**Winter Symposium Program** February 21, 2014, Nobel Hall

**Session I –Wallenberg Auditorium - 1:30-2:30pm**

1:30-1:50 **Oligomerization of gamma-glutamylcysteine ligase**

 Elizabeth Wiese (’15) and Katie Diederichs (’16)

Abstract: Gamma-glutamylcysteine ligase (γ-GCL) is the enzyme that catalyzes the rate limiting step in the synthesis of glutathione. Glutathione is important in detoxifying cells however has been found to be upregulated in cancer cells leading to chemotherapy resistance. Thus, investigation of γ-GCL and its structure can be applied toward therapeutic advancements. The goal of this project was to separate and characterize the different oligomeric forms of *E. coli* γ-GCL and analyze the role of disulfide bonds in the oligomerization process. The monomeric and dimeric forms had similar kinetic activities, however, the dimer was found to have greater stability as determined through fluorescence spectroscopy. The addition of the reducing agents dithiothreitol and β-mercaptoethanol resulted in the transition from the dimeric to a monomeric state. However, the addition of oxidizing agents ascorbic acid and hydrogen peroxide to both the monomer and dimer resulted in the transition to a higher order oligomer of unknown size. Future experimentation will involve an examination of the impact of other oxidizing agents on the transition between oligomeric states, determination of the molecular weight of the higher order oligomer, and analyzing the role of noncovalent interactions in the oligomerization process.

1:50-2:10 **Photodegradation of Imidazolinone Herbicides and Pesticides**

 Amy Christiansen (’14) and Rachel Meier (’16)

Abstract: Imazapic, imazamox, imazaquin, and imazethapyr are herbicides commonly used on corn and soybean plants in the Midwest. Photolysis has been shown in previous studies of imidazolinone herbicides to be a major pathway of degradation in the environment. The purpose of this study was to determine the rate at which each herbicide degraded under different conditions and to propose photoproducts of the degradation. Two different systems were used for study: irradiation of the herbicides in aqueous solution as well as on the epicuticular waxes of corn and soybean leaves. Using ultraviolet light, these herbicides were irradiated at several pH values or at a constant pH with varying amounts of natural organic matter (NOM). The rates of degradation were analyzed using a high-performance liquid chromatograph (HPLC). To identify preliminary photoproducts, liquid chromatograph-mass spectrometer (LC-MS) data from previous research was used. Future work will include obtaining more data on the corn and soybean waxes, identifying photoproduct pathways, and observing how the herbicides degrade when analyzed on intact corn and soybean plant leaves.

2:10-2:25 **Characterization of Therapeutic Monoclonal Antibodies using Two-Dimensional Liquid Chromatography**

 John Danforth (’16)

Abstract: Monoclonal antibodies (mAbs) are used as pharmaceuticals produced for their specificity in targeting diseased cells in the human body. The development of mAbs is becoming increasingly important in the treatment of cancer and autoimmune diseases because of their unique specificity. The complexity of these large biomolecules makes it difficult to produce consistent batches and so they must be rigorously characterized to determine their effectiveness and safety. Many methods involving liquid chromatography coupled with mass spectrometry such as LC-MS/MS have been used for the characterization of mAbs.

 This research focused on comparing conventional one-dimensional liquid chromatography (1DLC) techniques with two-dimensional liquid chromatography (2DLC), a method of chromatography using two orthogonal columns to separate complex mixtures. These methods were applied to separate digested and reduced samples of the mAb rituximab. Rituximab has been the subject of many characterization techniques making it a suitable test sample. In order to characterize rituximab several samples were analyzed using both 1D separation and 2DLC to compare the effectiveness of these methods and determine how the mAb breaks apart upon digestion and/or reduction.

 This presentation will include background regarding the characterization of mAbs and the importance of characterization, as well as issues with the initial papain digestions and possible solutions to the problem. Data presented will be mainly chromatograms compared to previous work that we intend to replicate before moving on to less characterized mAbs and their biosimilars and biobetters which discussed in more detail.

**Session I – Nobel 201 - 1:30-2:30pm**

1:30-1:40 **Brief Overview of Liquid Chromatography**

 Dr. Dwight Stoll

1:40-1:55 **Synthesis and Characterization of Fullerene Modified Silicas for Use in Liquid Chromatography**

 Jamie Brooks (’14)

Abstract: This work focused on the preparation of Fullerene-Modified Silicas (FMS) packed columns for Reverse Phase Liquid Chromatography. First, the reaction conditions for the silanization of the fullerene particles were optimized. In this reaction, the fullerene was reacted with the silica particles through the usage of an aminoalkyl tether. It was found that this reaction requires the usage of an inert environment, such as nitrogen gas. The inert environment helps reduce the amount of undesired radical reactions between the aminoalkyl tether and fullerene.

 After the silanization was optimized, the fullerenes were reacted with the silanized silica particles, which produced the FMS materials. The results of these reactions helped identify a key fact about the procedure as a whole: the reaction could take place at a temperature just below reflux without compromising the molecular structure of the fullerenes or the chromatographic resolution of the column. Additionally, the high temperature reactions (24 hours) are much more time efficient compared to a room temperature reaction (~10 days).

1:55-2:10 **Comparison of Graphite-Like and Related Stationary Phases for use in High Performance Liquid Chromatography using Linear Solvation Energy Relationships**

 Anna Krieger (’17)

Abstract: Six different carbon-based phases used for High Performance Liquid Chromatography were characterized using Linear Solvation Energy Relationships. Thirty-seven solutes with known and varying solute parameters were used to determine the corresponding stationary phase characteristics via multiple linear regression of solute parameters and their respective retention factors on each column. Interesting results included the observation of a large degree of variation in the "r" column coefficient, which quantifies the polarizability of the stationary phase material. Graphitic phases tended to have higher "r" values, suggesting that large systems of π electrons are responsible for this high degree of polarization.

2:10-2:25 **Characterization of New Stationary Phases for High Performance Liquid Chromatography using the Hydrophobic Subtraction Model**

 Mai Der Vang (’14)

Abstract: Column selectivity is key to successful chromatographic separations and varies depending on the chemical properties of the stationary phase material. A method specifically used to describe column characteristics is the Hydrophobic Subtraction Model. The database containing parameters determined using this model has data for over 600 commercially available columns. In this work, eight new columns were characterized and compared to columns already in the database. One of the new columns, Cosmosil 5PYE, appears to be truly unique compared to the existing materials, whereas the other seven column were similar to at least one other column already in the database. In addition to characterizing the new columns, we examined changes in diversity of stationary phases in the database over time, and observed a general increase in redundancy as more columns were characterized over time, as expected.

**Session II – Nobel 201 - 2:30-3:30pm**

2:30-2:50 **Impact of Mining on Methylmercury in the St. Louis River Using Dragonflies as a Bioindicator**

 Russell Krueger (’17), Rachel Weitz (’16), and Kris Reiser (’17)

Abstract: Methylmercury (MeHg) is a neurotoxin formed in anoxic environments by sulfate reducing bacteria when Hg(II), sulfate, and organic carbon are present. Once MeHg is formed it bioaccumulates in organisms and biomagnifies up the food chain. Mining activities in northern Minnesota releases sulfate into area water bodies, potentially impacting MeHg formation. In this study, we used dragonfly larvae as a bioindicator of MeHg contamination in streams and rivers within the St. Louis River watershed. Dragonfly larvae were chosen because they are obligate predators, widespread regionally, and individual larvae are restricted to the water body in which it was hatched. We analyzed 252 samples of dragonfly larvae from 12 sites and related MeHg levels in the larvae to MeHg contamination in the water. We did not find a correlation in 2013 between dragonfly larvae MeHg and dissolved MeHg concentrations in the water. By combining data collected in 2012 with our 2013 data, we did find a significant relationship between MeHg in the larvae and MeHg in the water. Further research is needed to assess some of the systems that appeared to be outliers. Increased sulfate concentrations did not appear to lead to increased MeHg levels in dragonfly larvae.

2:50-3:05 **Effect of Sample Buffer pH and Injection Volume on HPLC Column Performance under High pH Mobile Phase Conditions**

 Kelly O’Neil (’15)

Abstract: A prototype HPLC column capable of withstanding high pH mobile phases (e.g., pH > 10) was tested with the intent to study how varying the pH and volume of injected samples of amines affects the peak shapes for these compounds when the mobile phase is buffered at high pH. Initially, poor peak shape was observed for the amine-containing analytes, and several different experimental variables were investigated to find conditions that would provide good peak shapes under the conditions of this study. We find that both the pH and the ionic strength of the buffered mobile phase have a dramatic effect on peak shape, and that good peak shape can be obtained if the ionic strength is sufficiently high. These findings will now enable further work that addresses the initial goals of the study.

3:05-3:20 **Simulation of Sample Matrix Effects in Liquid Chromatography with Large Injection Volumes**

 Ray Sajulga (’17)

Abstract: High Performance Liquid Chromatography is an analytical chemistry technique that allows for the qualification and quantification of chemical compounds of unknown identity or amount. With the right equipment and knowledge, multiple experiments can be run at a given time. However, these experiments may have durations longer than necessary—therefore wasting material—with all the trial and error that comes with experimenting. With this simulation approach described in this work, it may be easier to predict when an analyte will elute given certain conditions. As a result, experiments can be fine-tuned to minimize the use of resources without sacrificing results.

 The way this simulation works relies on three variables: time, distance, and the concentration of analyte. These are represented in a matrix, having time on the downward vertical axis, distance on the rightward horizontal axis, and concentration occupying the cells. As the program runs, it propagates chemicals that are injected at the beginning of the column to the rest of the column over time, resulting in a diagonally lower-right directionality. To ensure the accuracy of the program, the time of the experiment and length are divided into thousands of units. To handle the enormity of this simulation, MatLab was chosen due to its accessibility and efficiency in handling with matrices.

 This concept of simulated chromatography isn't exactly new. A lot of the chemophysics and concepts needed to program this simulation was explained thoroughly in a paper by Martin Czok and Georges Guiochon. However, to take a step forward, this simulation was modified to handle gradient elution situations. Another step taken was the creation of a supplemental program that animates analyte in a real-time video clip as it travels through the column. In the future, there is hope for a website and a java program to assist chromatographers and to educate the curious.

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