calibrated pH probe hooked up to a Vernier LabQuest was placed into the vial with the acidic solution. The base was released dropwise from a biuret through a drop counter (also hooked up to the LabQuest) and into the vial, with constant mixing. The former method worked best as a visual learning experience; the latter worked better to provide data of how the acidity changes in the solution over time, regardless of pH range or solution concentrations.

<u>**Results**</u>

In total, five distinct molecularly imprinted polymers were made with benzoic acid as the template. The first polymer appeared to be the most picturesque, especially before the template was removed via ether wash.

Figure 1: Photograph of the first MIP-benzoic acid film made in this lab, immediately after peeling from the interior wall of the round bottom flask
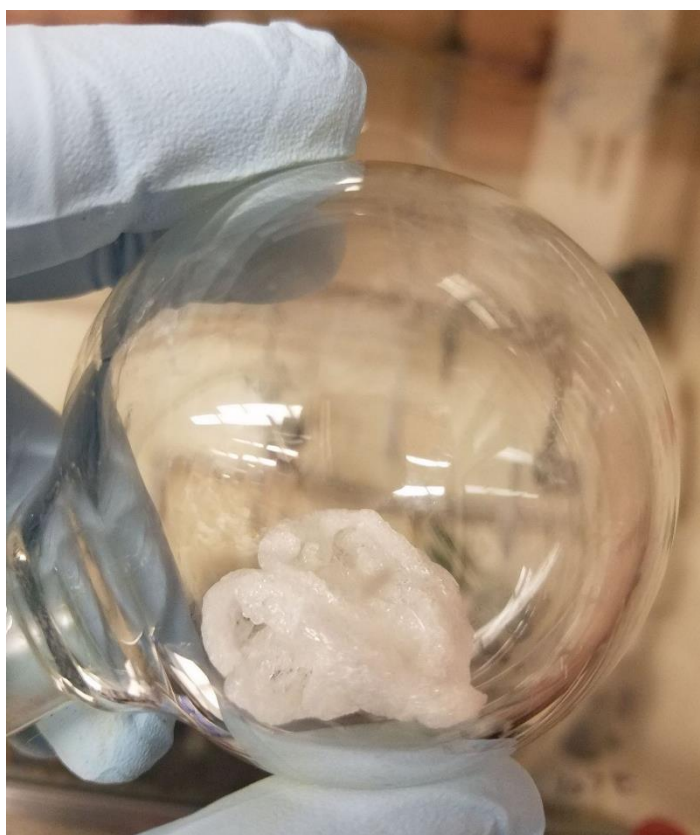
The quasi-plastic wrap appearance of this film makes sense, considering Saran plastic wrap is simply another type of polymer called polyethylene.[21] Remember that this MIP #1 film shown still contained the benzoic acid template. After washing with ether and before grinding, it still very much resembled what is shown in Figure 1, a used Saran wrap-like quality.

After being rotovapped, washed in ether, air-dried, and ground with a mortar and pestle, MIP #1 became a white powder. MIP #2 became light, tan, and flaky, with not much powder. All subsequently made MIPs resembled MIP #1. MIPs #1, #3, #4, and #5 were much easier to evenly spread around the inside of the frit used for vacuum filtration, compared to MIP #2.

At one point, the manufacture of a MIP without a template (also known as a "blank MIP," non-imprinted polymer, or NIP) was attempted, just to observe what would happen compared to any of the successful MIPs in this study. The NIP ended up being thick, hard, green, very translucent, and with an incredibly strong adhesion to the inside of the flask. No further study was done with the NIP, since it could not be removed from the flask without being submerged in a base bath, which consequently compromised its structural and chemical integrity.

For these first initial MIP observations with the vacuum filtration setup, we needed as much data from as many benzoic acid concentrations as possible. It was also critical to test substrates other than MIP #1 in this same experimental setup, to ensure that target molecules are being captured by MIP specificity and not another reason.

Table 1: Results of titration with ~0.10 M NaOH base and phenolphthalein indicator

Acid-Water Solution (9.9 mL each)	Which Acid?	Filtration Medium	Amount of 0.09 M NaOH Needed
0.05 M BEFORE	Benzoic Acid	N/A	5.7 mL
"0.05 M" AFTER	Benzoic Acid	0.126 g MIP #1	4.35 mL
0.025 M BEFORE	Benzoic Acid	N/A	2.75 mL
"0.025 M" AFTER	Benzoic Acid	0.127 g MIP #1	2.10 mL
0.0125 M BEFORE	Benzoic Acid	N/A	1.2 mL
"0.0125 M" AFTER	Benzoic Acid	0.127 g MIP #1	0.8 mL
0.006 M BEFORE	Benzoic Acid	N/A	0.5 mL
"0.006 M" AFTER	Benzoic Acid	0.127 g MIP #1	0.35 mL
0.003 M BEFORE	Benzoic Acid	N/A	0.2 mL
"0.003 M" AFTER	Benzoic Acid	0.128 MIP #1	0.1 mL
Ether Wash Control (Diluted Tenfold) BEFORE	Benzoic Acid	N/A	1 drop
Ether Wash Control (Diluted Tenfold) AFTER	Benzoic Acid	0.127 g MIP #1	½ drop
0.05 M BEFORE	Benzoic Acid	N/A	5.7 mL
"0.05 M" AFTER	Benzoic Acid	0.124 g PAA	5.65 mL
0.05 M BEFORE	Benzoic Acid	N/A	5.7 mL
"0.05 M" AFTER	Benzoic Acid	0.127 g Celite	5.3 mL

Even higher concentrations than listed were desired, which would have been fine in tenfold dilution, but it was difficult enough to get 0.50 M benzoic acid to dissolve in water. This was also the first indication that perhaps when performing the titration, water should not be used by itself, since benzoic acid and others are not very water soluble. It was not until much later on (testing terephthalic acid) when the change had to be made to incorporate 10% (v/v) ethanol in the water, since terephthalic acid is insoluble in ether.[22]

Figure 2: ^{1}H-NMR spectrum collected to check for presence of benzoic acid.
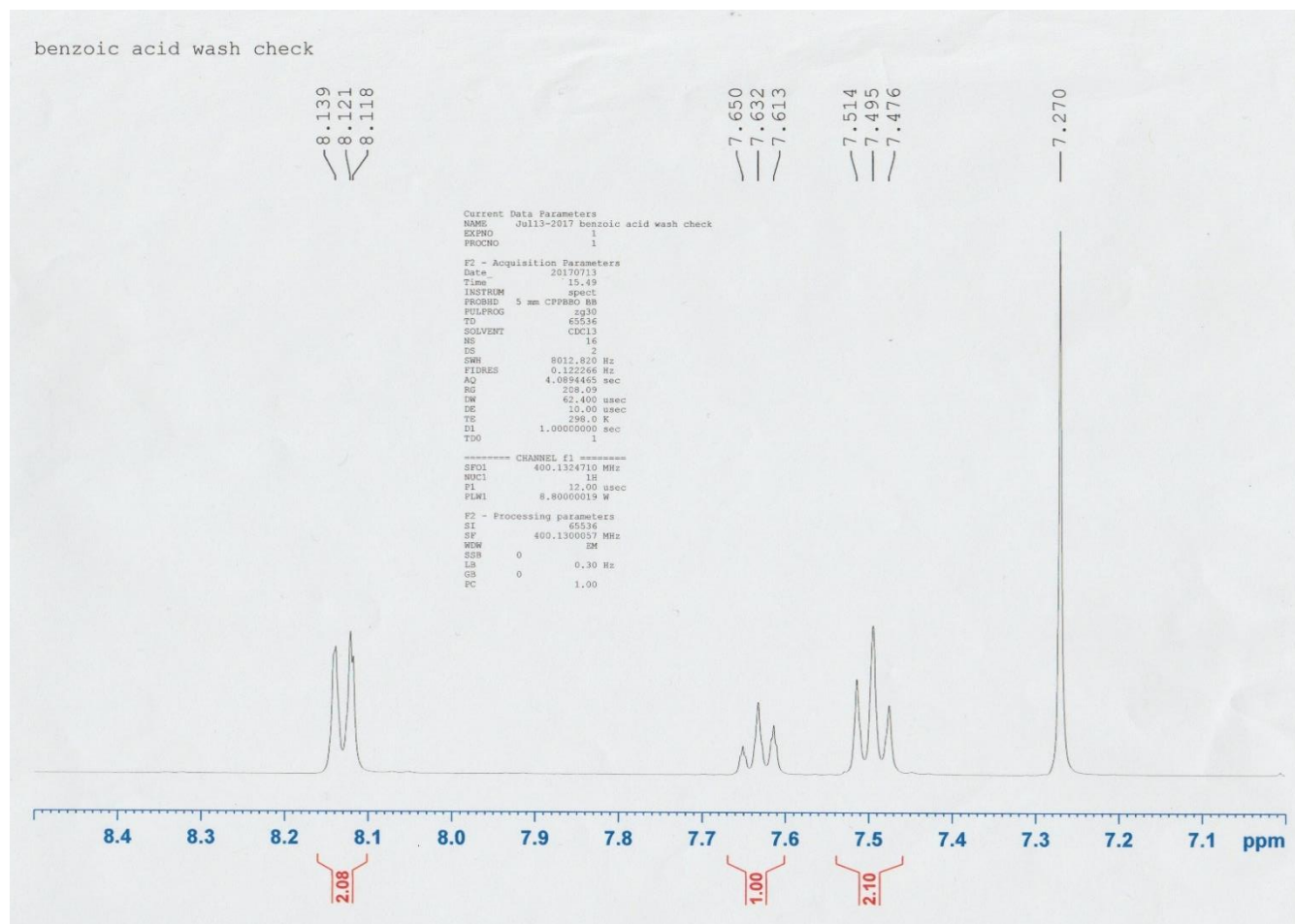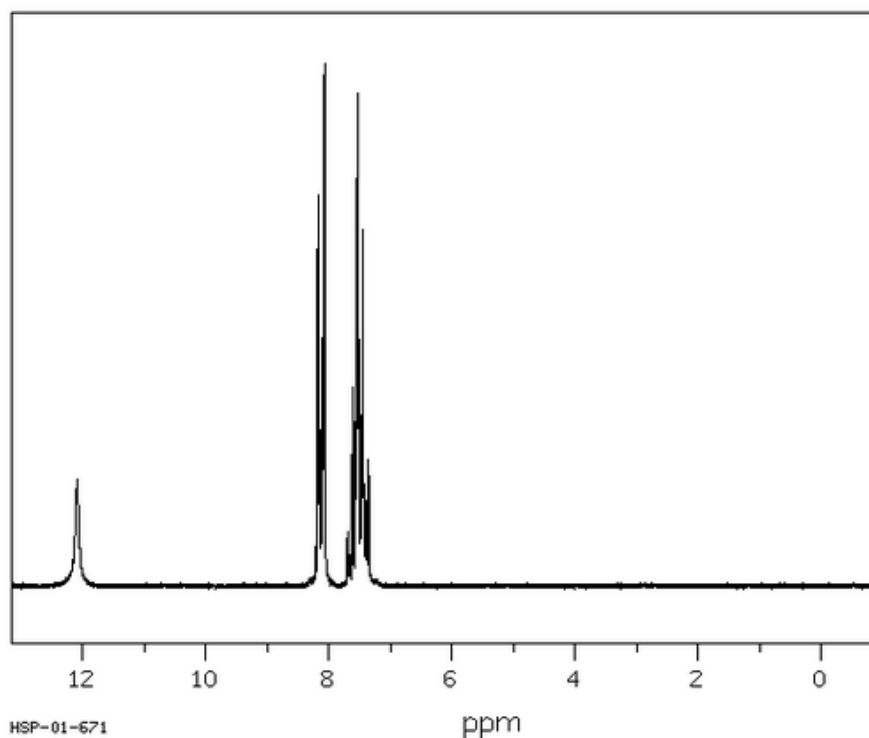

This initial NMR test, performed on some of the leftover white residue after vacuum filtration and after the ether had evaporated, was necessary to continue with the research in good faith. The residual resembled benzoic acid at first glance and was inferred as such. Still, reassurance that the solid was resoundingly BA and not some other white solid (ex: MIP, polyacrylic acid, a contaminant, et atl) was necessary before moving forward with any more MIP methods in similar manners to MIP #1.

Figure 3: Reference benzoic acid ^{1}H-NMR spectrum[23]

	Hz	ppm	Int.
	1083.07	12.094	203
	735.55	8.213	76
	732.98	8.185	645
	731.36	8.167	749
	729.74	8.149	267
	729.22	8.143	300
	727.26	8.121	246
	725.12	8.097	706
	723.84	8.083	356
	723.16	8.075	1000
	689.66	7.701	103
	688.04	7.683	86
	682.74	7.624	253
	681.88	7.614	381
	679.24	7.585	194
	677.70	7.567	440
	676.84	7.558	231
	675.82	7.547	781
	674.96	7.537	746
	674.37	7.530	943
	673.85	7.525	821
	672.74	7.512	473
	670.09	7.483	297
	668.55	7.465	329
	667.53	7.454	508
	667.02	7.448	684
	666.50	7.442	549
	665.56	7.432	196
	664.79	7.423	128
	664.45	7.420	134
	662.49	7.398	140
	661.04	7.381	241
	660.01	7.370	142
	658.30	7.351	211

89.56 MHz

0.040 g : 0.5 ml CDCl$_3$

HSP-01-671

ppm

This BA spectrum from literature lines up well with the spectrum collected in Figure 2, confirming some benzoic acid passed through the MIP and frit. The large peak on the right side of Figure 2 must be a CDCl$_3$ solvent peak.

This confirmation allowed for the manufacture of MIP #2 and MIP #3, two polymers that were dried in different ways and looked distinctly different to the naked eye. Divergent properties were hypothesized before any vacuum filtration occurred with either.

Table 2: Results of titration with ~0.10 M NaOH base and phenolphthalein indicator

Acid-Water Solution (10.0 mL each)	Which Acid?	Filtration Medium	Amount of 0.09 M NaOH Needed
0.05 M BEFORE	Benzoic Acid	N/A	5.70 mL
"0.05 M" AFTER	Benzoic Acid	0.128 g MIP #2	5.25 mL
0.025 M BEFORE	Benzoic Acid	N/A	2.25 mL
"0.025 M" AFTER	Benzoic Acid	0.128 g MIP #2	2.10 mL
0.05 M BEFORE	Acetic Acid	N/A	4.50 mL
"0.05 M" AFTER	Acetic Acid	0.128 g MIP #2	4.10 mL
0.025 M BEFORE	Acetic Acid	N/A	2.20 mL
"0.025 M" AFTER	Acetic Acid	0.128 g MIP #2	2.05 mL
0.05 M BEFORE	Benzoic Acid	N/A	5.30 mL
"0.05 M" AFTER	Benzoic Acid	0.128 g MIP #3	1.60 mL
0.025 M BEFORE	Benzoic Acid	N/A	2.70 mL
"0.025 M" AFTER	Benzoic Acid	0.128 g MIP #3	1.10 mL
0.05 M BEFORE	Acetic Acid	N/A	0.85 mL
"0.05 M" AFTER	Acetic Acid	0.128 g MIP #3	0.80 mL
0.025 M BEFORE	Acetic Acid	N/A	0.75 mL
"0.025 M" AFTER	Acetic Acid	0.128 g MIP #3	0.75 mL

Benzoic acid was tested each time as a MIP was made, acting as a control, since BA was the

template used to make the BA-MIP. If the MIP did not properly capture much of the template,

like in the case of MIP #2, then something must be wrong with the MIP itself. The testing of

MIP #2 could have stopped after all the benzoic acid dilutions were titrated, but since the acetic

acid dilutions were already set to go, they were tested as well. MIP #3 fared much better, with

the benzoic acid working as intended and seemingly not much acetic acid being captured. It was

after this round of testing that it was deemed appropriate to move on from this simpler, more

visual method of titration, with phenolphthalein as an indicator, and go forth with an instrumental method that could produce graphs.

Figure 4: Results of titration with 0.10 M NaOH base and pH probe
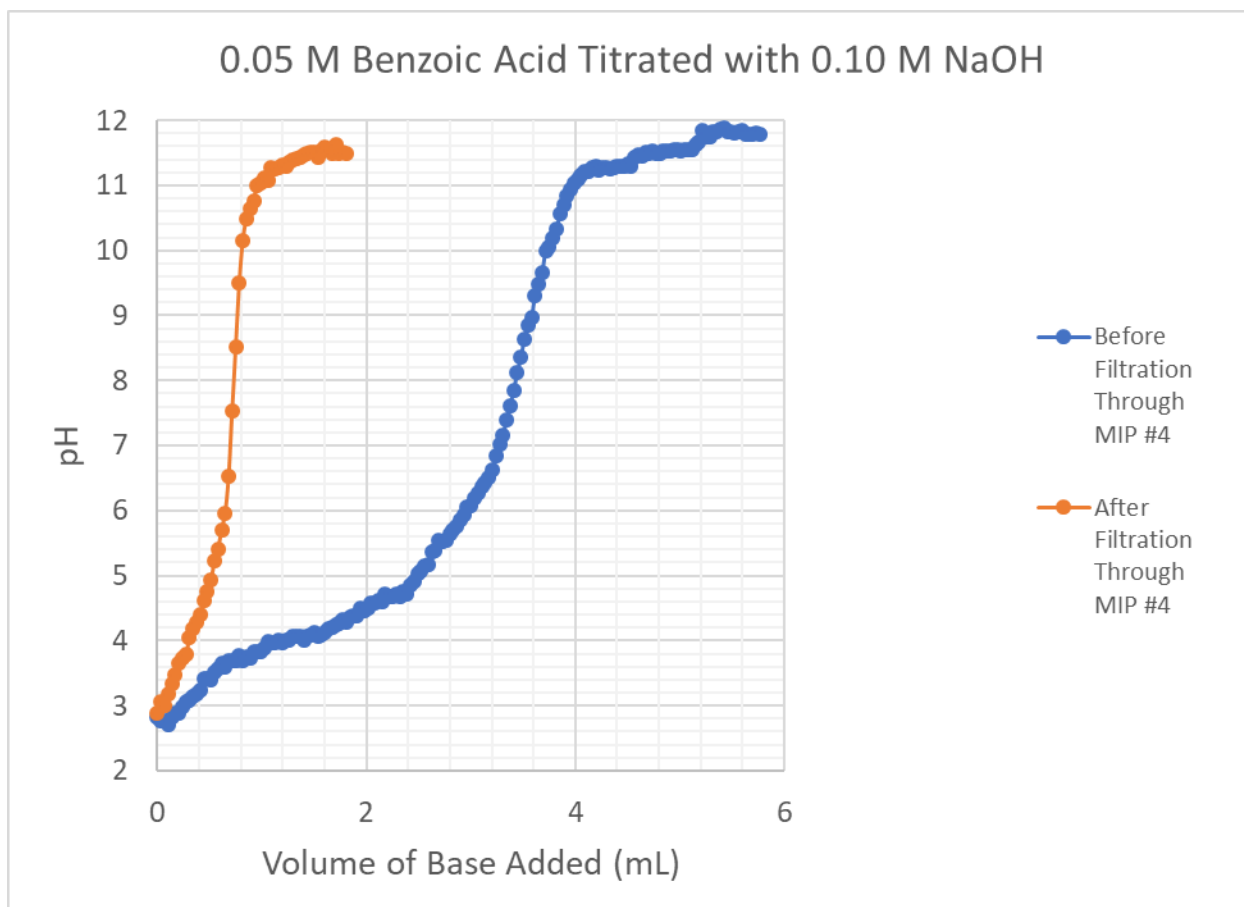
A couple of values in Tables 1 and 2 showed that the MIP was able to capture its template as a target. However, being able to visualize in this manner with more data really solidifies the perspective. Additionally, a calibrated pH probe is much more accurate and efficient compared to a chemical indicator, since indicators only work within a certain pH range and are more preliminary rather than confirmatory. It is much easier to see that the dilution of the "before

filtration" solution takes far longer to reach the equivalence point than the dilution of the "after" solution.

**Figure 5: Results of titration with 0.10 M NaOH base and pH probe**
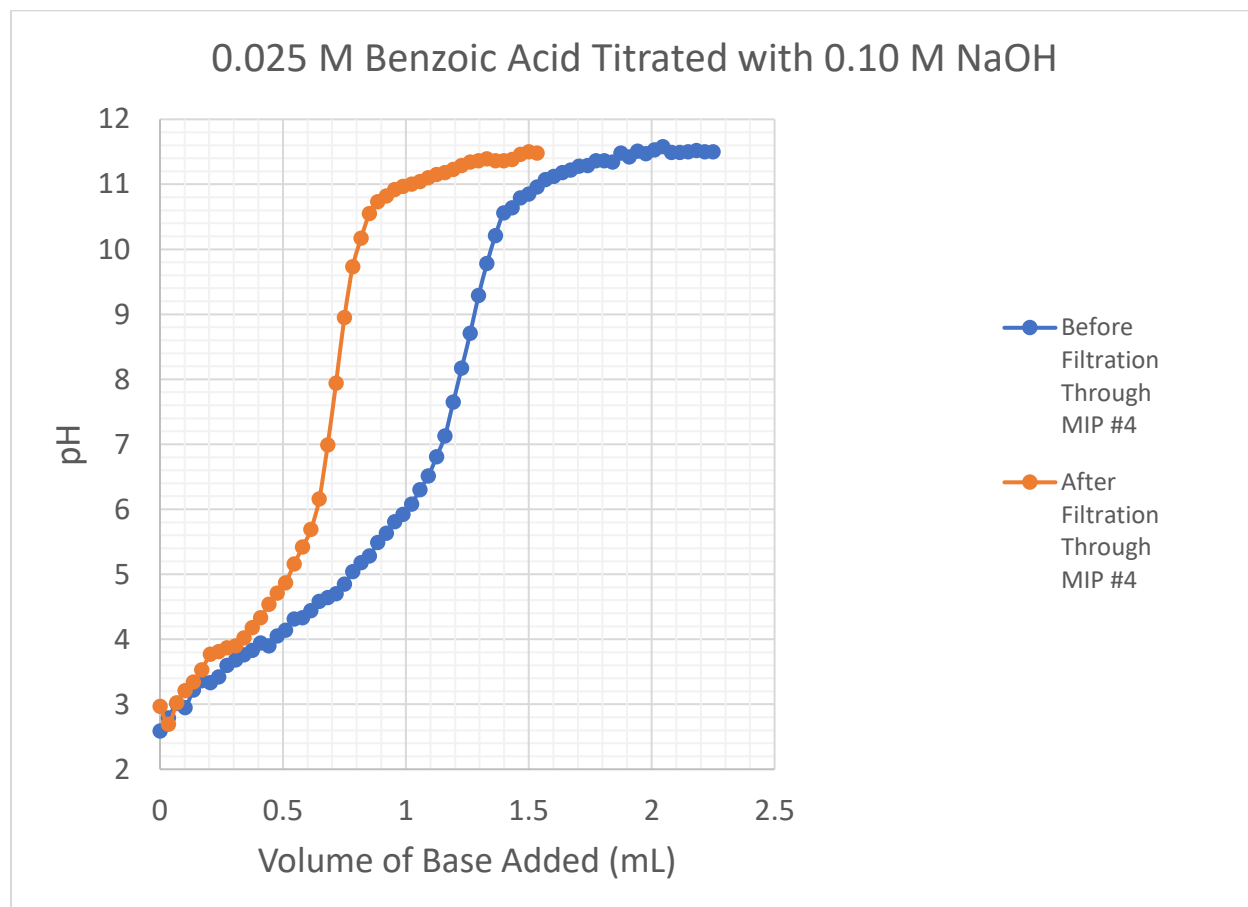
Compared to the graph in Figure 4, which was testing a solution of target molecule initially twice as concentrated as this one, the "before" and "after" of this solution are not as far apart, but there is still enough to show a stark difference in plotlines.

Phenol was the next logical molecule to test as a target against BA-MIP, due to its structural similarities with benzoic acid, namely the benzene ring.

**Figure 6: Results of titration with 0.10 M NaOH base and pH probe**
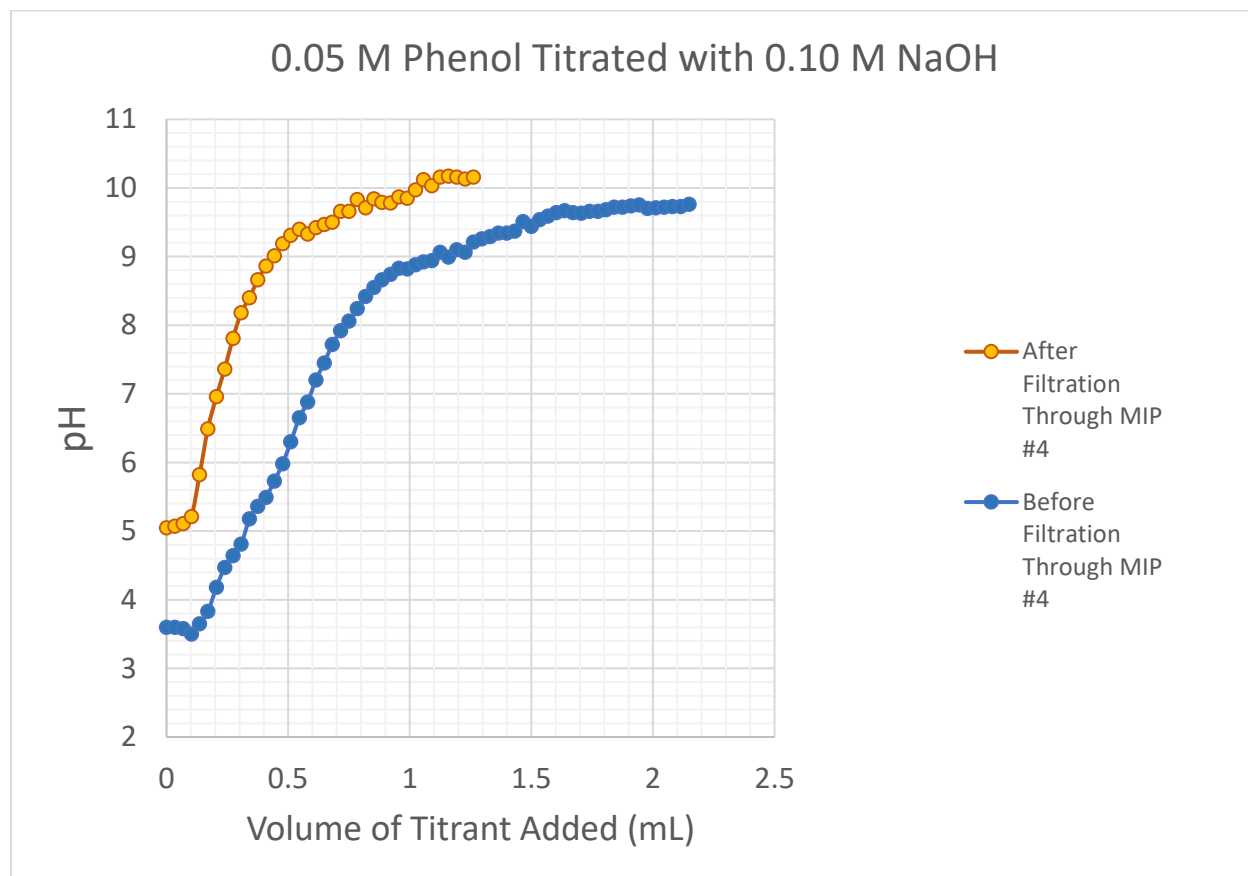
It is interesting to observe in Figures 6, 7, and 9 how the two solutions start off as drastically different pH levels, according to the probe. More importantly, it looks like these two plotlines are so close in proximity and shape that they could virtually be superimposed on one another.

**Figure 7: Results of titration with 0.10 M NaOH base and pH probe**
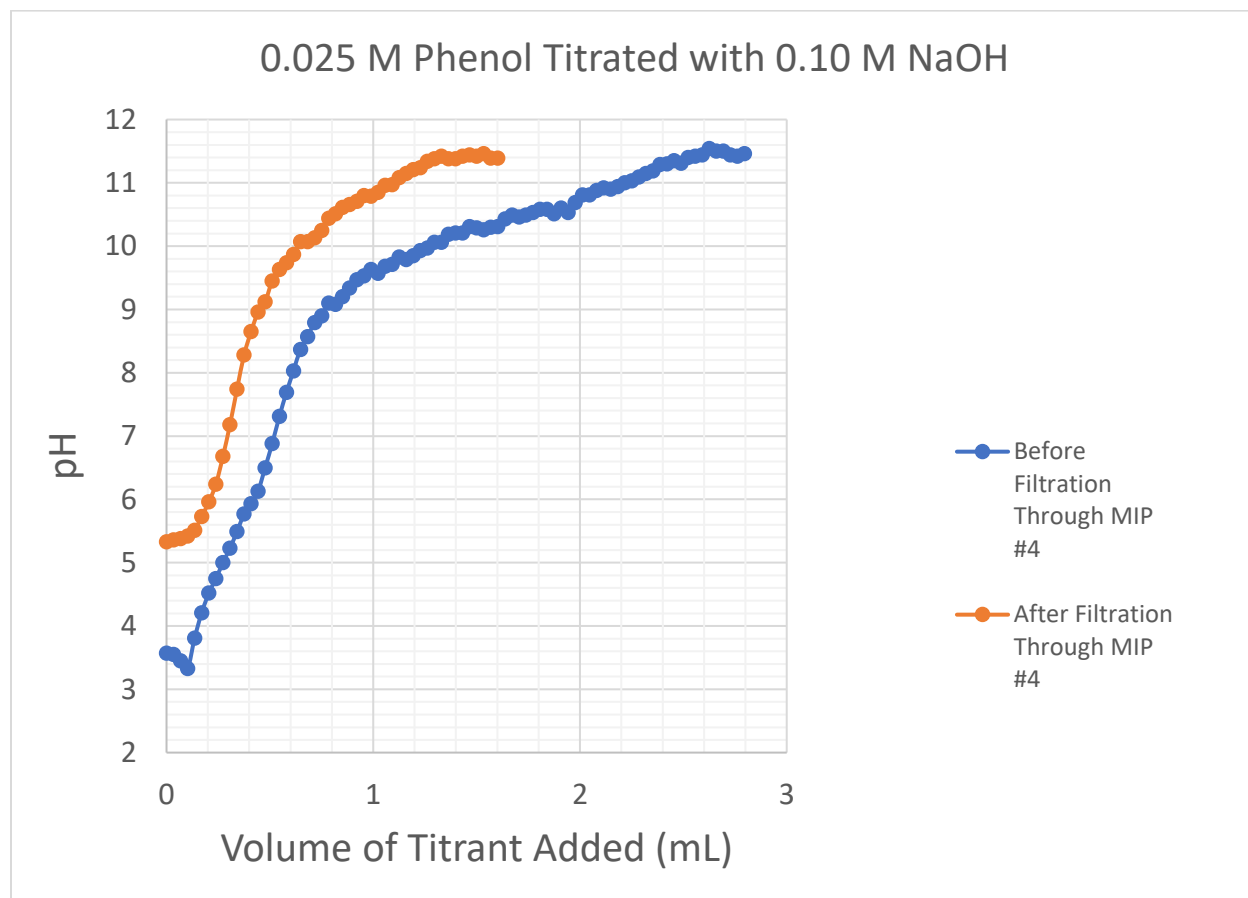
Similar to the difference between different benzoic acid concentrations in Figures 4 and 5, the "before" and "after" plotlines look even closer to each other than the previous graph with the higher concentration of target solution. For all subsequent MIPs after this, with the exception of any new targets (i.e. terephthalic acid), it seemed like it was no longer warranted to test more than one concentration of a solution. 0.50 M solution (then 0.050 M dilution for the titration) was made the standard concentration for these tests.

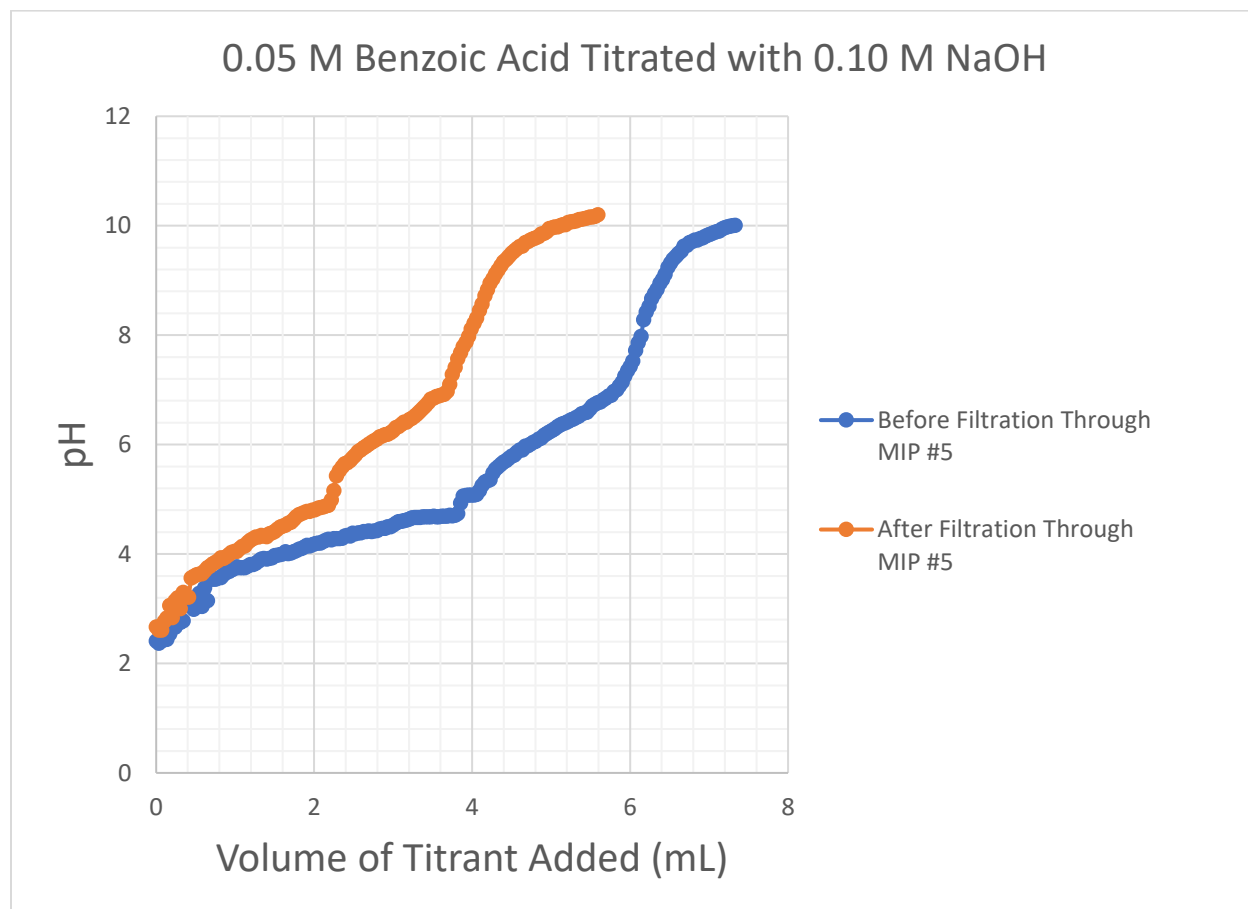**Figure 8: Results of titration with 0.10 M NaOH base and pH probe**
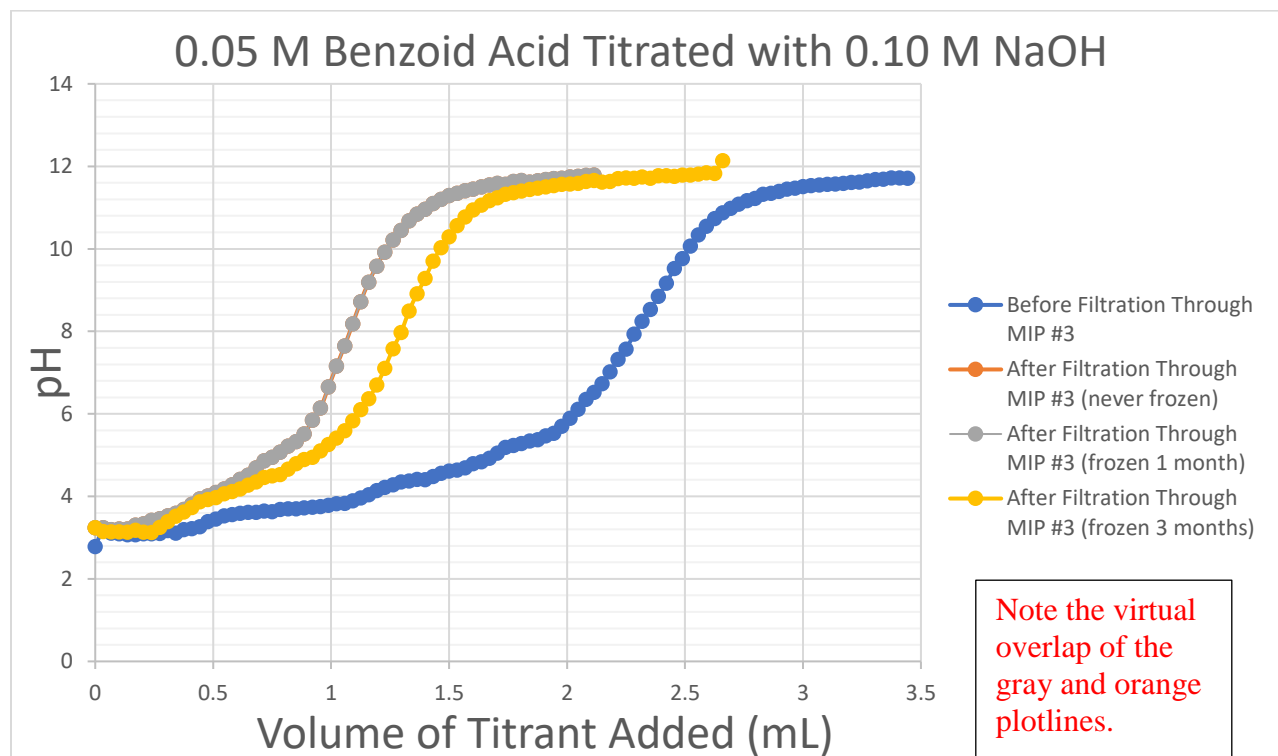
This is the sole graph that was developed in this study to examine the stability of the MIP in different temperature environments for different lengths of time. There was much hesitation to do a heat study with this MIP, given what happened to MIP #2 after being in an oven. So, for these titrations, neutral and cold environments were focused on. The standard BA-MIP sample used to filter through the orange line "after" solution was stored at room temperature for four months. The BA-MIP sample for the gray line "after" solution was stored in a freezer for one month, and then stored at room temperature for three months. On the other hand, the BA-MIP sample for the blue line "after" solution was stored in a freezer for three months, and then stored at room temperature for one month. The assigned solutions were then passed through the MIP samples, and titration was carried out on the dilutions.

Note that the orange and gray lines virtually overlap.

**Figure 9: Results of titration with 0.10 M NaOH base and pH probe**
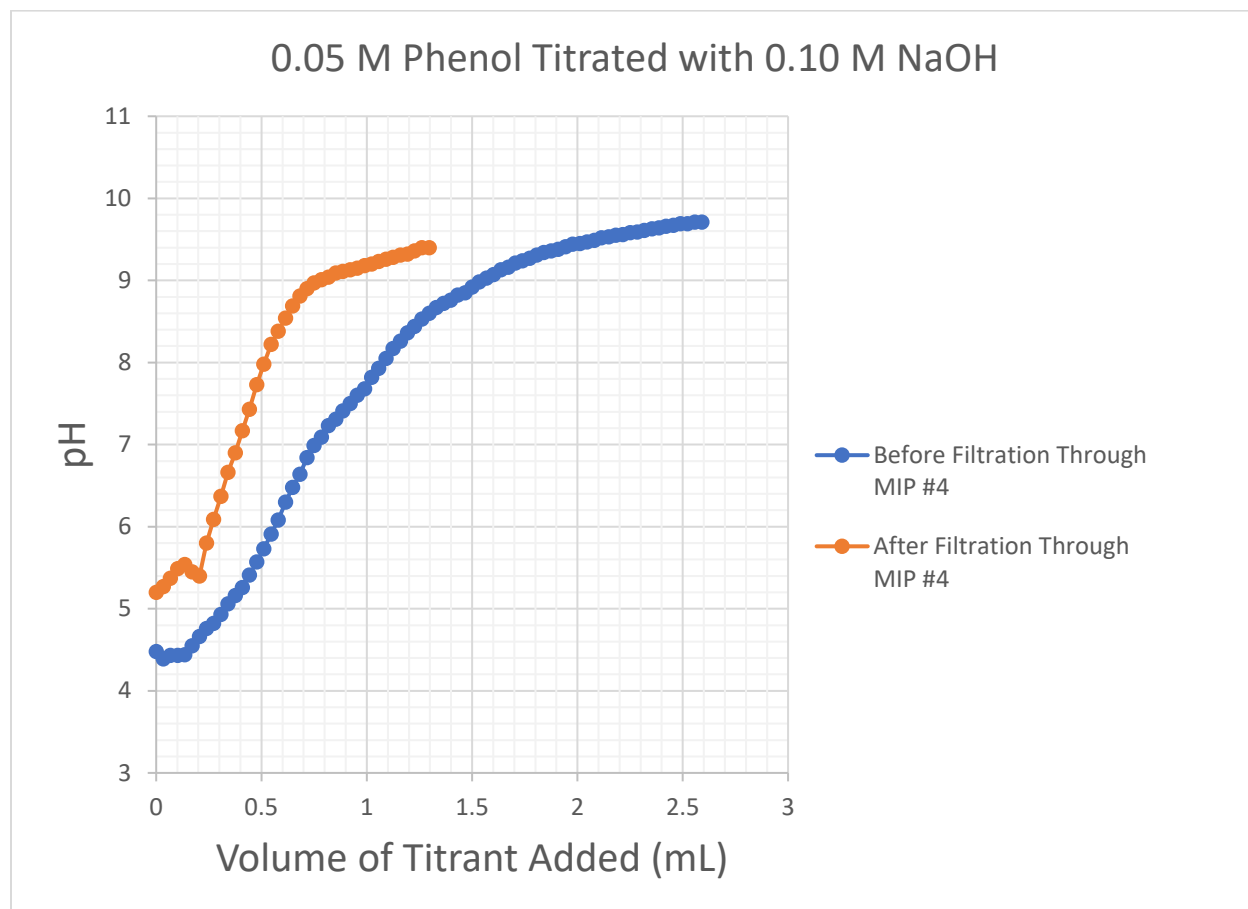
Phenol testing was repeated on MIP #4 to see if switching the titration solvent of the phenol

solution from water to 10% (v/v) ethanol in water would be able to bring the "before" and "after"

plotlines closer at the beginning. Evidently, this did not occur, but the titrations were as relatively

successful as they were in Figure 6. As such, for all remaining trials, the titration solvent of the

solution being tested (i.e. not the sodium hydroxide) was 10% (v/v) ethanol in water.

**Figure 10: Results of titration with 0.10 M NaOH base and pH probe**

This was the first set of titrations performed with regard to filtering through MIP #5. This served

as control to ensure the MIP behaved as intended for at least one known molecule, the template.

Figure 11: Results of titration with 0.10 M NaOH base and pH probe
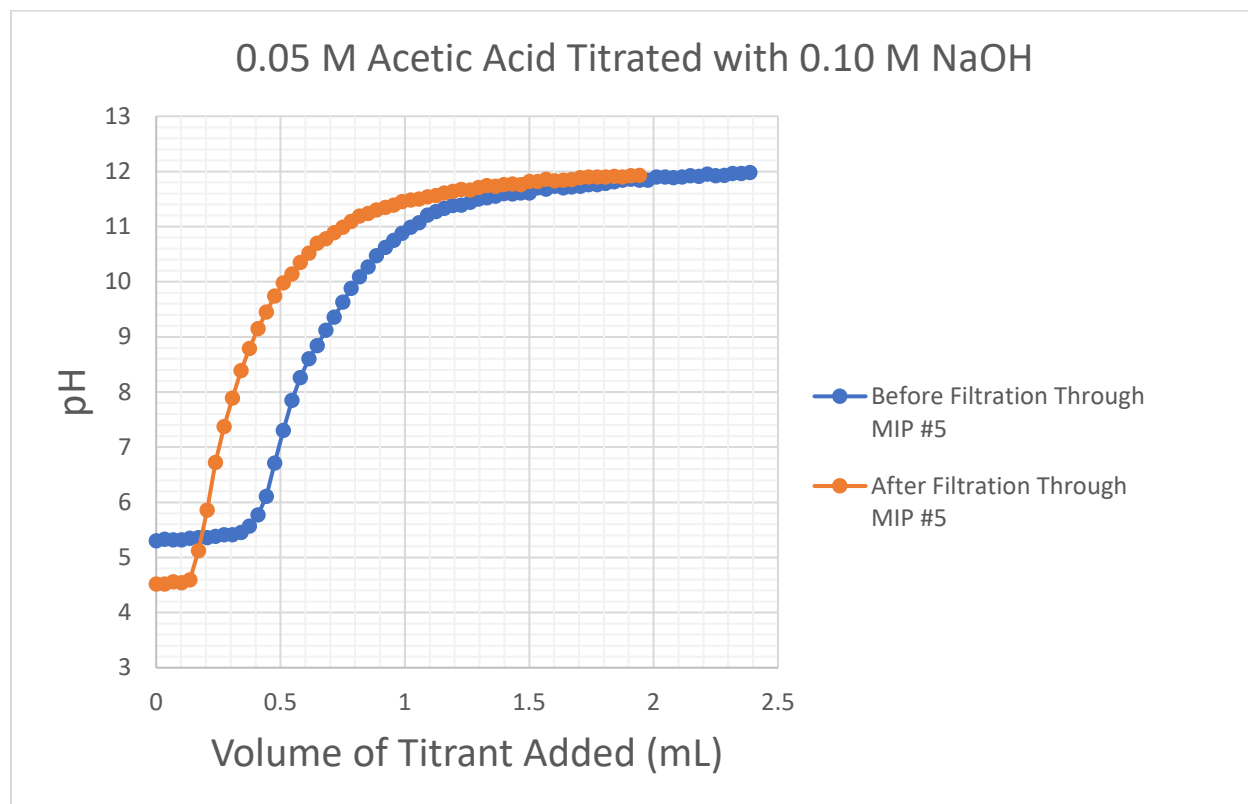
This set of titrations was carried out because acetic acid and its titration were never graphically illustrated earlier during this study. Results seem consistent with findings from MIP #3 in Table 2. Results also seem similar to findings with phenol in Figures 6, 7, and 9. In this case, the two titrations were able to reach the endpoint at around the same volume of base added. The two plotlines not only have extremely similar shapes, but even partially superimpose on one another.

The final set of titrations in this study focused on a new target that is somewhat similar in structure to benzoic acid: terephthalic acid. Essentially, terephthalic acid is simply BA with another carboxylic acid region *para-* to the original. Figures 12 & 13 (next page) are very similar to Figures 4, 5, and 10, and are remarkable in how different the "before" and "after" plotlines are from each other on each respective graph, as well as their relative large distance from each other.

Figures 12 & 13: Results of titration with 0.10 M NaOH base and pH probe

0.05 M Terephthalic Acid Titrated with 0.10 M NaOH

0.025 M Terephthalic Acid Titrated with 0.10 M NaOH
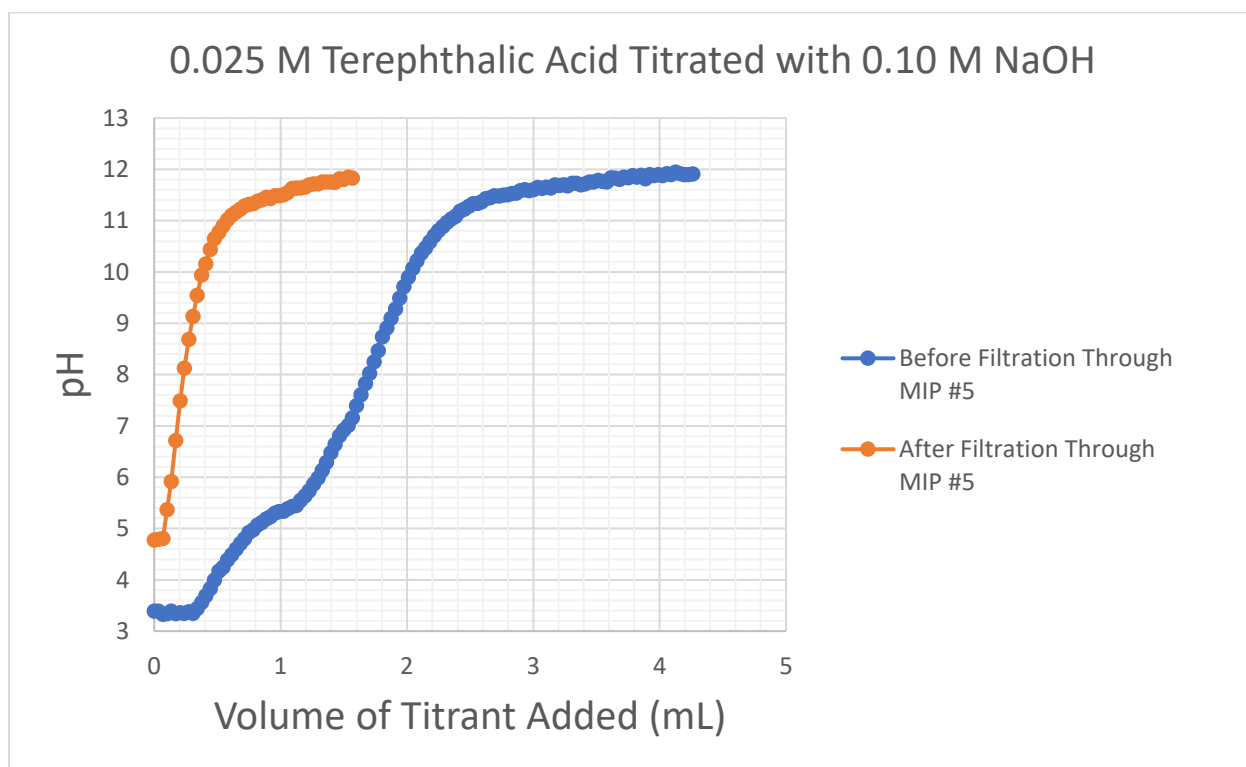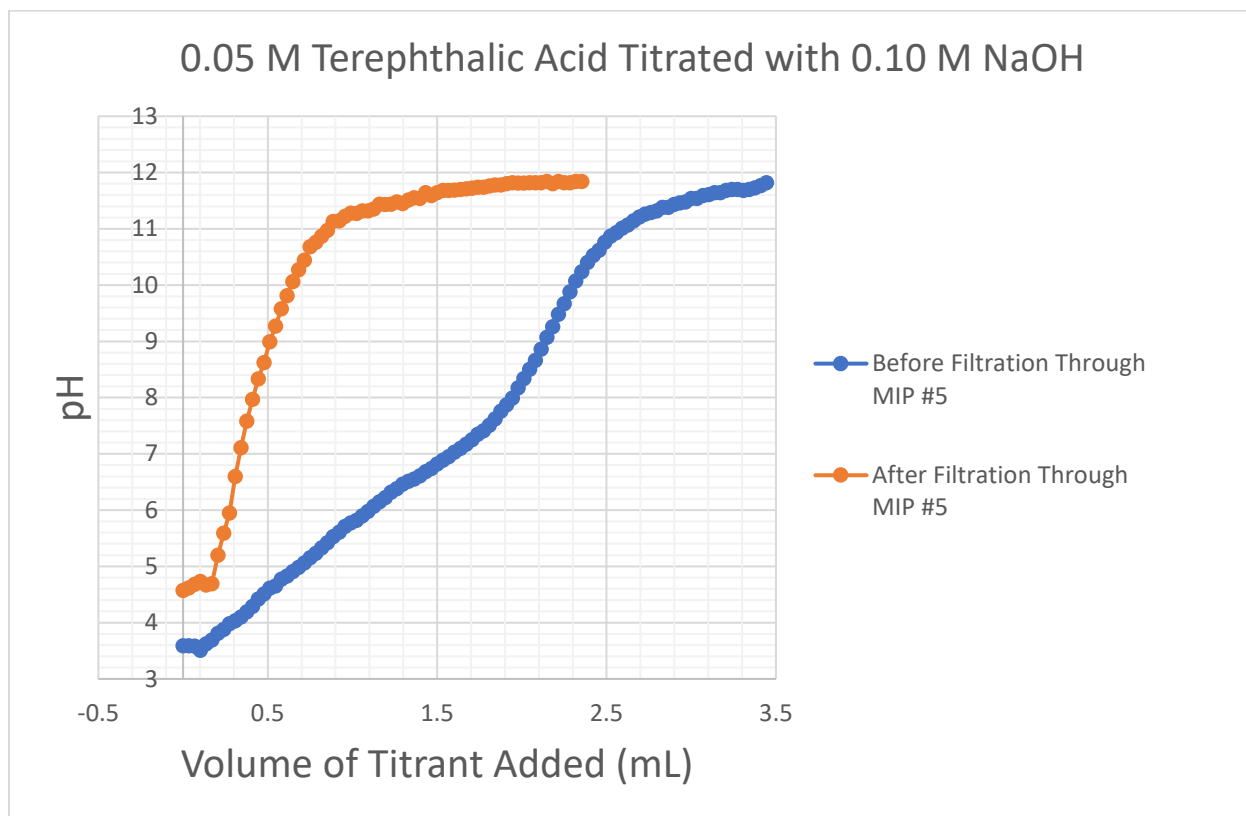
Discussion

Significant portions of benzoic acid appeared to be captured by the receptor sites of every one of the BA-imprinted MIPs explored. This confirmed the utility of MIPs for small molecular substrates such as benzoic acid. Specifically, it seems the BA target molecules were able to fit into the sites imprinted by the previously extracted BA template molecules. At the very least, this suggests that hydrogen bonding exists between target substrates and the "molded" PAA receptor sites on the carboxylic acid region.[20] The sole exception to the successful capture of BA by the prepared MIPs was MIP #2. These results differed from any runs done with any other MIP sample in this study. Considering the discoloration and unusual texture compared to the other MIP batches, it is believed that the heat treatment destroyed the imprinted sites in this imprinted polymer. In retrospect, this is not surprising, since the melting point of the MIP is under 100ºC.[24]

Previous MIP studies on similarly sized targets have predicted that aldehydes corresponding to their respective carboxylic acids (e.g. benzaldehyde corresponding to benzoic acid) will show at least this same hydrogen bonding affinity.[20] The only difference between benzoic acid and aldehyde is that instead of the carboxylic acid region that benzoic acid has, benzaldehyde has an aldehyde region, a formyl substituent. This means the only difference between those two regions is the presence (or lack) of one oxygen atom, depending on which molecule was being referred to. Consequently, this means the receptor sites in this test would have theoretically also captured the intended aldehyde MIP target, benzaldehyde.

Figure 14: Graphic depiction of two similar molecules highly relevant to this study

benzoic acid   benzaldehyde
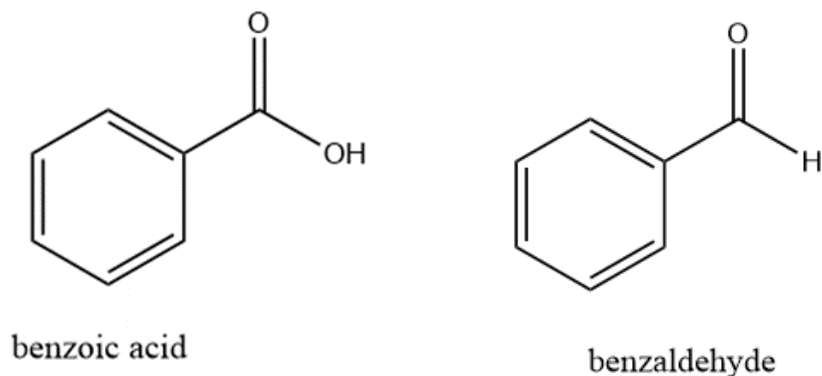
Calculations were performed on the relative success of each MIP in capturing benzoic acid molecules. This was done in order to see around how many moles of target molecule could feasibly be captured by a MIP sample, were it to be put on a QCM and made into a sensor with a specified, practical application (e.g. surveillance, defense, etc.). The theory is that the moles of the target will be at least equivalent (1:1 ratio) to the number of active sites available. Sample solution calculations, from before and after filtration through each MIP sample, are displayed below.

"Before" benzoic acid (BA) solutions, in theory:

$$\frac{10.00\ mL\ BA}{} \times \frac{0.05\ mol\ BA}{1\ L\ BA} \times \frac{L}{1000\ mL} = 0.50\ mmol\ BA$$

MIP #1:

$$\frac{5.70\ mL\ NaOH}{} \times \frac{0.09\ mol\ NaOH}{1\ L\ NaOH} \times \frac{L}{1000\ mL} \times \frac{1\ mol\ BA}{1\ mol\ NaOH} = 0.51\ mmol\ BA$$

$$\frac{4.35\ mL\ NaOH}{} \times \frac{0.09\ mol\ NaOH}{1\ L\ NaOH} \times \frac{L}{1000\ mL} \times \frac{1\ mol\ BA}{1\ mol\ NaOH} = 0.39\ mmol\ BA$$

Total amount of benzoic acid captured: 0.12 mmol

MIP #2:

$$\frac{5.70 \; mL \; NaOH}{} \times \frac{0.09 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.51 \; mmol \; BA$$

$$\frac{5.25 \; mL \; NaOH}{} \times \frac{0.09 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.47 \; mmol \; BA$$

Total amount of benzoic acid captured: 0.04 mmol [insignificant]

MIP #3:

$$\frac{5.30 \; mL \; NaOH}{} \times \frac{0.09 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.48 \; mmol \; BA$$

$$\frac{1.60 \; mL \; NaOH}{} \times \frac{0.09 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.14 \; mmol \; BA$$

Total amount of benzoic acid captured: 0.34 mmol

MIP #4:

$$\frac{4.00 \; mL \; NaOH}{} \times \frac{0.10 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.40 \; mmol \; BA$$

$$\frac{1.50 \; mL \; NaOH}{} \times \frac{0.10 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.15 \; mmol \; B$$

Total amount of benzoic acid captured: 0.25 mmol

MIP #5:

$$\frac{6.20 \; mL \; NaOH}{} \times \frac{0.10 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.62 \; mmol \; BA$$

$$\frac{4.70 \; mL \; NaOH}{} \times \frac{0.10 \; mol \; NaOH}{1 \; L \; NaOH} \times \frac{L}{1000 \; mL} \times \frac{1 \; mol \; BA}{1 \; mol \; NaOH} = 0.47 \; mmol \; BA$$

Total amount of benzoic acid captured: 0.12 mmol

Non-imprinted PAA and Celite were both found to not capture significant portions of benzoic acid. Originally, they were tested to ensure that the MIP acted not only as a physical filter, but more importantly, as one which chemically captures target molecules. If either of these media had similar results to the MIP after vacuum filtration, it could be implied that the MIP is not very chemically specific and that non-imprinted PAA or Celite could be used instead, saving time, money, and effort. Fortunately, this did not occur.

In order to test the BA-MIP for binding of similarly sized and functionalized species, a number of BA-similar molecules were tested for the BA-MIP recognition. It was found that insignificant portions of acetic acid were captured by the BA receptor sites of MIP #3, as shown by Table 2 and Figure 11. This means the imprint of the MIP is specific enough to virtually exclude acetic acid, giving insight into how the PAA monomers form around the benzoic acid template. Similarly, but to a lesser degree, insignificant portions of phenol appeared to be captured by the receptor sites of MIP #4, as shown by Figures 6, 7, and 9. This means the imprint of the MIP is specific enough to virtually exclude phenol, giving further insight into how the PAA monomers form around the benzoic acid template.

However, a significant portion of terephthalic acid molecules were indeed captured by the BA receptor sites of MIP #5, as evidenced by Figures 12 & 13, revealing the final piece of the puzzle into how specific these BA-MIP receptors are. The "before" and "after" plotlines were so far apart from each other, aside from the starting positions, that it seems quite evident that terephthalic acid can easily fit into this molecularly imprinted polymer. We know that binding happens on the carboxylic acid region of the benzoic acid molecule, but not very well on the same region for the acetic acid molecule. In this case, the acetic acid must lack something that

benzoic acid has: a benzene ring. Likewise, phenol is unsuccessful despite having a benzene ring, due to the fact it had an alcohol attachment and not a carboxylic acid or aldehyde attachment. (Figure 15)

Therefore, terephthalic acid is able to fit in the BA-MIP receptor site because it has a carboxylic acid branch and its base around a benzene ring. The other carboxylic acid region on the terephthalic acid molecule likely either goes into another receptor site, depending on how the polymer is situated, or just hangs free outside of the imprinted receptor site. The latter is akin to two copies of the same key with different bows. The parts hanging out of the lock, the bows, can be different shapes and functionalities, but the blades, which go inside the lock, both fit perfectly.[25] (Figure 15)

Figure 15: A graphic estimation of the important binding regions in BA-MIP receptor sites and how well some target molecules seemingly fit

[Hydrogen atoms have been omitted for clarity in the hand-drawn part of this diagram.]

Key: ▬▬▬▬ = wall of the MIP receptor site   ✖ = missing a key binding region

● = key binding region satisfied

benzoic acid

acetic acid

phenol

terephthalic acid
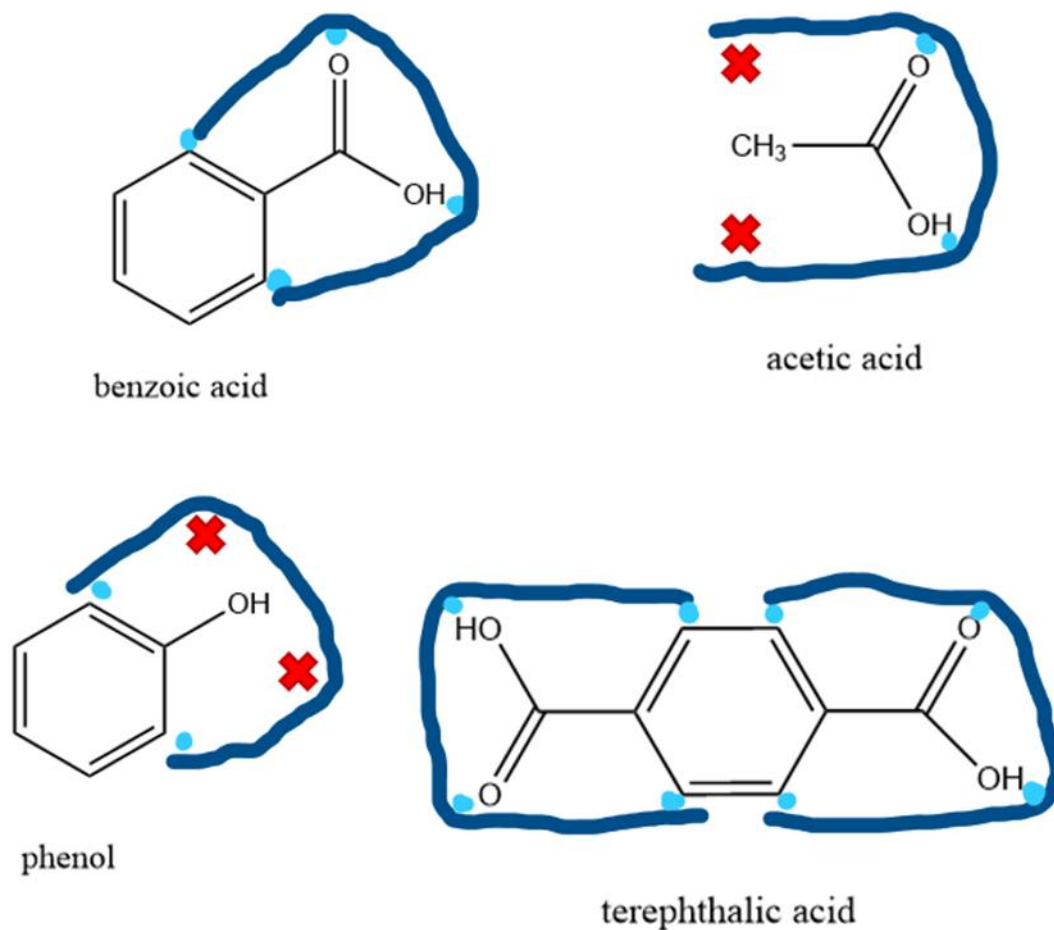
In order to truly know what exact amount and configuration of bonding there is in BA-MIP receptor sites, some advanced instrumental methods may have to be explored, such as SEM, AFM, or computational chemistry.

Regarding the stability of this MIP, it appears to operate best when stored in a specific temperature range. According to Figure 8, there was virtually no difference in performance, or ability to capture target molecules, between the sample of MIP stored for five months in room temperature conditions and the sample of MIP stored in a 9ºC freezer for the first of those five months. There is a slight drop in performance with the sample of MIP stored in the 9ºC freezer for three of those five months, but not significant enough to determine whether natural variation in samples and/or extended storage in a chilled environment caused this phenomenon. Regardless, all MIP samples were still effectively able to carry out their intended purpose of capturing a large amount of benzoic acid molecules during vacuum filtrations of solutions. With this knowledge, as well as previously attained knowledge of the MIP losing functionality when heated in a 120ºC oven, one can deduce that benzoic acid-templated MIPs operate best when stored in a dry environment between 9ºC and (far below) 120ºC.

Summary and Future Work:

There is a dearth of published literature concerning the specificity and stability of small molecularly imprinted polymers (MIPs). Five batches of a MIP made with PAA monomers and benzoic acid template were tested for specificity with various solutions passed through samples in a vacuum filtration, followed by titration to analyze. Also, some MIP samples were stored for extended periods of time in varied conditions and then put through the aforementioned methods to test for stability. Taking controls for the substrate and template solution into account, it is now clear that a BA-MIP can capture benzoic acid, benzaldehyde, and terephthalic acid very well, but not phenol and acetic acid. Furthermore, they work best after being stored in a dry environment above 9ºC but far below 120ºC, with shelf-lives of at least a few months minimum.

More studies should be conducted on these MIPs with a focus on stability at various temperatures, not just to create a more complete picture of optimal storage conditions, but to predict *where* future engineered sensors utilizing these types of MIPs could be effectively used. For example, a MIP made primarily of polyacrylic acid templated to detect Sarin gas molecules for defense purposes may only work appropriately in environments of certain temperatures. Temperate microclimates similar to "room temperature" conditions in modern buildings will more than likely be fine, but certain deserts and forests are different scenarios entirely that have yet to be part of lab analysis and field testing.

A more refined MIP manufacture process can also be developed. Waiting for a solvent to dissolve a template out of a MIP over three washes is unfavorably time-consuming. Using heat to remove templates have been shown possible in the past,[20] but caution must be taken to prevent overheating. 120 degrees Celsius heat for 90 minutes in an oven is clearly too high and/or long, as displayed by the functionally-ruined nature of MIP #2. A heating mantle with 70-80ºC with close monitoring, or some similar situation, is suggested.

Furthermore, this MIP should be tested for longer retention times (i.e. allowing the MIP more than a minute to take in benzoic acid molecules before the vacuum of the filtration commences) and eventually be tested in the gaseous phase (what these types of MIP-based sensors will be majorly used for). There are likely more advanced target molecules that can be tested for BA-MIPs as well, and plenty of other small molecules that can be used as templates to make different MIPs in a study similar to this.

References

(1) Bragazzi, N. L.; Amicizia, D.; Panatto, D.; Tramalloni, D.; Valle, I.; Gasparini, R. Quartz-Crystal Microbalance (QCM) for Public Health: An Overview of Its Applications. *Advances in Protein Chemistry and Structural Biology*. [Online] **2015**, *101,* 153-154 (accessed June 12, 2017).

(2) Wolfbeis, O. S. Springer Series on Chemical Sensors and Biosensors: Methods and Applications. *Piezoelectric Sensors*. [Online] **2007**, *4* (accessed October 1, 2017).

(3) Ophardt, C. E. Mechanism of Enzyme Action. Virtual Chembook, Elmhurst College. http://chemistry.elmhurst.edu/vchembook/571lockkey.html (accessed September 1, 2017).

(4) Piletsky, S.A.; Piletsky, S.; Chianella, I. Chapter 14 – MIP-Based Sensors. *Molecularly Imprinted Sensors*. **2012.** 339–354.

(5) Chen, L.; Xu, S.; Li, J. Recent Advances in Molecular Imprinting Technology: Current Status, Challenges and Highlighted Applications. *Chemical Society Reviews.* [Online] **2011**, *40.5,* 2922 (accessed June 6, 2017).

(6) Holthoff, E. L.; Bright F. V. Molecularly Templated Materials in Chemical Sensing. *Analytica Chimica Acta* [Online] **2007,** *594,* 147–161 (accessed June 15, 2017).

(7) Benz, M.; Benz, L.; Patel, S. V. High Temperature Mass Detection Using a Carbon Nanotube Bilayer Modified Quartz Crystal Microbalance as a GC Detector. *Anal. Chem.* [Online] **2015**, *87,* 2779-2787 (accessed June 20, 2017).

(8) Bunte, G.; Hürttlen, J.; Pontius, H.; Hartlieb, K.; Krause, H. Gas Phase Detection of Explosives such as 2,4,6-Trinitrotoluene by Molecularly Imprinted Polymers. *Analytica Chimica Acta* [Online] **2007,** *591,* 49-56 (accessed June 28, 2017).

(9) Liu, N.; Li, X.; Ma, X.; Ou, G.; Gao, Z. Rapid and Multiple Detections of Staphylococcal Enterotoxins by Two-Dimensional Molecularly Imprinted Film-Coated QCM Sensor. *Sensors and Actuators B: Chemical* [Online] **2014**, *191,* 326–331 (accessed June 7, 2017).

(10) Schofield, A. L.; Rudd, T. R.; Martin, D. S.; Fernig, D. G.; Edwards, C. Real-Time Monitoring of the Development and Stability of Biofilms of Streptococcus Mutans Using the Quartz Crystal Microbalance with Dissipation Monitoring. *Biosensors and Bioelectronics* [Online] **2007**, *23,* 407–413 (accessed June 12, 2017).

(11) Lu, W.; Xue, M.; Xu, Z.; Dong, X.; Xue, F.; Wang, F.; Wang, Q.; Meng, Z. Molecularly Imprinted Polymers for the Sensing of Explosives and Chemical Warfare Agents. *Current Organic Chemistry* [Online] **2015,** *19.1,* 62–71 (accessed June 9, 2017).

(12) Özkütük, E. B.; Diltemiz, S. E.; Özalp, E.; Gedikbey, T.; Ersöz, A. Paraoxon Imprinted Biopolymer Based QCM Sensor. *Materials Chemistry and Physics* [Online] **2013**, *139*, 107–112 (accessed on June 14, 2017).

(13) Pradhan, S.; Boopathi, M.; Kumar, O.; Baghel, A.; Pandey, P.; Mahato, T. H., Singh, B.; Vijayaraghavan, R. Molecularly Imprinted Nanopatterns for the Recognition of Biological Warfare Agent Ricin. *Biosensors and Bioelectronics* [Online] **2009**, *25*, 592–598 (accessed on June 8, 2017).

(14) Chulov, M. Sarin used in April Syria attack, chemical weapons watchdog confirms. *The Guardian*, June 30, 2017 (accessed July 1, 2017).

(15) Gilley, C.; MacDonald, M.; Nachon, F.; Schopfer, L. M.; Zhang, J.; Cashman, J. R.; Lockridge, O. Nerve Agent Analogs That Produce Authentic Soman, Sarin, Tabun, and Cyclohexyl Methylphosphonate-Modified Human Butyrylcholinesterase. *Chemical research in toxicology* [Online] **2009**, *22.10*, 1680–1688 (accessed September 5, 2017).

(16) Erikson, H. P. Size and Shape of Protein Molecules at the Nanometer Level Determined by Sedimentation, Gel Filtration, and Electron Microscopy. *Biol Proced.* [Online] **2009**, *11*, 32–51 (accessed on September 1, 2018).

(17) Virus: Size and shape. *Encyclopædia Britannica*. [Online] **2018.** https://www.britannica.com/science/virus/Size-and-shape (accessed on September 1, 2018)

(18) Particle Size Chart- Merv Rating Chart. https://www.coloradoci.com/bin-pdf/5270/ParticleSize.pdf (accessed on September 1, 2018)

(19) Samah, N. A.; Sánchez-Martín, M.; Sebastián, R. M.; Valiente, M.; López-Mesas, M. Molecularly Imprinted Polymer for the Removal of Diclofenac from Water: Synthesis and Characterization. *Science of The Total Environment* [Online] **2018**, *631–632*, 1534–1543 Contents (accessed on July 21, 2018).

(20) Jha, S. K.; Hayashi, K. A Quick Responding Quartz Crystal Microbalance Sensor Array Based on Molecular Imprinted Polyacrylic Acids Coating for Selective Identification of Aldehydes in Body Odor. *Talanta* [Online] **2015**, *134*, 105–119 (accessed on July 12, 2017).

(21) Saran Frequently Asked Questions. http://www.saranbrands.com/faq.asp#10 (accessed on September 4, 2018).

(22) Properties of substance: terephthalic acid. http://chemister.ru/Database/properties-en.php?dbid=1&id=1327 (accessed on August 2, 2018).

(23) Benzoic acid(65-85-0)^{1}HNMR. ChemicalBook.
    https://www.chemicalbook.com/ChemicalProductProperty_EN_CB8698780.htm
    (accessed on July 14, 2017).

(24) Melting Points of Polymers. Crystallographic Data and Melting Points for Various
    Polymers.
    http://nguyen.hong.hai.free.fr/EBOOKS/SCIENCE%20AND%20ENGINEERING/
    MECANIQUE/MATERIAUX/COMPOSITES/Polymer_Handbook/66286_38c.pdf
    (accessed on November 7, 2018).

(25) Master Locksmith Association. Glossary of Locksmith Terminology.
    https://www.timpsonlocksmiths.co.uk/locksmith-terminology/ (accessed on August 29,
    2018).

Curriculum Vitae

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EDUCATION

Syracuse University   Syracuse, NY 13210
Master of Science, College of Arts and Sciences, Chemistry
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University of New Haven   West Haven, CT 06516
Bachelor of Science, College of Criminal Justice and Forensic Sciences, Forensic Science
Bachelor of Science, College of Engineering, Chemistry
Completed May 2016

PROFESSIONAL EXPERIENCE

DEPARTMENT OF CHEMISTRY, Onondaga Community College & Utica College
Adjunct Professor (Aug. 2018 – Present)

- Serve as course instructor for four sections of general chemistry lab: one section of Basic Chemistry for the Health Sciences Laboratory I & one section of General Chemistry I Lab at OCC, and two sections of General Chemistry I Lab at UC.
- Deliver pre-lab lectures that are informative in regards to connection to lecture material, laboratory safety, and proper procedures.
- Grade all lab course assignments and provide timely feedback & guidance to diverse groups of students.

DEPARTMENT OF CHEMISTRY, Syracuse University
Graduate Researcher/Teaching Assistant (Aug. 2016 – Dec. 2018)

- Carry out research on piezoelectric QCM sensors specific to biologically relevant molecules, as well as assist in various laboratory practices and projects.
- Served as a TA for three sections of CHE 107 (General Chemistry I Lab) for the Fall 2017 semester, in order to educate students of various backgrounds on basic lab safety, fundamental experimental methods, and the foundations for understanding chemical concepts in a hands-on environment.
- Ensured the chalkboard for the pre-lab lecture was written in a detailed and eye-catching manner, and that the laboratory was prepared with all necessary supplies and procedural notes for the upcoming experiment.

THE DAILY ORANGE, Syracuse University
"Orange STEM" Podcast Creator/Producer/Host (Aug. 2017 – May 2018)
- Recorded and led an educational biweekly podcast for an online independent newspaper, based around STEM discussion & news relevant to local students and CNY residents (premiered September 2017).
- Scheduled multidisciplinary guests, including undergraduate & graduate students from SU & SUNY-ESF, to appear on the show in some format.
- Crafted outlines of one or two topics per episode, with specific questions to discuss with the guest(s). Edit each episode for speech quality and length (down to 7-15 minutes).
- Available for listening at http://dailyorange.com/tags/orange-stem

REU CHEMISTRY, University at Buffalo, State University of New York
Undergraduate Researcher, Velarde Group (June 2015 – Aug. 2015)
- Researched, designed, and led an original, physical chemistry-based, laboratory experiment to determine the behavior of acetone molecules in water at a silica interface, by observing net dipole moments at various solution concentrations via nonlinear spectroscopy.
- Communicated the findings of the aforementioned research project in various formats, including a research paper that was at one time being prepared for submission to a scientific journal.
- Enforced proper laboratory safety protocols in both a wet chemistry lab and a laser lab.

PRESIDENT'S PUBLIC SERVICE FELLOWSHIP, University of New Haven
Public Service Fellow, Connecticut Yankee Council, BSA (May 2014 – Aug. 2014)
- Created and implemented six distinct educational day camp curricula for children aged 5-11, entirely focused on learning STEM concepts in the wilderness while enforcing Cub Scout morals. Served as point of contact and leader for project from brainstorming to implementation.
- Founded and developed a professional relationship between the University of New Haven and the Connecticut Yankee Council of the Boy Scouts of America, with the intent of having the council utilize university facilities and faculty in order to instill KSA (both science-based and career-based) into young men. Served as sole liaison between various UNH offices and CYC executives.
- Documented all activities in daily log & weekly essay prompts for submission to supervisors.

PROFESSIONAL PRESENTATIONS

- **Syracuse Center of Excellence Symposium 2017** (group poster session): "Chemically Functionalized Acoustic Wave Devices for Indoor Air Quality Monitoring" with Harvey N. Mosher and Elizabeth L. Clifford

- **University of New Haven Honors Thesis** Presentation (oral presentation & poster session): "Classification of International Black Ballpoint Ink Evidence via Non-Destructive and Destructive Forensic Instrumental Methods"

- **University at Buffalo REU Program** Presentation (oral presentation & poster session): "Using Nonlinear Spectroscopy to Determine Molecular Interactions at the Interface of Acetone-Water Mixtures and Silica"

- **Connecticut Yankee Council, BSA** & University of New Haven President's Public Service Fellowship Presentation (two oral presentations): "Connecticut Yankee Council Cub Scout STEM Day Camp Curricula"

AWARDS

Chancellor's Award for Public Engagement & Scholarship (CAPES), Syracuse University	April 2018
Alpha Chi Sigma Scholar Award Honorable Mention	April 2018
Graduate Fellow, Syracuse University	Aug. 2016 – July 2017
WNHU Broadcaster of the Year, Univ. of New Haven	May 2016
John D. Hatfield Scholar, Univ. of New Haven	Fall 2014 – Spring 2015
Honor Society for Experiential Education, Univ. of New Haven	April 2014 – May 2016
Alpha Lambda Delta First-Year Honor Society, Univ. of New Haven	Feb. 2013 – May 2016
Eagle Scout, Revolutionary Trails Council, BSA	Dec. 2010

PROFESSIONAL ORGANIZATIONS

WAER Syracuse Public Media- Volunteer Producer/Host	Dec. 2017 – Present
American Association for the Advancement of Science (AAAS)	Sept. 2017 – Present
Science Olympiad- Volunteer Guest Judge/Assistant	Mar. 2017 – Present
Alpha Chi Sigma Professional Chemistry Fraternity- Pi Chapter	Nov. 2016 – Present
American Academy of Forensic Sciences (AAFS)- Student Affiliate	April 2016 – Present