Covalent Organic Frameworks as Customizable Vehicles for Controlled Drug Release

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Abstract

This study explores the use of covalent organic frameworks (COFs) as drug carriers which can be modified and customized for non-toxic, resilient, controllable drug delivery applications. We identified COFs as a possible polymer candidate among other porous polymers and reviewed the literature to design a preliminary study investigating the toxicity, stability, and controlled drug delivery property of COFs. In this study we selected COF-Ph (COF1) as the reference COF and compared its performance to that of COF-TpPa (COF2), and COF-TfpbPa (COF3) to understand the effect of hydrophobicity/hydrophilicity and pore size on their drug release. Ibuprofen (IBU) was selected as the target drug for loading and release experiments given its prevalence in release studies reported in previous literature. IBU was loaded into each COF and released into phosphate buffer solution (PBS). Release profiles for each COF were characterized by nanodrop UV-vis. The stability, loading, and porosity of COFs were characterized by powder x-ray diffraction (PXRD) and N₂ adsorption/desorption isotherms with Brunauer-Emmett-Teller (BET) analysis. Toxicity was evaluated by treating HeLa human cervical cancer cells with COFs of varied concentrations. At the end of this project, no solid conclusion can be drawn, and more questions arose than what were answered yet suggestive conclusions were distilled. COFs with different structures have different characteristics for drug delivery. COFs’ toxicity is likely dependent on intra and extracellular interactions with the COF. COFs are likely stable through release into PBS, but their porosity and crystallinity may change throughout the experiment and differ from batch to batch. This study is the beginning of a project whose goal is to develop a controllable drug delivery system that can be used to match patients’ metabolism, significantly extending and controlling the release time and location, thus creating an opportunity for shifting the medication from patient administration of standard medications to professionally administered personalized medicines.
Acknowledgements:

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This thesis is dedicated to Dr. Otto Zivko Sellinger and Myriam Aróstegui Sellinger
Introduction

Patients are required to take too many pills in order to treat their medical problems or comply with current medicine protocols. From birth control to bacterial infections to advanced cardiovascular disease, pills are an integral part of modern medical treatment. Today, pills are designed and produced according to protocols of standard care and standard dosing, however, patients are not standard. Their symptomology, pathology and, metabolisms are not all standard either. Patients have varying preferences, tolerances, and personal uniqueness. They should not be treated with standard medicine, but instead with personalized medicine.

As much as patients are personal, they are also imperfect. They are forgetful, inconsistent, and sometimes abuse their medications.\(^1\)-\(^3\) All these examples are under the umbrella of patient non-compliance. Non-compliance is very detrimental to patients and the health care system as a whole costing time, resources, money and lives. In the US alone, 33-69% of medication related hospitalizations are associated with poor medication adherence and end up costing the patients and healthcare system around $100 billion each year.\(^3\) The World Health Organization, (WHO) reported that 40% of patients in developed countries are non-compliant with treatment protocols, 50% of chronic prescriptions are taken incorrectly, and each year there are around 125,000 avoidable deaths from treatable diseases because patients do not adhere to their medication regimens.\(^4\) Immediate release (IR) medications release the active drug immediately after oral administration. IR medications are often prescribed once a day, once in the morning and once at night, or with every meal and result in rapidly varying drug plasma concentration (Figure 1). These protocols turn into 30-90 chances each month for a patient to miss a dose of their medication which can minimally or severely impact a person’s health. Instead, one pill a week, once a month, or administered at each check-up, longer acting medications and sustained release (SR) drug delivery systems could significantly reduce the number of missed doses and abused medications.\(^3\),\(^5\)
Figure 1. Idealized drug plasma concentration versus time profile of an immediate release drug protocol. The drug is only effective when above the minimum effective concentration and below the minimum toxic concentration. The time which the drug is above the minimum concentration is longer for sustained release than that of the immediate release profile. More drug is dosed to the system, yet plasma concentrations remain below the minimum toxic limit due to slow release.

Current sustained release mechanisms include osmotic pumps, capsule engineering, and ion exchange resins.\textsuperscript{6–8} These are all designed to prolong the drug release from the delivery system and extend the time the drug is within the therapeutic range (between the minimum effective concentration and minimum toxic concentration). Yet these systems are not made for personalized medicine. Each of these systems has one point of tunability - diffusion rates of osmotic pumps, or pore size with capsule engineering, or thermodynamics with ion exchange resins.\textsuperscript{6–8}

Sustained and personalized drug delivery systems must have many points of tunability, and polymer chemistry has opened many new options. Controlled drug release (CR) is the idea that not only is the time the drug is released for is controlled but the location, rate, and change in rate of drug release can be controlled.\textsuperscript{9} Proprietary porous materials with potential as controlled drug delivery capabilities are metal organic frameworks (MOFs), mesoporous siliceous nanoparticles (MSNs), and covalent organic frameworks (COFs). Research has shown that these different materials have the ability to absorb and release small molecules at controlled rates.\textsuperscript{10–16} MOFs, COFs, and MSNs have also been the subject of research for ion and gas storage and separation, catalyst supports, and other applications which has led to
an abundance of MOF, COF, and MSN R&D in academia and industry. However, MOFs and MSNs may be constrained in future use due to potential toxicity issues of the metals used in the framework and the metabolism of that compound after exposure. Aside from the toxicity of the materials in the drug delivery systems to the human body, the metabolism and excretion of drugs and delivery systems is a relatively new area of research and must be explored due to observations of the impact of drug metabolites on the ecosystem. COFs, on the other hand, can be comprised of relatively benign forms of carbon, nitrogen, and oxygen, reducing future toxicity and environment concerns. Examples of drug release studies using MOFs, COFs, and MSNs are shown in Figures 3-5.

**Figure 2.** A) Calcein release during the first 5 hours and B) 30 days from crystalline MOF, UiO-66, black closed circles, and amorphous MOF, amUiO-66, red open circles. Black solid and red dotted lines represent the kinetics of delivery, fitted using non-linear regression on UiO-66 and amUiO-66, respectively.

**Figure 3.** The physicochemical characterization of MSNs. A) TEM images of MSNs with different particle sizes: MSNs120 (A1), MSNs200 (A2) and MSNs360 (A3). B) SAXRD patterns of MSNs with different particle sizes: MSNs120 (B1), MSNs200 (B2) and MSNs360 (B3) (D) In vitro DOX release profile of DMNs 120. (E) In vitro DOX release kinetics of DMNs120.
Covalent organic frameworks (COFs) represent a novel class of porous polymers comprised of organic building blocks arranged in uniform skeletons with ordered pores and channels. COFs can be functionalized by tuning pore characteristics such as pore size, backbone functional groups and by adding thermoresponsive and/or pH responsive functional groups inside the pores. COFs demonstrated the ability to absorb and release drugs coupled with many reports in the literature describing their customization and tuning, may lead to an impactful research area for controlled drug release.

While the ability of COFs to absorb and release small molecules is well documented, the interactions that affect loading capacity and release rate have not been identified. By studying the
COF/drug host-guest interactions we can begin to identify how to control the release profile of the drug from the COF. The abundance of COFs and published modifications to COFs combined with the variety of small molecule drugs currently available allows for the potential of this protocol to increase patient compliance, drug efficacy, and prognosis as well as reduce secondary and tertiary issues from patient noncompliance. This protocol would increase patient compliance by reducing the number of drug administrations through longer release period, support consistency in patient’s drug plasma concentration by metabolism matching and reduce the opportunity for active intentional patient noncompliance and abuse by constructing an in-office administration protocol around controlled extended drug delivery.

*Figure 4.* Idealized drug plasma concentration versus time profile of a controlled release drug protocol over the course of several days. The time which the drug is above the minimum concentration is longer than that of the immediate release profile and sustained release profile. In practice the concentration will eventually decrease as the drug is metabolized and depleted. The key difference is the lack of a bell curve profile and the concentration plateaus between the minimum effective and minimum toxic concentrations.

The ultimate goal of this research area is to demonstrate that COFs can be customizable, non-toxic, stable in physiological conditions, and functional as drug carriers for controlled release. Then by employing COF pore customization and COF-drug host-guest interaction, a treatment protocol may be developed to tailor drug release to the patient’s metabolism of the medication. In this project, we began to investigate whether COFs are safe and stable polymers that integrate small molecules into their pores. In addition, we began to explore the impact of pore and drug characteristics on release of the small molecule from COFs.
Project design rationale

Figure 5. COFs COF-Ph, COF-TpPa, and COF-TfpbPa and their respective building block monomers that will be used in this project.
COF-Ph (COF1), COF-TpPa (COF2), and COF-TfpbPa (COF3) are selected for this study because they are structurally simple COFs, well studied, and have well documented synthetic methods and characterization. They can be synthesized through the well-known Schiff-base reaction of aldehydes and primary amines to form a stable imine-based COF networks with water as the byproduct. Although not the focus of this thesis, more information on COFs can be found in many review articles. COF1 will act as the reference COF. COF2 will be used to explore whether increased hydrophilicity can cause variations in drug loading capacity and release profile, while COF3 will be used to show differences in loading and release profile differences due to its larger pore size.

Synthesis of monomer building blocks CHO1, CHO2, and CHO3 were performed according to previous literature methods and are available in the Appendix. Building blocks were characterized by thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) for structure conformation and purity assessment. COFs COF1, COF2, and COF3 were synthesized using adapted literature methods and characterized using powder X-ray diffraction (PXRD) for crystallinity, N2 adsorption/desorption isotherms for pore size and surface area determination using Brunauer-Emmett-Teller (BET) analysis, and scanning electron microscopy (SEM) for imaging morphology (in Appendix). The monomer NH1 was commercially available.

Previous literature has also shown that certain COFs can uptake and release drugs, mainly ibuprofen (IBU), for 1-7 days and sometimes up to 30 days. To continue on this research path, we wanted to see how different COF pore characteristics change the release profiles of drugs already studied to try to explain other literature and begin looking at mechanisms for controlling drug delivery. We hypothesize that COFs and the drugs they uptake and release have collaborative effects on host-guest interactions, and by matching COFs to drugs of varying characteristics we may fine-tune the release profile of the drug from the COF. This was studied using UV-vis spectroscopy to observe and loading and release of the drug into and from the COF.
Previous literature has proposed the new application for COFs as viable SR drug delivery systems but still questions whether such COFs have negligible or minimal toxicity to humans. Therefore, part of this project was to begin the study of toxicity of COFs to humans using HeLa human cervical cancer cells treated with at various concentrations and exposure periods.

Methods for drug loading and release in previous literature are available and acknowledged, however, across the literature there is little consistency to the characterization of loading and release. The loading and release methods used in this study were adopted from a careful review of the literature on this topic.

Methods

Preliminary Release study drug loading and release characterizing: COF samples were crushed into fine powders using a mortar and pestle. 10 mg of COF1, COF2 and COF3 were placed in a 15mL centrifuge vial. The COFs were then suspended in 10 mL of 0.1M IBU in hexane. Samples were allowed to absorb at room temperature for 4 hours without stirring. The whole sample was then isolated by centrifugation and removal of supernatant. Another 5 mL of PBS was added to each. Samples were placed in a warm room at 37˚C. Samples were taken at intervals by centrifuging COFs to pellet and UV-Vis of supernatant by nanodrop (~10 µL/sample). Test was performed in triplicate for each COF.

Final release study drug loading and release characterization: COF samples were crushed to a fine powder using a mortar and pestle. 10 mg of COF were suspended in 10 mL of 0.1M ibuprofen in hexane solution in a 20 mL scintillation vial. Vial was sealed with a lid and parafilm and set at room temperature for 3 hours, stirring. Drug-loaded COFs (DL-COFs) were isolated by vacuum filtration and washed once with ~5 mL of hexane. DL-COFs were then dried under vacuum. 5 mg of DL-COF were placed in a centrifuge vial along with 5 mL of PBS. Samples were placed in a warm room at 37˚C. Samples were
taken at intervals by centrifuging COFs to pellet and UV-vis of supernatant by nanodrop (~10 µL/sample). Test was performed in triplicate for each COF.

**Nitrogen adsorption/desorption of COFs:** COF samples were crushed to a fine powder with a mortar and pestle. 50 mg of COF were suspended in 1:1 mg of COF to mL of 0.1M ibuprofen in hexane solution in a 20 mL scintillation vial or beaker. Vial or beaker was sealed with parafilm and set at room temperature for 3 hours, stirring. Drug-loaded COFs were isolated by vacuum filtration and washed once with ~50 mL of hexane. DL-COFs were then dried under vacuum. 50 mg samples were given to Dr. Brian Trewyn for N₂ adsorption/desorption, BET, and BJH analysis at the Colorado School of Mines.

**Cytotoxicity Assay of COF-Ph on HeLa cells:** 20,000 HeLa cells were plated in 15 wells of a 24-well plate (3x5). Cells were cultured for 24 hours. Cells were then treated with COF solutions of concentration 0.3, 0.1, 0.033, or 0.011 mg COF/mL. Negative control was no COF treatment. After 48 hours after treatment cells were harvested and counted by hemocytometer.

**Cytotoxicity Assay of COF1 and COF2 to HeLa cells:** 20,000 HeLa cells were plated in 16 wells of a 24-well plate (4x4). Cells cultured for 24 hours. Cells were then treated with solutions 1, .1, or .01 mg COF/mL of COF1 or COF2. Negative control was no COF treatment After treated for 5 days, cells were collected and counted by hemocytometer.

**Stability of COF materials in PBS Buffer over 1 week:** 10 mg of each unloaded COF were placed in a 15 mL centrifuge vial. The vial was sealed and left at rest for 7 days at which point the COFs were isolated by vacuum filtration then washed with water then acetone. The COFs were then placed in a 20 mL scintillation and dried for 4 hours at 80 ºC under vacuum. After which point the samples were sealed and submitted for PXRD. PXRD was run by Yiming Hu of the Zhang Group in the Department of Chemistry at the University of Colorado, Boulder.
Results

Preliminary Release study drug loading and release characterizing:

This preliminary COF release study was performed over 48 hours. Loading of the COFs was done by immersion in a hexane solution for 4 hours after which the solution was removed by decantation and no wash of the COF was performed. Results of the study are summarized in Table 1 below. Total mass released was calculated using a standard curve to convert absorption of the IBU UV-vis characteristic peak at 264 nm to a solution concentration of IBU and then to a mass. Loading capacity (LC%) is calculated by the total mass of drug in the system divided by the total mass of the mixture. In this case:

\[
LC\% = \frac{\text{Total Mass Released}}{\text{Total Mass Released} + 10 \text{ mg of COF}}
\]

Percent Released is the calculated by the mass released during a certain time interval divided by the total mass released.

Table 1. Summary of preliminary release study data. Total masses were derived from standard curve and association with UV-vis 264 nm characteristic peak of ibuprofen.

<table>
<thead>
<tr>
<th>COF</th>
<th>Total Mass Released (mg)</th>
<th>Loading Capacity (%)</th>
<th>Percent released after 24hr (%)</th>
<th>Percent released after 24-48hr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COF1</td>
<td>5.22</td>
<td>34.31</td>
<td>83.69</td>
<td>16.31</td>
</tr>
<tr>
<td>COF2</td>
<td>5.35</td>
<td>34.86</td>
<td>78.11</td>
<td>21.89</td>
</tr>
<tr>
<td>COF3</td>
<td>5.22</td>
<td>34.31</td>
<td>84.51</td>
<td>15.49</td>
</tr>
</tbody>
</table>

Positive percent releases indicate release of drug from the COF while negative percent releases would indicate an absorption of drug from solution. Only during the interval from 23.1 hours to 27.1 hours did COF3 demonstrate a negative percent release which was -42%. 


Final release study drug loading and release characterization:

This final COF release study was performed over 142 hours as opposed to the preliminary study which was only for 48 hours. Loading of the COFs was done by immersion in a hexane solution for 4 hours after which the COFs were isolated by vacuum filtration and washed with a small amount of hexane. 5 mg of drug-loaded COF was then transferred to a centrifuge vial for the release study. Total mass released was calculated using a standard curve to convert absorption of the IBU UV-vis characteristic peak at 264 nm to a solution concentration of IBU and then to a mass. Loading capacity (LC%) is calculated by the total mass of drug in the system divided by the total mass of the mixture. In this case:

\[
LC\% = \frac{\text{Total Mass Released}}{5 \text{ mg of Drug loaded COF}}
\]

Percent Released is the calculated by the mass released during a certain time interval divided by the total mass released at the peak release (120 hours after immersion).

*Table 2. Summary of final release study data. Total masses were derived from standard curve and association with UV-vis 264 nm characteristic peak of ibuprofen.*

<table>
<thead>
<tr>
<th>COF</th>
<th>Total Mass Released (mg)</th>
<th>Loading Capacity (%)</th>
<th>Percent released 0-24hr (%)</th>
<th>Percent released 24-48hr (%)</th>
<th>Percent released 48-96hr (%)</th>
<th>Percent Released 96hr-120 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COF1</td>
<td>2.93</td>
<td>36.93</td>
<td>86.66</td>
<td>-2.93</td>
<td>10.57</td>
<td>5.69</td>
</tr>
<tr>
<td>COF2</td>
<td>3.26</td>
<td>39.47</td>
<td>78.32</td>
<td>-0.59</td>
<td>16.87</td>
<td>5.40</td>
</tr>
<tr>
<td>COF3</td>
<td>3.30</td>
<td>39.74</td>
<td>94.51</td>
<td>3.11</td>
<td>-1.08</td>
<td>3.47</td>
</tr>
</tbody>
</table>
Figure 8. A) Preliminary study of Ibuprofen released to PBS from COFs over 48 hours, B) final study of Ibuprofen released to PBS from COFs over 142 hours, C, D) analysis of the release rate during intervals, E, F) 1st derivative analysis of A and B release profiles.
Cytotoxicity Assay of COF1 on HeLa cells: Following the above protocol, cells were counted by hemocytometer then the percent growths were calculated and normalized to the negative control. After 3 days of incubation COF1 treated cells of concentrations 0.011, 0.033, 0.1, and 0.3 mg COF1/mL media yields +9.8%, +7.8%, -42.2%, and -97.1% normalized cell growth, respectively. 0% growth would indicate growth matched that of the negative control while +, - each represents a percent increase or decrease in growth compared to the negative control.

Cytotoxicity Assay of COF1 and COF2 on HeLa cells: Following the above protocol, cells were counted by hemocytometer then the % growths were calculated and normalized to the negative control (n=6 of control as opposed to n=2 for treated cells). After 3 days of incubation with COF-treated wells with 0.01 mg COF/mL media COF1 and COF2 both showed mean 43.6% increase in HeLa cell growth. At 0.1 mg COF/mL media COF1 and COF2 yielded a 25.1% and 37.7% decrease in cell growth, respectively. At 1 mg COF/mL media COF1 and COF2 showed a 96.4% and 99.1% decrease in cell growth.

![Graph A](A) Normalized Growth of HeLa cells treated with COFs for 3 days
![Graph B](B) Normalized Growth of HeLa cells treated with COF1 for 3 days

*Figure 6. A) Growth of HeLa cells treated with increasing COF concentrations of COF1 and COF2 normalized to negative control, B) Growth of HeLa cells treated with increasing concentrations of COF1 normalized to negative control.*
Characterization of COF backbone stability in PBS by PXRD: COF1 shows peaks at 5, 9 and 16.5 degrees. COF2 shows peaks at 5 and 8 degrees while COF3 does not show any peaks before immersion. This means COF1 and COF2 have some crystallinity before immersion in PBS for 7 days but COF2 loses its crystallinity through the study and COF1 retained crystallinity.

![COF PXRD Stability Characterization](image-url)

Figure 7. Overlay of COF1 (A), COF2 with post-immersion reference peak inset (B), and COF3 (C) PXRD before and after immersion in PBS for 7 days.
Nitrogen Absorption/Desorption Isotherms: Nitrogen absorption/desorption and subsequent BET analysis was obtained by the Dr. Brian Trewyn Group in the Department of Chemistry at the Colorado School of Mines. COF1 and COF3 preloaded samples had anomalously low BET surface area therefore the loaded samples were not run. COF2 preloaded and loaded samples yielded single point surface areas of 577.3 m$^2$/g and 54.64 m$^2$/g, respectively, a 90.5% decrease. Overall pore total pore volume of COF2 before and after loading were 0.223859 cm$^3$/g and 0.010187 cm$^3$/g.

\[\text{Figure 8. } N_2\text{ adsorption data for COF2. A) Single point and BET surface area as well as BJH analysis of absorption and desorption surface area of pores. B) Single point and BJH analysis of absorption and desorption pore volume.}\]
Discussion

Cytotoxicity studies of COF1 and COF2 are inconclusive at this point due to broad confidence intervals and the low number of studies performed yet may suggest a range where the toxic concentration limit lies. COF in media concentrations above 0.1 mg COF/mL media appear to be toxic to HeLa cells, growth inhibiting, or a physical interference to growth and adherence of the cells to the well. Wells of concentrations 0.3 mg COF/mL media and above noticeably congested and showed very low cell growth which toxicity and adherence interference are likely both occurring. Positive percent growth in both studies may be accounted for by technical inconsistency or by COFs indeed having a positive impact on cell growth. Future studies should be performed at concentration lower than 0.1 mg COF/mL to better construct a survival curve for HeLa cells and to investigate positive cell growth in lower concentrations. These studies should also be performed on many COFs and various cell lines to determine structural factors that may be more or less cytotoxic to certain types of cells.

The preliminary and final release studies had one notable difference which highlighted an interesting result. In the preliminary study, COF3 released 84.5% of its total release amount in the first 24 hours while in the final study COF3 released 94.5% in the same interval. However, between immersion and the first measurement COF3, in the preliminary study, had already released 66.3% of its total released mass while in the final study by the first measurement COF3 had only released 50.7%. The 15.6% difference between the two studies highlights high release rates just following immersion. These release rates could be explained by weak interactions between the pore and drug allowing it to be released very quickly or loading of the drug outside of the pore in which case there would be no interaction between the pore and the drug. Another interesting occurrence in the release studies was the negative percent in intervals of preliminary and final release studies. This result is likely due to ibuprofen degradation in solution. One the other hand, it is important to remind that the forces responsible for loading and release are not understood; therefore, the reabsorption of ibuprofen after release is important to note and continue to research. In both the preliminary and the final release study, COF3 released the IBU faster than the
other two COFs while there did not seem to be much of a difference in the release rate for COF1 and COF2. This suggests that pore size may have a larger impact on release rate than hydrophobic hydrophilic interacting. If it is true a larger pore fits more drug, then it is also true that the drug at the center of the pore has less interaction with the pore than a molecule of drug at the edge of the pore next to the COF. Further research should be conducted to confirm the hypothesis that smaller pores encourage more interaction between the COF and the drug. This could be done by using more COFs with pore sizes of less than 2 nm for drug adsorption and release with drugs of similar molecular size as ibuprofen. Then using a ratio of molecule size to pore size scaling up drug size and pore size proportionally while maintaining pore and drug characteristics it would be expected that the release profile would be similar.

The PXRD data suggests that COF1 and COF2 are crystalline at the beginning of the study but that COF2 loses its crystallinity during immersion in PBS for 7 days. This suggests that the crystalline structure of COF2 degrades due to interaction with PBS. Conversely, COF1 seems to show crystallinity throughout immersion. This could be the reason COF2 released more IBU in the studies while COF1 does not, but it is not conclusive as we do not know if COF1’s lower release is due lower loading or high retainment. In the future, separately characterizing the loading amount the release would answer this question.

COF2 was the only framework which stability, functionality, and toxicity data were reliably obtained with success despite poor nitrogen absorption data on COF1 and COF3 (see Supplemental Figure 3). Nitrogen adsorption data for COF1 and COF3 showed very low porosity and nearly no change before and after loading. This suggests that IBU did not integrate within the COFs because there was no porosity to the COF. However, these COFs were shown to be crystalline by PXRD. Nitrogen adsorption and PXRD are often used in tandem to characterize the quality of the framework. In this case, PXRD and nitrogen adsorption yield contradictory conclusions. The reason for these contradictions is beyond the
scope and expertise of this project therefore conclusions about IBU integration to COF pore will be limited to COF2.

The decreased surface area and pore volume indicates that ibuprofen is integrating within the pore of the COFs. The loading capacity of the COFs reported in the UV-vis characterized release of ibuprofen was 39.49%. IBU, at a density of 1.03 g/cm$^3$, would, with a loading capacity of 39.49%, in 5 mg of loaded COF would take up $1.92 \times 10^{-3}$ cm$^3$ while the 3.0255 mg of COF in that loaded COF sample would only have an available $6.75 \times 10^{-4}$ cm$^3$ of pore volume available. Therefore, the ibuprofen cannot physically all be fitting within the pores of this COF. The remaining 64.8% of the ibuprofen would have two locations to feasibly reside: on the surface of the COF crystals or between domains of COF. Additionally, we cannot determine the level of impregnation of the drug into the COF. Furthermore, N$_2$ adsorption/desorption is not able to identify the pore volume or surface level directly but calculated indirectly through the isotherm behavior. If IBU is loaded just to the few layers at each end of a pore channel the whole channel and pore loses its volume which is the same result as if IBU is loading within the entire channel of COF. If we cannot determine the level of impregnation, we cannot then make a conclusion as to whether or not the differing pore characteristics have an effect on host-guest interactions. If location of the drug within the pore or direct quantification of drug loading into the COF is not known, the level of loading and variation of loading at a methodological and COF characteristics could play a much larger role in the release profiles obtained.

Using one batch of COF to measure loading capacity, release profile, N$_2$ adsorption, PXRD is crucial as COF crystallinity and overall quality may play a larger role in the characterization of COF drug delivery capabilities. As shown in Figure 4, amorphous MOF have significant difference in release profile so it is reasonable to suggest that the crystallinity of each COF batch would have a variation in crystallinity and account for variations in release characteristics.
Conclusion

Each of these projects research sections (loading and release, stability, and toxicity) are all suggestive but not conclusive. Future research is needed to repeat these studies to assess the fidelity of the protocol and results. In addition, many of these experiments asked more questions than they answered. Loading and release studies raise the possibility of reabsorption of drugs after release. The potential of this phenomenon is COFs potentially acting as physical buffer systems between drug concentrations in the media outside the COF. In the future, experiments should look at the range and flashpoint in medium drug concentration at which COFs stops absorbing drug and begins release and vice versa. It is crucial to do this study with many COFs, drugs, and media to observe behavioral variations and which forces play a role in determining the point at which release and absorption switches.

Cytotoxicity studies observed an increase in cell growth at low concentrations. While these studies were done in few replicates, this phenomenon may suggests that COFs interaction with HeLa cell is not just extracellularly by inhibiting adherence but also intracellular and affecting cell growth.

The stability of COFs before and after immersion in PBS as well as the molecular location of ibuprofen within the drug loaded COF required much more research. Changes in PXRD intensity, surface area, and FT-IR should be investigated at every point along the loading and release experiments. Additionally, studies of COFs and their interaction with growth media should also be performed to continue to address effects of COFs in cell growth. As for the low N$_2$ adsorption of COF1 and COF3 before loading suggests issues with synthesis and purification. In future studies, confirmation of porosity should be done on each batch prior to continuation of experiments involving each batch.

This project was designed to explore the feasibility of COFs as controllable drug carriers. This field requires significantly more expertise, time, depth, and breadth to draw any conclusions of significance in developing COFs as controllable drug delivery systems. However, this project does provide an outline for
a larger interdisciplinary project between organic chemistry, molecular biology, biomedical engineering, pharmacokinetics, and medicine. The quality of COF synthesis is of paramount importance to the rest of the study and should therefore be undertaken by experienced and dedicated synthetic chemists with a wealth of knowledge in organic and dynamic covalent chemistry. Determining toxicity and feasibility from a health and safety standpoint requires advanced knowledge of cell toxicity studies as well as future access to mammalian and other larger organisms to assess toxicity as the project grows. Finally, if COFs are determined to be feasible from a functionality and safety standpoint, a mechanism to keep COFs in a patient’s system for 7-30 days would require development of new technologies or adaptation of current technologies through novel biochemical and biomedical engineering. Overall, this was an invaluable learning experience for all members of this project and may be at the beginning of another long road of research and innovation to help propel healthcare toward personalized medicine.
References:


Appendix

Experimental procedures for the synthesis precursor monomers and final COFs.

**Synthesis of 1,3,5-tris(hydroxymethyl)benzene (S2):** Under nitrogen atmosphere, a solution of trimethyl-1,3,5-benzenetricarboxylate (S1) (100 mg, 0.4 mmol, 1 eq) in dry THF (1.8 mL) was added dropwise through a pressure-equalized addition funnel to LiAlH₄ (60 mg, 1.6 mmol, 4 eq) in dry THF (0.41 mL) at 0 °C. After complete addition, the reaction mixture was refluxed for 24 hours and cooled to room temperature. The reaction was quenched by slow addition of water (18 mL). The reaction mixture was filtered, and the residue was washed first with MeOH (18 mL) then with THF (18 mL). The solvent was removed under reduced pressure to give 3.3 g (95%) of 1,3,5-tris(hydroxymethyl)benzene as a pale-yellow solid. 1H NMR (300 MHz, D₂O): δ = 7.35 (s, 3H, ArH), 4.69 (s, 6H, -CH₂OH) ppm. The synthesis was adapted from previous literature and analytical data are consistent with those from literature.²³

**Synthesis of 1,3,5-triformylbenzene (CHO1):** 1,3,5-Tris(hydroxymethyl)benzene (100 mg, .6 mmol, 1 eq) was suspended in DCM (6.3 mL). Pyridinium chlorochromate (PCC, 380 mg, 1.8 mmol, 3 eq) and Celite (200 mg) were added and the mixture stirred for 6 hours at room temperature. Subsequently the reaction mixture was diluted with ether (5 mL) and was allowed to stir for further 30 minutes. The reaction mixture was filtered, and the filtered reaction mixture was extracted with DCM (200 mL). The extract was concentrated to dryness by rotary evaporation. Crude product solid was then was purified by column chromatography (DCM) 1H NMR (300 MHz, CDCl₃): δ = 10.21 (s, 3H, ArH), 8.64 (s, 3H, -CHO) ppm. The synthesis was adapted from previous literature and analytical data are consistent with those from literature.²³

**Synthesis of 1,3,5-triformylphloroglucinol (CHO2):** Hexamethylenetetramine (7.50 g, 53.5 mmol, 2.25 eq), phloroglucinol (3.00 g, 23.8 mmol, 1.0 eq) and trifluoroacetic acid (45 mL) were added to a 250 mL round bottom flask. The solution mixture was allowed to stir in a preheated oil bath at 100 °C temperature for about 2 h under nitrogen atmosphere. The reaction mixture was then subjected to vigorous stirring for 1 h after slow addition of (75 mL) 3 M hydrochloric acid. After reaction completion, the resultant solution mixture was filtered through a fritted filter funnel, and the filtrate was extracted with dichloromethane (75 mL 4 times) and dried with sodium sulfate. The light orange solid product was collected after solvent evaporation. 1H NMR(300MHz, d6-DMSO): δ =14.15 (s, 3H, Ar-OH), 10.82 (s, 3H, CHO). The synthesis was adapted from previous literature and analytical data are consistent with those from literature.²⁴
Synthesis of 1,3,5-tris(formylphenyl)benzene (CHO3): Tribromobenzene (1.00 g, 3.1 mmol), 4-formylphenyl boronic acid (2.85 g, 19 mmol), potassium carbonate (2.63 g, 19 mmol) were added to a 125 mL Schlenk tube equipped with a stir bar. The flask was evacuated and backfilled with Ar three times in the glove box entrance chamber before being brought into a glove box to add Pd(PPh₃)₄ (0.184 g, 0.16 mmol). Under N₂ in the hood, dioxane (27 mL) and H₂O (5.7 mL) were mixed and then added to the flask. The reaction mixture was then heated to reflux for 48 h. The reaction mixture was cooled to rt, after which a precipitate formed that was washed with ethyl acetate and collected. This solid was saved and identified as pure. The filtrate was washed with H₂O, then brine, finally dried with MgSO₄, filtered, and the solvent was removed. The crude product isolated from the supernatant purified by column chromatography. 1H NMR (400 MHz, CDCl₃) δ 10.11, (s, 3H), 8.02 (AA’BB’, 6H), 7.91 (3H, s), 7.87 (AA’BB’, 6H). The synthesis was adapted from previous literature and analytical data are consistent with those from literature.²⁵

Synthesis of COF-Ph (COF1): To a 5 mL glass ampule, 1,3,5-triformylbenzene (CHO1) (12.6 mg, 0.08 mmol, 1 eq), p-phenylenediamine (12.6 mg, 0.12 mmol, 1.5 eq) and 1 mL of dioxane. The mixture was then sonicated to make a homogenous suspension. 0.2 mL of 3M acetic acid was added and mixture was sonicated again for 10 minutes. Ampule was sealed using the Zhang group freeze-pump-seal method. Ampule was then placed in an oven at rt, heated to 120 ºC over 1 hour and left for 3 days undisturbed. After 3 days ampule was broken open and solids were isolated by vacuum filtration. COFs were purified by Soxhlet extraction by THF for 3 days. Method was adapted from previous literature and modified to fit lab capabilities and at suggestions of senior members who have previously synthesized this COF of the lab.²⁶

Synthesis of COF-TpPa (COF2): To a 5 mL glass ampule, 1,3,5-trihlorogluclinol (CHO2) (18.9 mg, 0.09 mmol, 1 eq), p-phenylenediamine (24.9 mg, 0.135 mmol, 1.5 eq) and 1 mL of 1:1 1,4-dioxane/mesitylene were added. Mixture was sonicated for 10 minutes to a homogenous dispersion. 0.15 mL of 3M acetic acid was added to the vial and then it was sonicated for another 10 minutes to homogenous suspension. Ampule was sealed using the Zhang group freeze-pump-seal method. Ampule was then placed in an oven at rt, heated to 120 ºC over 1 hour and left for 3 days undisturbed. After 3 days ampule was broken open and solids were isolated by vacuum filtration. COFs were purified by Soxhlet extraction by THF for 3 days. Method was adapted from previous literature and modified to fit lab capabilities and at suggestions of senior members who have previously synthesized this COF of the lab.²⁷

Synthesis of COF-TfphPa (COF3): To a 5 mL glass ampule, 1,3,5-tris(formylphenyl)benzene (17 mg, 0.04 mmol, 1 eq), p-phenylenediamine (6.5, 0.06 mmol, 1.5 eq) and 1 mL of 1:1 1,4-dioxane/mesitylene were added. Mixture was sonicated for 10 minutes to a homogenous dispersion. 0.2 mL of 3M acetic acid was added to the vial and then it was sonicated for another 10 minutes to homogenous suspension. Ampule was sealed using the Zhang group freeze-pump-seal method. Ampule was then placed in an oven at rt, heated to 120 ºC over 1 hour and left for 3 days undisturbed. After 3 days ampule was broken open and solids were isolated by vacuum filtration. COFs were purified by Soxhlet extraction by THF for 3 days. Method was adapted from previous literature and modified to fit lab capabilities and at suggestions of senior members who have previously synthesized this COF of the lab.²⁸
Zhang Group freeze-pump-seal method: The glass ampule neck was heated to mobility and stretched to an extra 1-1.5 cm in length and neck diameter of 2-3 mm. Nalgene 180 clear PVC VI grade tubing was heated on the inside and expanded to be attached to the stem of the ampule. Ampule and contents were frozen in LN$_2$, attached through tubing to vacuum line, evacuated to vacuum. Under vacuum thinnest portion of the next was heated to using a blow torch to seal.

SEM

*Supplemental Figure 1 (S2): A) SEM of COF-Ph with inset region B, B) Expanded view of inset region*

*Supplemental Figure 2 (S3): A) SEM of COF-TpPa with inset region B, B) Expanded view of inset region*
Nitrogen Adsorption

Supplemental Figure 3 (S1): Nitrogen Adsorption Isotherm of A) COF-Ph, B) COF-TpPa and C) COF-TfpbPa