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EDGE ENHANCED DISCOVERY THROUGH GUIDED EXPLORATION

Creative Inquiry:

The process of exploring issues, objects or works through the collection and analysis of evidence including combining or synthesizing existing ideas, products, or expertise in original ways to answer an open-ended question or achieve a desired goal.

About the Cover:

This year's cover of the Journal of Creative Inquiry focuses on the theme of "systems" and features a set of human lungs sculpted out of branches and roots, permeating the blood vessels, bronchi, and trachea. The respiratory system, comprised of multiple organs working together to achieve a common goal, epitomizes the mission of JCI. Hopefully, this cover will act as a symbol for the interdisciplinary happenings at Tennessee Tech University and inspire JCI readers to breathe deep.

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Guest Editorial

"Systems" is the driving theme of this issue of the Journal of Creative Inquiry. Contributors of diverse backgrounds independently explored complex systems like chemical reactions, the human body, nanoparticles, societal roles, online communities, cycles of substance abuse, adult learning and more. These studies of delicately balanced and complex systems connect the unique journeys of our student and faculty scholars. Universal systems found in nature and unique systems experienced as people weave throughout the pages of this issue. Siloing is common for academic disciplines at universities, but the systems in which our disciplines operate are interrelated. We as scholars can build and foster relationships by finding intersections and designing interactions within our systems. The role of this issue of JCI is to illuminate the universal significance of systems and to spark interactions and connections across Tennessee Tech University.

Jeremy Blair, *Guest Editor*

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The Human Body and Visual Art: Three Perspectives

Jenna Lee Stevens, Melissa Geist and Kimberly Winkle

ABSTRACT

In the spring of 2017, Professor Melissa Geist, Professor Kimberly Winkle, and Vice President of Student Affairs Mr. Marc Burnett co-instructed HONORS 4013-002: The Human Body and Visual Art, an interdisciplinary Honors Colloquium supported by the Tennessee Tech University EDGE QEP Grant for creative inquiry. The course employed a variety of art mediums and formats to explore anatomy and physiology through creating art. By challenging students to think critically and learn using multiple modalities, the course ultimately encouraged a deeper understanding of human anatomy and physiology and art.

The Human Body and Visual Art

Students intending to graduate *in cursu honorum* take up to two Honors Colloquia, interdisciplinary courses designed exclusively for Honors students by Tennessee Tech faculty. Offering thought-provoking topics that vary each semester, Colloquia challenge students to open their minds beyond the scope of their majors. In the spring of 2017, twenty students from diverse backgrounds joined in pushing the boundaries of their comfort zones; they enrolled in what one might argue to be the most unique Colloquium offered yet: HONORS 4013-002 The Human Body and Visual Art.

Dr. Melissa Geist, Professor of Nursing and member of the Tennessee Tech Board of Trustees; Professor Kimberly Winkle, the School of Art, Craft, and Design Director; and Mr. Marc Burnett, the Vice President of Student Affairs, team-taught The Human Body and Visual Art. Collaboratively, they designed the Colloquia to facilitate creative thinking and problem solving, as well as imaginative design, for Honors students of any major, not only those in healthcare or art. The course employed a variety of art mediums and formats to explore anatomy and physiology through creating art. By challenging students to think critically and learn using multiple modalities, the course ultimately encouraged a deeper understanding of human anatomy and physiology. As part of Tennessee Tech's Quality Enhancement Plan (QEP), the Enhanced Discovery through Guided Exploration (EDGE) Grant supported this course for creative inquiry.

This once-a-week class worked in cycles. First, Dr. Geist taught a new anatomical system, although these meetings were far from an ordinary lecture-style class. In fact, Dr. Geist surprised students during their first lesson with cow hearts to examine and dissect for a closer look at the cardiac system. For the respiratory system, students inspected pig lungs, one black from cigarette exposure and the other healthy, and observed as special equipment caused the lungs to "breathe" to exemplify the damaging effects of smoking. Using virtual reality simulators in the Tennessee Tech Library's iCube, students explored the chambers of the heart and travelled through the respiratory system as a molecule of oxygen. During the second week of a given system, Prof. Winkle and Mr. Burnett taught students new art techniques and vocabulary. Their diverse perspec-tives as a trained artist and self-taught artist, respectively, were both inspiring and useful in finding one's own, unique artistic styles. Students gained exposure to a variety of mediums, including water-color pencils and water-soluble pastels on cold press paper, collage, acrylic on canvas, and mixed media. In the final week of a system, students submitted a written analysis and all convened for a formal critique, presenting on both how the individual completed artworks communicate a concept from the assigned anatomical system and the visual elements and principles of design employed in its creation. This cycle was repeated four times to cover the cardiac system, respiratory system, renal system, and endocrine system. The semester culminated with a student exhibition in the Joan Derry-berry Art Gallery, during which friends and family joined for student led final presentations of their favorite artwork created during the semester long inquiry.

Jenna Lee Stevens' Perspective: Student

As an accounting major, I have a very analytical mind. The anatomy and physiology concepts came relatively easy to me, but learning to apply this material in a creative way required me to challenge my traditional method of thought. For each system, I dedicated significant time to brainstorming how I might apply various concepts, processes, or aspects of my personal life to Dr. Geist's anatomy and physiology lesson and then how to construct a visual representation that effectively communicates this correlation. Actively engaging both the left and right brain way of thought allowed me to analyze in a more creative and perceptive manner than ever before. As the semester progressed, I grew more comfortable finding an expressed relationship between anatomy and physiology and art, and I discovered that this creative method of problem solving ultimately gave me a deeper understanding of the anatomy and physiology concepts. I turned my ideas from this unique critical thinking process into preliminary sketches, which often inspired new ideas and further brainstorming. After receiving constructive feedback on my sketches from the three instructors and learning new techniques for the assigned medium and format, I finally began the art making process, allowing my creative side to take over. In the days leading to the formal critique, I carefully crafted my artwork, of which an example is seen in *Figure 1*. In this work, entitled *Endocrine System*, I was expressing the power and beauty of the female endocrine system. Through use of color, symbolism, iconography and scale, I was able to not only communicate my belief in women's reproductive rights but also capture the magnificence and magnitude of the specific anatomical system. The work of both an artist and a surgeon requires patience and a steady hand, as Mr. Burnett once suggested, which I certainly found to be true in creating my art. The formal critique was an engrossing and special process. In addition to the satisfaction of sharing my own art on which I worked tirelessly, my classmates' insights and artistic abilities always captivated me, especially considering many of us did not have prior experience with either art or anatomy and physiology.

I am immensely thankful for this Honors Colloquia, as it is undoubtedly the most fascinating, enriching course I have had the pleasure of taking here at Tennessee Tech. Dr. Geist, Prof. Winkle, and Mr. Burnett offered ample amounts of motivation throughout the

Figure 1. Endocrine System, acrylic and collage on canvas, 24" x 30", Jenna Lee Stevens, 2017.

semester and established a welcoming, positive classroom environment that allowed us to feel comfortable sharing personal aspects of our lives in our artwork. This class served as both an academic challenge and an outlet for self-expression. It challenged me to use critical thinking to apply scientific concepts to art and then effectively communicate how my visual representation expresses some aspect of an anatomical system while using proper art terminology. I looked forward to this class each week and enjoyed learning to analyze in a new, compelling way.

Dr. Geist's Perspective: Nursing

The challenge for the course designers (Prof. Kimberly Winkle, Mr. Marc Burnett, and Dr. Melissa Geist) was to build a course to en-gage students from diverse majors (Accounting, Elementary Educa-tion, Biochemistry, and Music plus more) with complex topics for which they did not have any foundational knowledge. From an outsider's view it might seem impossible or even "silly" to bring together anatomy and physiology with art in a cross-disciplinary class, but when we pitched the idea to Dr. Rita Barnes and the Honors Council, they immediately saw the value of such a pairing. Four main factors led to the success of the course.

1. The faculty members' genuine interest to learn from each other

2. A clear pedagogical structure that allowed students to fail safely

3. Formative peer review and collaboration that encouraged students to tap into one another's strengths for the success of all

4. Fostering student buy-in with an art gallery exhibition requiring them to demonstrate a deep understanding of science and art

One of the best aspects of this course was spending time watching incredibly talented individuals teach subjects for which they are so passionate. Each class period, dedicated to a different art medium, provided a new learning experience for me. I knew very little about the different artistic mediums introduced in the course (watercolor, acrylic paints, pencil, collage, etc.) and I, along with the students, learned how to push fears and inhibitions aside as we delved into each new visual artistic style. Critiquing art was also new to most students in the class. Professor Winkle developed a guide for peer critique that prompted all of us to look beyond the "I like it" surface treatment of works of art. Throughout the semester we heard non-art majors discussing focal point, use of line, shape and texture, color scheme, and context, and we now all possess a more disciplined and knowledgeable approach to viewing art.

Because the faculty understood that the students in the course possessed different types of background knowledge, formative assessments were built into the class to provide ongoing feedback from peers and faculty. For example, the students took an anatomy and physiology quiz for each body system (cardiac, respiratory, renal, endocrine, etc.) covered in class. The students first took the quiz individually, and then they were placed in pairs, with one person in each pair having completed coursework in anatomy and physiology. The two students compared answers and, using a different color of ink, changed their responses if necessary. The faculty could see what an individual student scored before and after he or she discussed the quiz with a more knowledgeable peer. This approach decreased anxiety for students who were apprehensive about the science in the course. There was similar support for the visual art content of the class. The students engaged in small group peer review of their artistic creations before the required presentation to the entire class.

The pinnacle of the course was an art exhibit beautifully curated by students and Prof. Winkle. During the exhibition, each student picked one of their creations from the semester and discussed the artistic merits of the piece as well as the anatomy and physiology related to the design. It is hard to capture the magic of that evening adequately. The students' presentations were imaginative, deeply personal, and scientifically on point.

The Human Anatomy and Visual Art class represents the best of what is possible when dedicated faculty, talented students, and a supportive academic structure come together to push the boundaries of traditional college experiences.

Professor Winkle's Perspective: Art

As a firm believer in the creative inquiry learning model, I was excited to have the opportunity to implement it in a unique course offering, which combined two disparate academic disciplines: Anatomy and Art. While the disciplines are quite distinct, the ability to ex-plore problem solving through divergent and convergent means is useful to all disciplines. It is this mode of thinking that we encouraged and students employed as they learned about human anatomy and art. The honors colloquia course was populated by students from all disciplines, which provided wonderfully diverse perspectives and responses to the problems that were posed. However, the unifying element was their genuine enthusiasm and acceptance of the course and subject matter. Initially, I wasn't sure how receptive or serious students would be to the art-making process. Often people who have little experience with art may not understand or appreciate the challenge of creating compelling works of art. Some may have the opinion that art should be "pretty" and "pleasant" and not realize or accept that art can exist in any form or style and that its creation is time consuming and tedious. While I was quite pleased by how fully students embraced the art-making process, what is more important and satisfying is how well they explored the idea-tion process of seeking and finding connections between the anatomical systems and visual expression. Students were not tasked with creating direct anatomical models, which doesn't encourage abstract thinking and exploration. Instead, they were pushed to see beyond the expected representation by gaining a deeper understanding of the system: how it works, where it resides, what it affects, etc. Through this understanding, students were able to connect aspects of the anatomical system to larger concepts of which they represented through a variety of artistic mediums. For instance, Muzakhir Amanzholov's artwork (*Figure 2*) represents the cardiac system. In his explanation of the work, he cited the relationship of the cardiac system to an engine, which requires efficiency, proper timing and interdependence amongst parts. His representation is a stylized heart, which includes various mechanical elements to re-contextualize the function from biological to mechanical. His use of color is symbolic, representing oxygenated and deoxygenated blood and his use of line suggests not only arteries but also the interconnectedness requisite of both a functioning heart and a motor. In order to create this artwork, Muzakhir needed an acute understand-ing of the anatomical system. He needed to know much more than how a heart appears, but also how it functions, why it functions and what its function supports. Through this understanding, he was able to synthesize these concepts into his compelling work of art. All twenty of our students (*Figure 3*) engaged weekly in this rigorous endeavor, inspired first by Dr. Geist's experiential teaching methods, then with brainstorming, research and sketching, then artwork creation and finally the project critique and analysis. Through this creative inquiry process, I am confident that students gained a much deeper and thorough understanding of anatomy and developed a heightened appreciation of art.

This course was a joy to teach; I enjoyed the challenge of creating a unique course with my colleagues and I learned a lot from my colleagues and our students in the process of implementing it. I am grateful for the opportunity and the support to be able to collaborate with colleagues across campus to provide unique, valuable and, sometimes unusual, learning experiences for our students.

Figure 2. Cardiac System, watercolor on paper, 18" x 24", Muzakhir Amanzholov, 2017.

Conclusion

The Human Body and Visual Art offered a unique academic experience and opportunity to open one's mind to subject matter which might otherwise be absent in one's chosen field of study, ultimately contributing to a well-rounded collegiate career. It is truly remarkable what a classroom full of diverse majors and backgrounds can accomplish in a semester with the right guidance and inspiration.

Figure 3. Students and faculty in Human Anatomy and Art, 2017

Examining the Landscape of Innovation and Entrepreneurship at a University: Evidence from an Experiential Perspective

Jonathan C. Abbotoy and Ann Boyd Davis

ABSTRACT

Given that jobs will change and that careers will develop and transform, students must not only understand the topic-based hard skills in a particular college or major but also the innovative and entrepreneurial skills that strengthen and support those topic-based skills. Traditionally, these innovation and entrepreneurship skills are taught in a classroom setting that students tend to find boring or routine. Some universities are introducing these skills using games or intense summer programs. Our study examines students' perspectives of the introduction of innovation and entrepreneurship when little to no barriers are introduced. In our study, the University introduced and encouraged various innovative and entrepreneurship activities throughout campus with experiential learning activities outside the classroom. Student performance and involvement in innovation and entrepreneurship outside of the classroom setting allows for involvement and awareness. The entrepreneurship initiatives on campus have improved student awareness of resources and shown these resources to be beneficial to the student body. The student body also tends to be innovative and desire to tinker with the development of new ideas, solidifying the importance of campus activities.

Introduction

According to the 2017 Kauffman Index of Startup Activity, business startup activity continued a three-year increase reaching pre-Recession levels (Kauffman 2017). Students also demonstrate an interest in innovation and entrepreneurship. In fact, according to Kauffman Foundation, over half of young people (ages 18-34) would like to start their own businesses (Kauffman Foundation, 2011). Keenly aware that careers will grow and develop, students see the importance in developing not only the topic-based hard skills in a particular college or major but also the innovative and entrepreneurial skills that strengthen and support those topic-based skills. Soft skills may also follow along with these hard skills.

Traditionally, these innovation and entrepreneurship skills are taught in a classroom setting that students tend to find boring or routine. Recently, universities are teaching these skills using games or intense summer programs (Robinson 2013, Robinson et al. 2014). While these recent environments provide more exciting activities for students and allow them to encounter risk at different levels requiring team work, critical thinking and creativity, they still introduce parameters that can be restrictive to students. Our study examines students' perspectives of the introduction of innovation and entrepreneurship when little to no barriers are introduced.

The University in our study introduced and encouraged various innovative and entrepreneurship activities throughout campus. Specifically, the University Innovation Fellows worked together to immerse the entire campus in innovation and entrepreneurship by organizing a student club focused on Social Entrepreneurship, gathering speakers for presentations, encouraging participation in Eagleworks (the University-sponsored pitch competition where students pitch their business ideas and products), utilizing the Makerspace in iCube for activities, etc. These activities were also encouraged by many faculty, staff, and administration at the University. While classes were offered to support these activities and a subsequent undergraduate Certificate program was developed, the momentum for learning and developing innovative and entrepreneurial skills centered around experiential learning activities. This study examines students' perspectives of a University landscape with regards to innovation and entrepreneurship both before and after the year in which many of these activities were introduced. To our knowledge, this study is the first to document the importance of learning activities outside the classroom or a structured program related to innovation and entrepreneurship.

Background on University Innovation Fellows

The University Innovation Fellows (UIF) program is a global initiative to change the innovative and entrepreneurial culture and mindset of university students. The program consists of university students from across the country acting as change agents at their respective campuses. The program itself consists of a six-week training program where students learn to gauge their universities' innovative and entrepreneurial landscapes and examine the effectiveness of the existing programs and resources. As part of the training process, the fellows select a project that they believe will impact their university based on the evaluation of their campuses' innovative and entrepreneurial ecosystem. Fellows, after successful completion of the program, focus on changing their university through design thinking, ideation strategies, and project implementation.

The focus of the UIF program is to use students as the primary leaders and advocates for innovation and entrepreneurship. The goal is to create an interdisciplinary, innovative, and entrepreneurial culture. The benefit of the less-structured, student-lead programs and initiatives is that they help to break down the barriers often created by courses and programs specific to one field of academic study. This approach focuses on the importance of involvement from all colleges across the university.

Tennessee Tech University began its involvement in the UIF program in 2014. Tennessee Tech now has ten Fellows who have been working to spread innovation and entrepreneurship on their campus. These Fellows have been involved with the development of the University's MakerSpace, implemented campus-wide innovation and entrepreneurship awareness projects, and continuously interacted with hundreds of students and university stakeholders to create an innovative and entrepreneurial culture.

Various universities have many programs, resources, and opportunities for innovation and entrepreneurship, including competitions, speaker series, and tours of incubator programs. However, these resources and programs appear to be disconnected and unused by the general student populace, with many resources and programs in innovation and entrepreneurship never making it out of the building in which they originated. Paramount to innovation and entrepreneurship is an interdisciplinary focus. Tech is a school in which the Colleges of Business and Engineering are two of the larger Colleges on campus and present the opportunity for this interdisciplinary focus. However, the general student body needs to capitalize on the resources offered at Tech and expand its ecosystem to further foster an innovative culture across campus.

Prior Literature

Over the past decade, we have experienced periods of global economic recession. Entrepreneurship activities are often touted as the solution to these recessions due to the economic and employment outcomes (Jones and Colwill 2013). College graduates, generally, face the challenge of a portfolio career including paid employment, non-work, and self-employment. Acquiring an innovative and entrepreneurial mindset helps prepare students for this portfolio career and the changing economic environment (Rae et al. 2011).

In higher education research, the debate continues on the effectiveness of taught innovation and entrepreneurship education. Charney and Libecap (2000), in their research titled Impact of Entrepreneurship Education, examined entrepreneurship in higher education in the U.S. Here, they find that entrepreneurship education produces successful business leaders, enhances wealth creation, and develops champions of innovation. For those graduates focusing on entrepreneurship versus non-entrepreneurship graduates, this translates into business leaders that generated five times the sales and employment growth, 27 percent higher annual average income, and a 13 percent higher probability of being associated with a high-tech firm and a 9 percent higher probability of developing new technological products (Charney and Libecap 2000). However, despite the potential impact of entrepreneurship education on careers, much of the literature focuses on traditional classroom experiences.

Studies have shown that the risk-taking element of entrepreneurship and innovation is more likely to occur in less formal settings. Robinson (2013) examined the benefits of games in the classroom setting and the student response to risk. It was found that students were less likely to take risk when a grade is involved and more likely when it was a part of a game. Additionally, Robinson asserts that "research on risk-taking has shown that uncertainty in a learning game enhance players' experience… including changes in brain chemistry and activity." These risk-taking games act as a stimulus for creativity. Since there is always some element of risk to expressing a new idea or solution, risk-taking and creativity are integrally related (Robinson, 2014). This research suggests that there might be several benefits to taking innovative and entrepreneurial education outside of the classroom, where students are more likely to take risks and show creativity.

Our study extends the current research by addressing the outcomes of innovation and entrepreneurship education when it occurs outside the traditional classroom in an experiential learning setting. Specifically, our research looks at the results of student lead initiatives and the various outcomes on the student body. As previous research indicates, entrepreneurship has greater association with business, and innovation is more closely associated with engineering. Traditional classroom settings appear to be a driving factor in this correlation between innovation and entrepreneurship and their perspective fields of study. One of the objectives of this paper is to exam the benefits of an interdisciplinary approach to innovation and entrepreneurship and the numerous opportunities afforded to students in an academically diverse and experiential setting.

Data and Results

Survey and Data

With funding from the Summer 2015 URECA Grant, the authors developed a survey to further investigate the innovation and entrepreneurship landscape of the University campus. The study focused on two factors regarding the innovation and entrepreneurship landscape: student interest and student awareness. During this time, the University was aware of the lack of student involvement in these activities but was unaware of the potential momentum behind innovation and entrepreneurship. As stated earlier, the University had several programs set to launch during this year, including a Tech Talk, the Makerspace at iCUBE, and the growth of Eagleworks. These events and programs were designed to alleviate the problem of students not taking advantage of the current innovation and entrepreneurial resources.

The development of the survey questions and ultimately the regression models were based on prior research by Jones and Colwill (2013) where they assess the impact of a young enterprise program. We also examined the types of questions being asked in the National Survey of Student Engagement. The survey was administered in two parts: a pre-survey and a post-survey. The pre-survey was administered at the beginning of the fall 2015 semester in conjunction with the innovation and entrepreneurship initiatives. The pre-survey was designed to gauge the current level of involvement by examining the interest and awareness of the student body. The post-survey was administered at the end of the spring 2016 semester. Both surveys were sent out to the entire undergraduate student population. The overall response rate for the pre-survey and post-survey was 9.2 percent and 5.7 percent, respectively.

Because the dependent measures are ordered variables, we employ the following ordered logit model to assess student interest in innovation and entrepreneurship:

$$INTEREST_i = \alpha 0 + \beta 1 INNOVATIVE_i + \beta 2 ENTREPRENEURIAL_i + \beta 3 START\_BUSINESS_i + \beta 4 TINKERING_i + \beta 5 WHAT\_WORKS_i + \beta 6 COLLEGE\_ED_i + B7 FAMILIAR_i + \beta 8 RESOURCES\_AVAIL_i + \beta 9 FUTURE\_CAREER_i + \beta 10 RESOURCES\_BENEFICIAL_i + \beta 11 COLLEGE_i + \beta 12 YEAR_i + \beta 13 GPA_i + \beta 14 GENDER_i + \varepsilon_i$$

INTEREST is a student's perspective of their involvement in innovation. INNOVATIVE, ENTREPRENEURIAL, START_BUSINESS, TINKERING, and WHAT_WORKS are all independent variables in the model. We are interested in the significance of these independent variables. We define all variables in Table 1.

TABLE 1

Dependent Variables:

INTEREST	How would you describe your interest/involvement in innovation? I don't see how innovation matters for my major. (Code=-1) I don't have much experience in innovation, but I am interested in learning more. (Code=0) Innovation is an important part of my major (Code =1)
AWARENESS	How would you rate Tennessee Tech's innovative/entrepreneurial environment? I don't have any idea. (Code=0) There are limited resources available to students at Tech. (Code=1) Tech has some resources available. (Code=2) Tech has great resources for innovation and entrepreneurship readily available to students. (Code=3)

Independent Variables:

These questions were answered on the following scale:

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
Coded:	2	1	0	-1	-2

INNOVATIVE	I tend to be innovative
ENTREPRENEURIAL	I have an entrepreneurial personality
START_BUSINESS	I plan to start a business
TINKERING	I spend my spare time tinkering with new ideas/projects
WHAT_WORKS	When working on a project I rather go with what works than find a better way to do it
COLLEGE_ED	Innovation and entrepreneurship are an important part of my college education
FAMILIAR	Since my time at TTU I have become more aware/interested in innovation and entrepreneurship
RESOURCES_AVAIL	The innovation and entrepreneurship resources at TTU are readily available to students
FUTURE_CAREER	Innovation and entrepreneurship have better enabled me to pursue my future career
RESOURCES_BENEFICIAL	The innovative and entrepreneurial resources at TTU are greatly beneficial to the student body

Control Variables:

COLLEGE	Coded 1 for Business, 2 for Engineering, 3 for Ag and Human Ecology, 4 for Interdisciplinary, 6 for Education, 7 for Arts & Sciences, 8 for Other, 9 for Nursing
YEAR	Coded 1 for Freshman, 2 for Sophomore, 3 for Junior, and 4 for Senior
GPA	Coded 1 for GPA less than 2.5, 2 for 2.5 to 3.0 GPA, 3 for 3.0 to 3.5 GPA, and 4 for greater than 3.5 GPA
GENDER	Coded 1 for Male and 0 for Female

To assess student awareness of the environment involving innovation and entrepreneurship, we employ a model similar to the one described above:

$$\begin{aligned} AWARENESS_i = {} & \alpha 0 + \beta 1 COLLEGE\_ED_i + B2 FAMILIAR_i + \beta 3 RESOURCES\_AVAIL_i \\ & + \beta 4 FUTURE\_CAREER_i + \beta 5 RESOURCES\_BENEFICIAL_i + \beta 6 INNOVATIVE_i \\ & + \beta 7 ENTREPRENEURIAL_i + \beta 8 START\_BUSINESS_i + \beta 9 TINKERING_i \\ & + \beta 10 WHAT\_WORKS_i + \beta 11 COLLEGE_i + \beta 12 YEAR_i + \beta 13 GPA_i \\ & + \beta 14 GENDER_i + \varepsilon_i \end{aligned}$$

AWARENESS is how students' rate the University's innovative and entrepreneurial environment. *COLLEGE_ED, FAMILIAR, RESOURCES_AVAIL, FUTURE_CAREER,* and *RESOURCES_BENEFICIAL* are all independent variables in the model. We are interested in the significance of these independent variables.

Descriptive Statistics

Table 2, Columns A and B presents descriptive statistics of the 1,004 responses from the pre-survey and the 619 responses from the post-survey, respectively. Column C provides the t-tests between the pre- and post-surveys. For the dependent measure of *INTEREST*, students tend to fall between innovative being important to their major and not having the experience but being interested on average. This is similar for both surveys with the t-test being insignificant. For *AWARENESS*, on average, students feel that the University has some resources available for innovation and entrepreneurship. This measure is marginally significant and higher in the post-survey as compared to the pre-survey. In untabulated results, AWARENESS is significantly higher in freshman as compared to seniors in the post-survey. Using univariate results, the efforts focused at younger students over the last year has positively impacted their perception of innovation and entrepreneurship on the University's campus.

In both surveys, the average student agreed with the survey statements regarding being innovative and having an entrepreneurial personality (*INNOVATIVE* and *ENTREPRENEURIAL*). Students tend to be neutral with planning to start a business and completing projects with what works (*START_BUSINESS* and *WHAT_WORKS*). Significantly more students in the post-survey as compared to the pre-survey spent time tinkering with new projects or ideas (*TINKERING*). Students agree in both surveys that innovation and entrepreneurship are part of their college education. Significantly more students in the post-survey were familiar or aware of innovation and entrepreneurship on campus and knew that resources were available to students (*FAMILIAR* and *RESOURCES_AVAIL*). On average, students agreed that innovation and entrepreneurship better enabled pursuit of a future career and that these resources greatly benefit the student body (*FUTURE_CAREER* and *RESOURCES_BENEFICIAL*). Overall, the students seem to be aware of the resources being placed behind innovation and entrepreneurship.

The students completing both surveys were, on average, in the middle of their sophomore year in college. The average GPA of students completing the surveys was a 3.0 to 3.5. Approximately, 50 percent of the survey participants were male.

Table 2
Descriptive Statistics, Pre- and Post-Survey

	Column A: Pre-Survey (1004 responses)				*Column B: Post-Survey (619 responses)*				*Column C:*	
Variables	Mean	Std Dev	Minimum	Maximum	Mean	Std Dev	Minimum	Maximum	t-test	
Dependent Variable										
INTEREST	0.511	0.643	-1.000	1.000	0.507	0.650	-1.000	1.000	-0.112	
AWARENESS	1.930	0.993	0.000	3.000	2.047	0.953	0.000	3.000	2.332	**
Independent Variable										
INNOVATIVE	0.853	0.754	-2.000	2.000	0.871	0.728	-1.000	2.000	0.478	
ENTREPRENEURIAL	0.490	0.975	-2.000	2.000	0.494	1.023	-2.000	2.000	0.085	
START_BUSINESS	-0.027	1.217	-2.000	2.000	0.032	1.220	-2.000	2.000	0.951	
TINKERING	0.440	1.038	-2.000	2.000	0.551	1.011	-2.000	2.000	2.107	**
WHAT_WORKS	0.009	0.978	-2.000	2.000	0.024	1.010	-2.000	2.000	0.302	
COLLEGE_ED	0.739	0.884	-2.000	2.000	0.759	0.890	-2.000	2.000	0.447	
FAMILIAR	0.442	0.983	-2.000	2.000	0.530	1.000	-2.000	2.000	1.733	*
RESOURCES_AVAIL	0.480	0.870	-2.000	2.000	0.601	0.912	-2.000	2.000	2.669	***
FUTURE_CAREER	0.414	0.891	-2.000	2.000	0.473	0.883	-2.000	2.000	1.300	
RESOURCES_BENEFICIAL	0.742	0.831	-2.000	2.000	0.763	0.852	-2.000	2.000	0.478	
Control Variables										
COLLEGE	4.308	2.754	1.000	9.000	3.871	2.604	1.000	9.000	-3.170	***
YEAR	2.659	1.136	1.000	4.000	2.788	1.080	1.000	4.000	2.263	**
GPA	3.031	0.878	1.000	4.000	3.011	0.922	1.000	4.000	-0.428	
GENDER	0.499	0.500	0.000	1.000	0.528	0.500	0.000	1.000	1.145	

Notes: ***, **, and * indicate significance for a two-tailed test at the 1 percent, 5 percent, and 10 percent levels, respectively. See Table 1 for variable definitions.

Regression Results

We present the results from the regression model using *INTEREST* as a dependent variable in Table 3. As expected, across both models, we find that *INNOVATIVE* and *TINKERING* are significant and positive (coefficient=0.6916, t-stat=4.58 and coefficient=0.2139, t-stat=2.08, respectively for the post-survey). *ENTREPRENEURIAL* is negative and marginally significant in the post-survey (coefficient=-0.2498, t-stat=-1.92). This result is not surprising given that engineering students tend to associate more in innovation than entrepreneurship and that the College of Engineering had the largest response rate. In the post-survey, *WHAT_WORKS* was highly significant and negatively related to *INTEREST* (coefficient=-0.2709, t-stat=-2.82). This finding is intuitive and supports innovation. Finding significance here further supports that the efforts on-campus during the year encouraged the students to think more specifically about innovation and entrepreneurship. Specifically, students seeking to find better ways to complete a task rather than just going with what has worked in the past fosters innovation.

Innovation and entrepreneurship being part of a student's college education and enabling a future career are both highly significant and positively related to a student's interest in innovation (*COLLEGE_ED* coefficient=0.7995, t-stat=6.03 and *FUTURE_CAREER* coefficient=0.3547, t-stat=2.53 for the post-survey). *GENDER* is also highly significant indicating that male students have a stronger interest in innovation than females in this study (coefficient=0.7025, t-stat=3.60 for the post-survey).

Table 3

Ordered Logit Model for INTEREST

$$INTEREST_i = \alpha_0 + \beta_1 INNOVATIVE_i + \beta_2 ENTREPRENEURIAL_i + \beta_3 START\_BUSINESS_i + \beta_4 TINKERING_i + \beta_5 WHAT\_WORKS_i + \beta_6 COLLEGE\_ED_i + \beta_7 FAMILIAR_i + \beta_8 RESOURCES\_AVAIL_i + \beta_9 FUTURE\_CAREER_i + \beta_{10} RESOURCES\_BENEFICIAL_i + \beta_{11} COLLEGE_i + \beta_{12} YEAR_i + \beta_{13} GPA_i + \beta_{14} GENDER_i + \varepsilon_i$$

	Column A: Pre-Survey			*Column B: Post-Survey*		
Variables	Coeff	z-stat		Coeff	z-stat	
Independent Variable						
INNOVATIVE	0.6448	5.57	***	0.6916	4.58	***
ENTREPRENEURIAL	0.0486	0.51		-0.2498	-1.92	*
START_BUSINESS	-0.1880	-2.50	**	-0.0865	-0.87	
TINKERING	0.1553	1.92	*	0.2139	2.08	**
WHAT_WORKS	-0.0425	-0.56		-0.2709	-2.82	***
Control Variables						
COLLEGE_ED	0.5663	5.54	***	0.7995	6.03	***
FAMILIAR	0.0749	0.78		0.0871	0.73	
RESOURCES_AVAIL	0.0692	0.67		-0.0105	-0.08	
FUTURE_CAREER	0.4412	3.99	***	0.3547	2.53	**
RESOURCES_BENEFICIAL	-0.1105	-1.03		0.1331	0.96	
COLLEGE	-0.0508	-1.87	*	-0.0820	-2.26	**
YEAR	-0.0009	-0.01		0.0539	0.62	
GPA	0.1367	1.67	*	0.0813	0.77	
GENDER	0.6857	4.47	***	0.7025	3.60	***
N	1004			619		
LR chi2	305.10			227.48		
R^2	0.1727			0.2076		

Notes: ***, **, and * indicate significance for a two-tailed test at the 1 percent, 5 percent, and 10 percent levels, respectively. See Table 1 for variable definitions.

Table 4 shows the regression results from the model using *AWARENESS* as the dependent variable. We find that across both surveys *FAMILIAR, RESOURCES_AVAIL,* and *RESOURCES_BENEFICIAL* are all significant and positive (coefficient=0.3754, t-stat=3.43, coefficient=1.0012, t-stat=8.13, and coefficient=0.2618, t-stat=2.09, respectively for the post-survey). Here, each of these significant coefficients were larger in the post-survey than the pre-survey indicating that students are becoming aware of the resources available to them and that their knowledge of the resources has perhaps strengthened over the year.

In the pre-survey, *YEAR* is highly significant and negative indicating that Freshman were more aware of the innovative and entrepreneurial environment than Seniors. *GENDER* is significant and positive indicating that men were more aware. In the post-survey, only GPA is significant and positive indicating that as GPA increases so does the awareness of the innovative and entrepreneurial environment on the University's campus.

Table 4
Ordered Logit Model for AWARENESS

$$AWARENESS_i = \alpha_0 + \beta_1 COLLEGE\_ED_i + B_2 FAMILIAR_i + \beta_3 RESOURCES\_AVAIL_i + \beta_4 FUTURE\_CAREER_i + \beta_5 RESOURCES\_BENEFICIAL_i + \beta_6 INNOVATIVE_i + \beta_7 ENTREPRENEURIAL_i + \beta_8 START\_BUSINESS_i + \beta_9 TINKERING_i + \beta_{10} WHAT\_WORKS_i + \beta_{11} COLLEGE_i + \beta_{12} YEAR_i + \beta_{13} GPA_i + \beta_{14} GENDER_i + \varepsilon_i$$

Variables	*Column A: Pre-Survey* Coeff	z-stat		*Column B: Post-Survey* Coeff	z-stat	
Independent Variable						
COLLEGE_ED	0.0368	0.39		0.1887	1.60	
FAMILIAR	0.3328	3.81	***	0.3754	3.43	***
RESOURCES_AVAIL	0.9754	10.33	***	1.0012	8.13	***
FUTURE_CAREER	0.0466	0.48		0.1682	1.34	
RESOURCES_BENEFICIAL	0.2587	2.78	***	0.2618	2.09	**
Control Variables						
INNOVATIVE	0.1153	1.10		-0.0184	-0.13	
ENTREPRENEURIAL	0.0393	0.46		-0.1070	-0.93	
START_BUSINESS	-0.0372	-0.55		0.0769	0.85	
TINKERING	-0.0913	-1.23		0.0064	0.07	
WHAT_WORKS	0.0462	0.69		-0.0426	-0.50	
COLLEGE	-0.0286	-1.15		-0.0277	-0.81	
YEAR	-0.1855	-3.18	***	-0.0687	-0.88	
GPA	-0.1106	-1.49		0.2009	2.09	**
GENDER	0.3228	2.34	**	0.2001	1.11	
N	1004			619		
LR chi2	363.91			278.52		
R^2	0.1558			0.1959		

Notes: ***, **, and * indicate significance for a two-tailed test at the 1 percent, 5 percent, and 10 percent levels, respectively. See Table 1 for variable definitions.

Conclusion

The Kaufman Foundation in its state of entrepreneurship address identifies three major trends in the entrepreneurial environment: demographic, map, and nature of entrepreneurship (Kaufman 2017). Entrepreneurship is becoming more diverse, occurring in mid-sized metros, and growing revenue for startups. The address outlines these trends as key areas of how the breakdown of a formal entrepreneurial structure has improved the entrepreneurship and innovation in the United States. The Foundation is built around the premise that entrepreneurship and innovation, by its very nature, is not traditional or formal.

As evidenced in this study, student performance and involvement in innovation and entrepreneurship outside of the classroom setting allows for greater involvement and awareness. The entrepreneurship initiatives on campus have improved student awareness of resources and shown these resources to be beneficial to the student body. The student body also tends to be innovative and desire to tinker with the development of new ideas, solidifying the importance of iCUBE and the Makerspace on the University's campus. Students demonstrate a desire to incorporate entrepreneurial and innovative concepts by seeking to find better methods or solutions when working on projects. While these skills can perhaps be taught in a class, the students experience the most growth when working on projects and ideas in an experiential learning environment. It appears that innovation and entrepreneurship are aiding future career choices and development. The efforts to support and expand entrepreneurship and innovation should continue as evidenced by this survey.

While we have attempted to evaluate the environment for entrepreneurship and innovation, future research studies should attempt to quantify the use of different units on campus. Perhaps by understanding what impacts the students the most, donors and University administration will be better prepared in supporting specific activities. It might also be important to measure the impact that these initiatives have on job placement and career development after graduation.

References

Charney, A. and Libecap, G.D. 2000. Impact of Entrepreneurship Education. Insights: A Kauffman Research Series, Kauffman Center for Entrepreneurial Leadership, Kansas City, MO.

Jones, Paul and Anne Colwill. 2013. Entrepreneurship Education: An Evaluation of the Young Enterprise Wales Initiative. Education + Training. 55 (8/9). 911-925.

Kauffman Foundation. 2017. Startup Activity Swings Upward for Third consecutive Year, Annual Kauffman Index Reports. May 18, 2017. http://www.kauffman.org/newsroom/2017/05/startup-activity-swings-upward-for-third-consecutive-year-annual-kauffman-index-reports

Kauffman Foundation. 2017. State of Entrepreneurship Address. February 17, 2017. http://www.kauffman.org/what-we-do/resources/state-of-entrepreneurship-addresses/2017-state-of-entrepreneurship-address

Kauffman Foundation. 2011. An Entrepreneurial Generation of 18- to 34-Year-Olds Wants to Start Companies When Economy Rebounds, According to New Poll. November 20, 2011. www.kauffman.org/~/media/kauffman_org/.../2011/11/millennials_study.pdf

Rae, David, Andy Penaluna, and H. Dhaliwal. 2011. Higher Education and Graduate Enterprise in the New Era, Graduate Market Trends, Winter, Higher Education Careers Service Unity, Manchester. 9-11.

Robinson, Sherry. 2013. Student Response to Risk in Classroom Learning Games. Academy of Educational Leadership Journal, 17 (4): 1-12.

Robinson, Sherry and Hans Anton Stubberud. 2014. Teaching Creativity, Team Work and Other Soft Skills for Entrepreneurship. Journal of Entrepreneurship Education, 17 (2): 186-208.

Using Animations to Visualize Olefin Metathesis Chemical Reactions

Savannah Hall and Janet Coonce

ABSTRACT

A series of animations and PowerPoint presentations have been created to help introductory chemistry students visualize the Chauvin mechanism for olefin metathesis chemical reactions. Olefin metathesis is the subject of the 2005 Nobel Prize for chemistry, and the reaction can be difficult to describe with static diagrams. For this reason, a team of high school and undergraduate chemistry students at Tennessee Technological University have created animations of olefin metathesis reactions to demonstrate how the atoms rearrange in the chemical reaction. The reaction animations are available with open-access for presenters to download editable files.

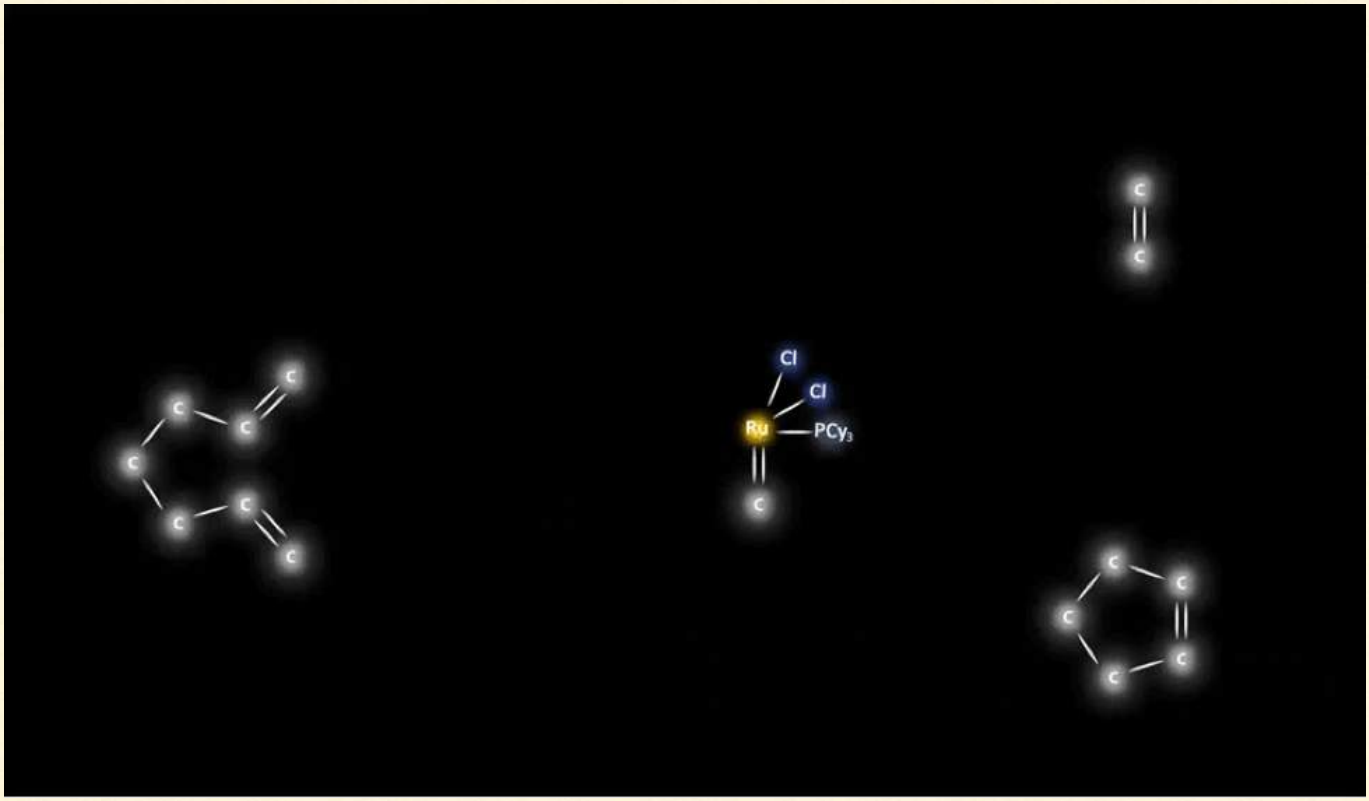

Figure 1. Animated .gif file of the catalytic cycle of the Ring Closing Metathesis (RCM) mechanism using Grubb's 1st generation ruthenium catalyst. Savannah Hall, 2017.

Introduction

The olefin metathesis chemical reaction has been studied extensively over the past forty years, and it is one of the most widely-used chemical reactions in industry and academics today.[1] An olefin is simply a compound that contains at least one carbon-carbon double bond, and another term for an olefin is an alkene. In an olefin metathesis reaction, one carbon of a carbon-carbon double bond changes places with another carbon of a second carbon-carbon double bond to form two new carbon-carbon double bonds.[2] The olefin metathesis reaction allows chemists to make new organic compounds including medicines, plastics, and other petroleum products that were previously slow, difficult, or unable to be synthesized.[3]

In 1971, French chemist Yves Chauvin explained the mechanism for how the atoms might rearrange in the metal-catalyzed olefin metathesis reactions.[4] Since then, new olefin metathesis catalysts and uses for the olefin metathesis reaction have been discovered and developed. Notably, Richard R. Schrock and his research group at Massachusetts Institute of Technology (MIT) have continued to produce efficient metal catalysts for metathesis using molybdenum (Mo^{6+}), tungsten (W^{6+}), and other metal centers.[5] Robert H. Grubbs and his research group at California Institute of Technology (CalTech) have continued to develop air-stable catalysts using ruthe-nium (Ru^{2+}) and other metal centers.[6] In 2005, the Nobel prize for chemistry was awarded jointly to Chauvin, Grubbs, and Schrock "for the development of the metathesis method in organic synthe-sis."[7]

Chauvin Mechanism

The Chauvin mechanism is the currently-accepted mechanism for the olefin metathesis reaction. This mechanism has been described as, "a pair-wise interchange of carbon atoms."[8] Figure 2 displays an example of the written Chauvin mechanism applied to a ring-closing metathesis (RCM) reaction of 1,6-heptadiene to form cyclopentene in the presence of a 1st Generation Grubb's catalyst. Unfortunately, written mechanisms such as these can be quite difficult for introductory chemistry students to initially visualize, especially without the necessary background knowledge or professional guidance. The goal of this creative inquiry project is to make simple animations of metathesis mechanisms in an easily-accessible format for students, presenters, and educators alike.

Animation and Powerpoint Progression

This project first began in the summer of 2014 when Tennessee Tech's Governor's School for Emerging Technology created simplified animations of (1) cross metathesis (CM), (2) Ring Closing Metathesis (RMC), (3) Ring Opening Metathesis (ROM), (4) Ring Opening Metathesis Polymerization (ROMP), (5) Acyclic Diene Metathesis (ADMET), and (6) Ethenolysis. Eight high school-level students worked in teams of two and three to research how these reaction worked, and then they explored Adobe Edge Animate to create scalable HTML5 web-based animations to help web viewers visualize the mechanism of the catalytic cycle of each metathesis reaction. These animations can be viewed at: https://animations.connectingchemistry.com/TNgovschool/

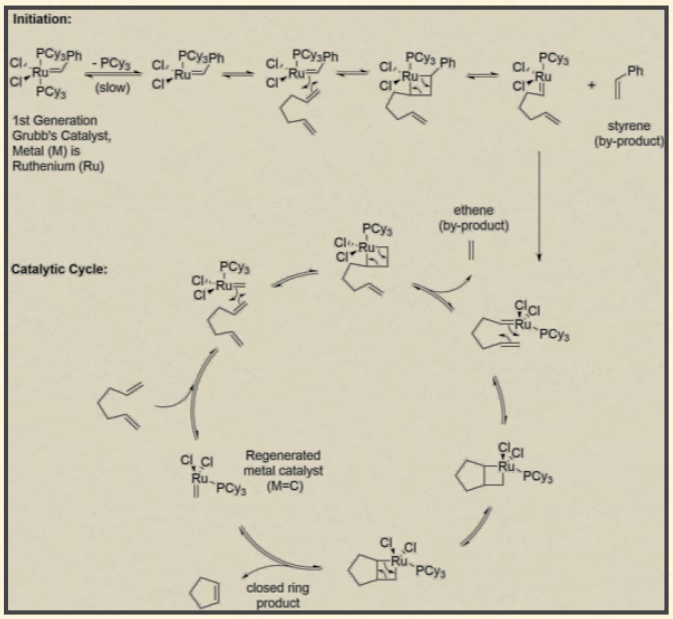

Figure 2. Chauvin mechanism of a simple Ring-Closing Metathesis (RCM) reaction, showing both the initiation and the catalytic cycle.[2,10]

After the Governor's School students published their animations online, the next step was to make the animations more accessible to educators who wished to use them offline. The animations were converted to animated .gif files and recreated for use in PowerPoint slides. The Governor's School animations depicted the metathesis catalyst (M=C) as a generic and simple metal-centered catalyst double-bonded to a carbon, without specifying ligands and alkyl groups. In the next set of animations, the generic gold-colored metal center (M) of the catalyst in the Governor's school student animations was specified as ruthenium (Ru) in the 1st Generation Grubb's Catalyst (Figure 3).

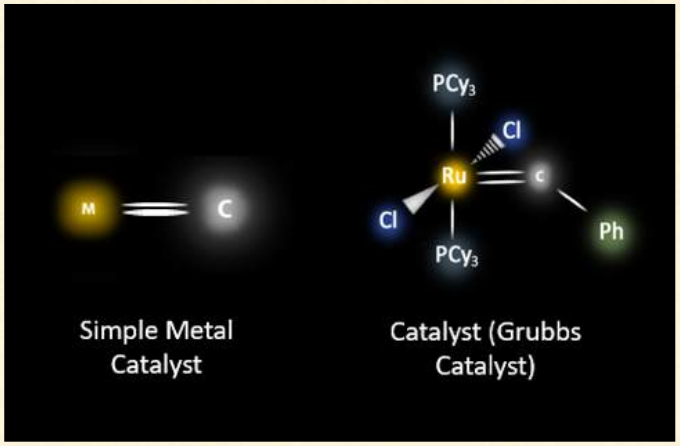

Figure 3. Comparison of initial metal catalyst used by the Governor's School students to the original first generation Grubb's Catalyst. PowerPoint slide by Savannah Hall, 2017.

Choosing to designate a specific Grubb's catalyst allowed us to animate the initiation step required to activate the catalyst. We were able to show the removal of a tricyclohexylphosphine (PCy_3) group and explain the minor product, styrene, CH_2CHPh (shown highlighted with a green Ph) in the animation. Even though much more effective catalysts [9] have been developed since the development of the 1st Generation Grubb's catalyst, this catalyst was chosen for simplicity's sake in order to demonstrate the mechanism of both the initiation and catalytic cycle. The new animated files were created to display the complete mechanism for the specific reaction of 1,6-heptadiene to form cyclopentene in the presence of a 1st Generation Grubb's catalyst (Figure 4).

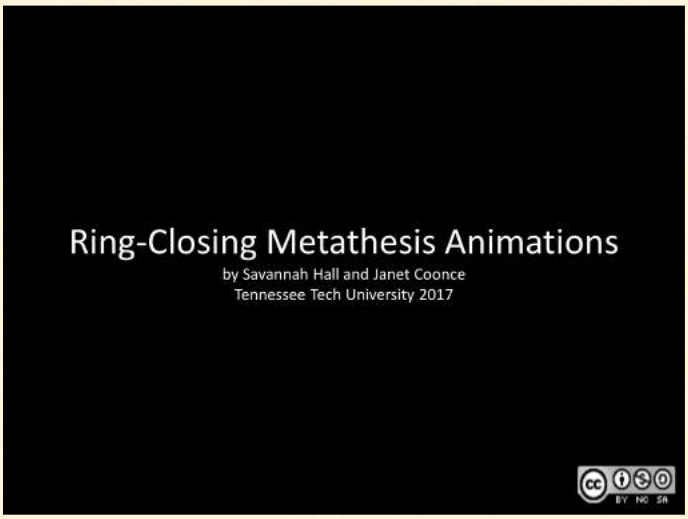

Figure 4. Initiation and catalytic cycle of the Chauvin mechanism for the ring closing metathesis reaction. Savannah Hall, 2017.

The new PowerPoint slides were used as rough draft precursors to developing a more fluid animation in Adobe Edge Animate for scalable HTML5 web browser viewing. Animations were made for both Ring-Opening and Ring-Closing Metathesis reactions, each having a version where the phenyl removal step was included (showing the initiation step and the catalytic cycle) and one where it was not (showing only the repeating catalytic cycle). However, using Adobe Edge to publish to the internet can be difficult if one is not familiar with HTML or JavaScript coding. These animations also need live internet access to show in a presentation. A solution to this problem was to turn the Edge file into a GIF file that could be easily downloaded and viewed in a presentation by using the recording software Camtasia Relay and Adobe Photoshop.

Each metathesis animation was recorded using Camtasia, and then Adobe Photoshop was used to export the video to a hi-definition GIF. The main advantages to using animated GIF files is that they can be easily shared and viewed in PowerPoint without internet access. The disadvantage is that the GIF files cannot be paused for discussion. To overcome this disadvantage, the animations can be viewed either as a step-by-step PowerPoint animation or as a more-fluid and powerful HTML5 animation file online at: https://animations.connectingchemistry.com/TTU/.

Conclusion

All metathesis animations, GIFs, and PowerPoint slides were successfully created, and they are available for all students, presenters, and educators to freely downloaded and modify. The intent of these animations is that they could be used in discussions in any language, and at many different levels of chemical understanding. All work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. This means all non-commercial parties may use, remix, transform, or build upon the material and freely distribute contributions with the attribution, "Savannah Hall and Janet Coonce, Tennessee Tech University, Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License © 2017." Our hope is that some or all of these animations will be useful to both students and presenters. Future work includes the animation of additional chemical reaction mechanisms.

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References

1. Grela, K. Olefin Metathesis: Theory and Practice. John Wiley & Sons. New Jersey, 2014.

2. Grubbs, et al. Handbook of Metathesis. Volume 1: Catalyst Development and Mechanism, 2nd edition. Wiley. New York, 2015.

3. Grubbs, et al. Handbook of Metathesis. Volume 2: Applications in Organic Synthesis, 2nd edition. Wiley. New York, 2015.

4. Chauvin, Yves. Catalyse de transformation des olefines par les complexes du tungstene. II. Telomerisation des olefines cycliques en presence d'olefines acycliques. Die Makromolekulare Chemie. 1971, 141(1):161-176.

5. Schrock, R.R. Massachusetts Institute of Technology. The Schrock Group Website. https://schrockgroup.mit.edu/publications (accessed Nov. 4, 2017).

6. Grubbs, R.H. California Institute of Technology. The Grubbs Group Website. https://grubbsgroup.caltech.edu/publications/ (accessed Nov. 10, 2017).

7. Press Release: The Nobel Prize in Chemistry 2005. Nobelprize.org. Novel Media AB 2014. Web. <http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2005/press.html> (accessed Nov. 10, 2017).

8. Grubbs, Robert H., et al. Consideration of the Mechanism of the Olefin Metathesis Reaction. J. Am. Chem. Soc. 1975, pp. 3265–3267.

9. Hartwig, John F. Chapter 21: Metathesis of Olefins and Alkynes. Organotransition Metal Chemistry-From Bonding to Catalysts. University Science Books. USA, 2010.

10. Ring Closing Metathesis. Organic-Chemistry.Org. November 2017 http://www.organic-chemistry.org/namedreactions/ring-closing-metathesis.shtm (accessed Nov. 10, 2017).

Synthesis of N-Ethyl-2-[1-(2-pyrazinyl) ethylidene] hydrazinecarbothioamide (APZ-ETSC) and Characterization by NMR Spectroscopy

Dylan M. Gardner, Kari L. Lawson, Shana T. Shaw, William R. Carroll and Edward C. Lisic

ABSTRACT

The synthesis of a new molecule, acetylpyrazine ethylthiosemicarbazone (APZ-ETSC) is presented and its structural characterization is described with NMR techniques using experiments from a 500 MHz Bruker Nuclear Magnetic Resonance (NMR) Spectrometer. The ^{1}H, ^{13}C-Distortionless Enhancement by Polarization Transfer (DEPT), ^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC), ^{1}H-^{13}C Heteronuclear Multiple Bond Coherence (HMBC), ^{1}H-^{15}N HSQC, and ^{1}H-^{15}N HMBC experiments were conducted with the NMR spectrometer, and the chemical shifts obtained by these experiments were correlated to confirm the assignments of the spectral peaks of the new molecule. These NMR experiments give evidence for the hypothesized structure of APZ-ETSC in solution.

Acronyms

APZ – 2-Acetyl pyrazine

ETSC – 2-Ethyl-4-thiosemicarbazide

NMR – Nuclear Magnetic Resonance

HSQC – Heteronuclear Single Quantum Coherence

HMBC – Heteronuclear Multiple Bond Coherence

APZ-ETSC – Acetylpyrazine Ethylthiosemicarbazone

Introduction

Thiosemicarbazones and their derivatives have been researched for many years, and have been shown to have a variety of pharmacological applications [1,2,3]. A subset of the thiosemicarbazones, named α-(N)-heterocyclic thiosemicarbazones, are of interest as chemotherapy agents [1,2]. Their specific α-(N)-heterocyclic backbone ring is designed to adopt a structure that allows them to bind to transition metals very efficiently, and they are tridentate ligands [1,2,4]. This allows them to interact with enzymes that contain transition metals, such as ribonucleotide reductase, and render them inoperative. One of these α-(N)-heterocyclic thiosemicarbazones is triapine, and it is currently used as a chemotherapy agent. The 2-acetyl pyridine thiosemicarbazones, which are similar to triapine, have been shown to inhibit a variety of microbial growths including Neisseria gonorrhoeae, Staphylococcus faecalis, Streptococcus faecalis, and D. Enterococcus [1,2,3]. 2-(α-hydroxyacetyl) pyridine thiosemicarbazone has been shown to inhibit both penicillin-sensitive and resistant strains of N. Gonorrheae, N. Menigitides, and Staphylococcus aureus [1,2,3]. It is hypothesized that these compounds have a higher activity when complexed with transition metals due to an increase in the lipophilicity [1]. Our research involves making analogues of triapine.

Literature shows that some 2-acetylpyrazine thiosemicarbazone (APZ-TSC) derivatives possess antifungal activity against several types of fungi including Candida albicans [4]. A structurally similar compound, 2-acetylpyridine thiosemicarbazone has shown antimicrobial activity against several microorganisms [5]. Past work in our laboratory focused on the synthesis and characterization of many thiosemicarbazones that share the common backbone with these previously reported compounds and their metal complexes [7,8,9].

There are currently pharmaceuticals employing metal-ligand complexes on the market such as cis-diammedichloridoplatium (II), also called Cisplatin, which are used to treat some types of cancers. However, these drugs have severe side effects, and are fairly limited in the type of tumors that can be treated with Cisplatin [6].

The target of these medications is usually DNA [6]. Cisplatin binds covalently to two Guanine base pairs preventing RNA synthesis and DNA repair [6]. New metal complexes of α-(N)-heterocyclic thiosemicarbazones [10] have anti-cancer activity like Cisplatin, however, these molecules target enzymes instead of DNA itself. The goal of our research is to synthesize new α-(N)-heterocyclic thiosemicarbazones molecules like APZ-ETSC. Use of these new molecules as a medicine requires that we fully elucidate the structure of these molecules in the solution state, so that we can predict how they interact with enzymes to understand their mode of action.

Materials and Methods

Starting materials, chemicals **[1]** (2-acetylpyrazine) and **[2]** (4-ethyl-3-thiosemicarbazide), were all purchased from Sigma-Aldrich and used without modification unless otherwise noted. NMR spectroscopy was carried out at the Center for Structural Chemistry, Tennessee Technological University (USA). The spectra reported here were measured with a Bruker Avance III HD 500 spectrometer at 500.13 MHz (^{1}H), 50.69 MHz (^{15}N) and 125.03 MHz (^{13}C) at 25 °C. For these measurements the substances were dissolved in deuterated dimethyl sulfoxide and the chemical shifts were referenced to the residual solvent peak. Coupling constants (J) are given in Hertz (Hz). ^{1}H NMR experiments were acquired using Bruker's standard Proton (zg30) NMR pulse sequence with the following parameters: Relaxation delay, 1s; 90° pulse, 12.0 μs; spectral width, 10,000 Hz; number of data points, 32K; and digital resolution, 0.153 Hz/point.

Synthesis of **[3]** APZ-ETSC,

N-Ethyl-2-[1-(2-pyrazinyl)ethylidene]hydrazinecarbothioamide

In a 50mL Erlenmeyer flask equipped with a magnetic stir bar, was placed **[1]** 2-acetylpyrazine, (3.03×10^{-3} mol, 0.403g), and **[2]** 4-ethyl-3-thiosemicarbazide, (3.03×10^{-3} mol, 0.393g) dissolved in 20mL of isopropanol with one drop of concentrated sulfuric acid catalyst. The reaction mixture was heated to 60 ^{0}C overnight. After 24 hours, the light-yellow product was filtered and dried to yield **[3]** APZ-ETSC , 2.18×10^{-3}mol, 0.528g, (66% yield).

^{1}H NMR (500 MHz, DMSO-d6) δ 10.41 (s, 1H), 9.64 (d, J = 1.4 Hz, 1H), 8.82 (t, J = 6.0 Hz, 1H), 8.69 – 8.40 (m, 2H), 3.74 – 3.51 (m, 2H), 2.35 (s, 3H), 1.16 (t, J = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, DMSO) δ 177.61, 150.10, 145.74, 143.79, 143.15, 143.04, 38.61, 14.39, 11.78

Results and Discussion

The acid catalyzed condensation of 2-acetylpyrazine and a thiosemicarbazide reagent in isopropyl alcohol to produce compound [3] is shown in Figure 1. The product **[3]** precipitated from isopropyl alco-hol and was separated by filtration with no further purification needed. This synthesis procedure can theoretically be applied to utilize any side chain on the thiosemicarbazide such as a benzyl, phenyl, tertbutyl, or dimethyl. Filtration at room temperature yielded 66% collection of **[3]**, cold filtration was found to increase the yield; however, the product is not as pure as in the first crop.

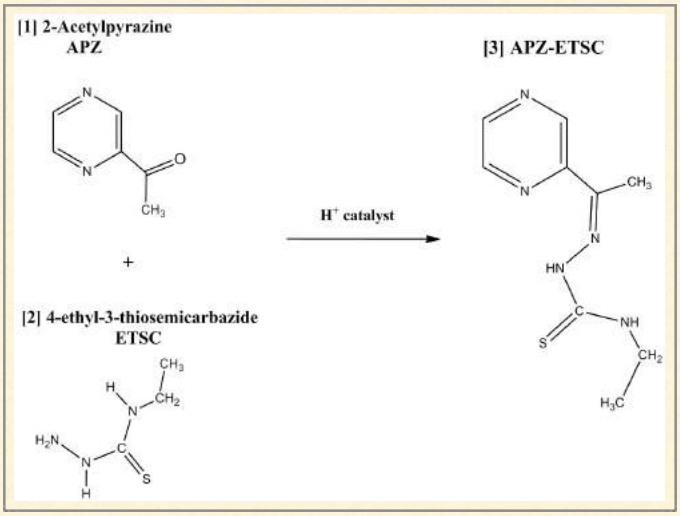

Figure 1. Synthesis drawing of the Title Compound [3] APZ-ETSC.

To characterize **[3]**, several spectra were acquired and used to assign the hydrogen, carbon, and nitrogen signals. Each carbon and nitrogen were numbered, 1-15, with the protons sharing a number with the atom to which it is directly bonded as seen in Figure 2.

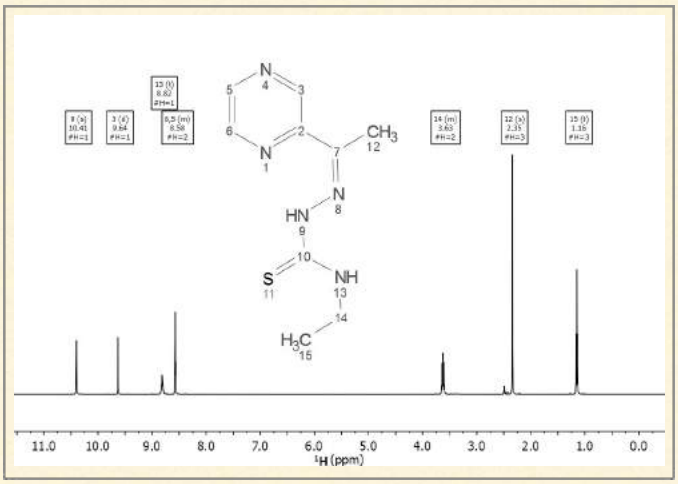

Figure 2. The [1]H-NMR Spectrum of APZ-ETSC in DMSO-d6.

The two one-dimensional spectra obtained were the ^{1}H-NMR (Figure 2) and the ^{13}C-NMR DEPTQ-135 (Figure 4) and used to propose initial assignments. From the ^{1}H-NMR, the number of protons are determined from the integration of the area under the peaks. The splitting and coupling patterns show the number of adjacent protons. In Figure 2, the most up-field signal is at 1.16 ppm and it integrates for 3 protons, and its triplet splitting pattern indicates there are 2 more protons 3 bonds from those in the signal. This is most likely the methyl group protons numbered 15 in Figure 2. The complex multiplet signal at 3.74 – 3.51 ppm integrates for 2 protons, and the complex splitting pattern indicates there is more than one coupling happening at different intensities. This would be expected of the protons numbered 14 with one of the coupling protons being attached to a nitrogen instead of a carbon atom. The singlet peak at 2.35 indicates an isolated methyl group that is not coupling with any neighboring protons; this must be the group numbered 12. The four peaks above 7 are all either bonded directly to a nitrogen or on the aromatic ring. The triplet at 8.82 has to be the proton on the nitrogen numbered 13 with the CH_2 (14). The singlet at 10.42 is likely the other nitrogen attached proton because of its lack of cou-pling. The other two are more ambiguous because the aromatic ring's Protons are in very similar environments. These may be determined using 2-dimensional spectroscopy.

The ^{13}C-NMR DEPTQ-135 provides very important information in the positive and negative phase distinctions and number of signals it contains. The DEPTQ-135 is a modified DEPT experiment that shows each carbon environment present in the molecule including quaternary carbons as an individual signal and the phase of these signals indicates the number of hydrogens attached. The positive peaks are carbon atoms in either CH or CH_3 groups; the negatives are carbon atoms in either CH_2 or fully substituted Carbons. Using this the most down-field signal at 177.61 ppm is most likely the Carbon numbered 10. The other two quaternary Carbons, 2 and 7 are harder to distinguish; the signal at 150.10 ppm is more likely to be 7 because of the electron withdrawing effects of the aromatic ring and Nitrogen groups. The signal at 145.74 ppm is more likely to be 2 because it is in the electronic environment of the aromatic ring where the delocalization of the electrons across the entire ring system shields the atoms within that system significantly putting the signals more up-field than would be usually expected.

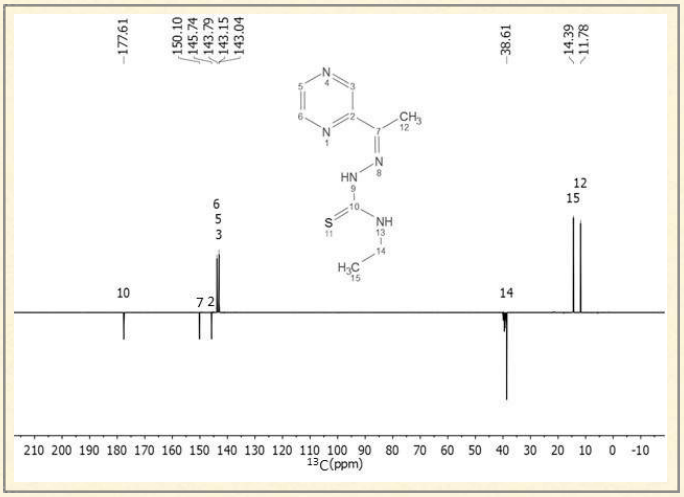

Figure 3. The ^{13}C-NMR DEPTQ-135 of APZ-ETSC in DMSO-d6.

To confirm and elaborate on the assignments made above, two dimensional spectra were used. The first type being Heteronuclear Single Quantum Coherence Spectroscopy (HSQC). HSQC showed two atoms directly bonded to each other, in this case ^{1}H-^{13}C or ^{1}H-^{15}N. This spectra is created by matching the 1H-NMR with the corresponding ^{13}C-NMR DEPT and ^{15}N-NMR spectra. These two dimensional spectra are plotted to appear like topographical maps with concentric rings indicating signals. The X-axis of HSQC spectra are set in line with the 1H-NMR spectra discussed above and the Y-axis was aligned with either ^{13}C or ^{15}N one dimensional spectra as appropriate. Signals that appear in these 2D spectra show that the ^{1}H and either ^{13}C or ^{15}N signals are connected by a single bond.

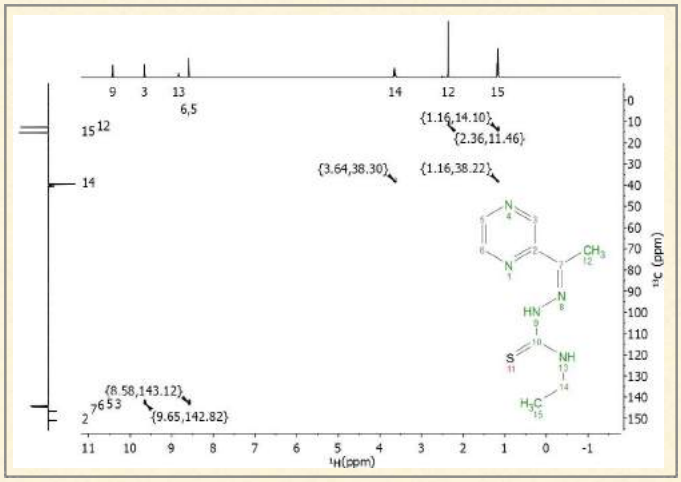

Figure 4. The 1H-13C HSQC NMR of APZ-ETSC in DMSO-d6.

As can be determined by Figure 4, the Carbon 15 has the signal at 14.39 ppm, Carbon 14 has the signal at 38.61 ppm, and Carbon 12 has the signal at 11.78 ppm. Each of these signals appear as a series of concentric rings between the attached carbon and proton NMR.

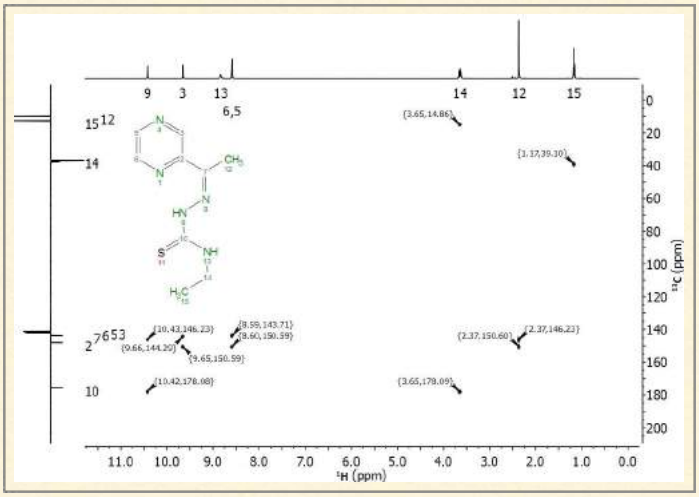

Figure 5. ^{1}H-^{13}C HMBC of APZ-ETSC in DMSO-d6.

HMBC spectra showed atoms that are 2-3 bonds away and come in the same varieties of ^{1}H-^{13}C and ^{1}H-^{15}N as HSQC. These spectra show signals as concentric rings between 1D spectra as in HSQC, but indicate a greater number of bonds separating them, typically 2 to 3 bonds. The ^{1}H-^{13}C HMBC shown in Figure 5 helped to determine exactly which signals were which for the aromatic Carbons 3, 5, and 6. It also helps to confirm Carbons 7 and 10 assignments where signals appear underneath hydrogen signals at 2.35 in line with a carbon at 145.74 indicating that they are 2 to 3 bonds apart in the case of carbon 7 and a signal is observed at 177.61 beneath a proton signal of 3.63 indicating a proximity of thiocarbonyl carbon 10 is 2 to 3 bonds from the proton on position 14. In each case a signal was observed under the corresponding proton signal that was in line with the carbon signal on the Y-axis indicating the atoms were 2 to 3 bonds apart. The exact numbers of these assignments are in Table 1.

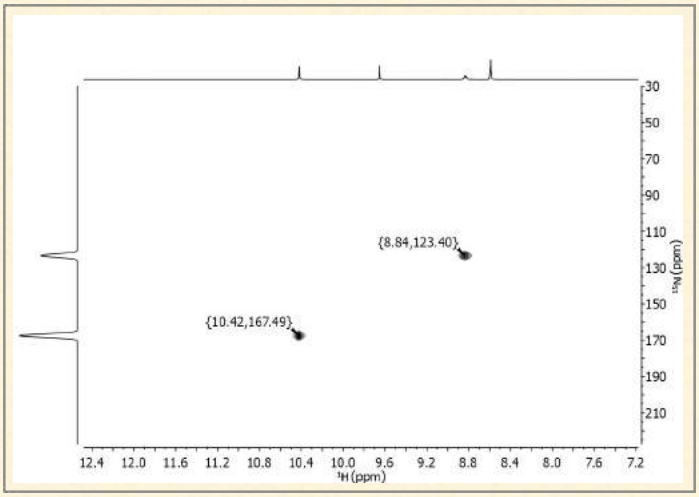

Figure 6. 1H-^{15}N HSQC of APZ-ETSC in DMSO-d6.

Using the ^{1}H-^{15}N HSQC in Figure 6, the assignments of the nitrogen atoms directly bonded to protons can be determined. Nitrogen 9 is more downfield due to the withdrawing effects of the adjacent nitrogen and thiocarbonyl, and nitrogen 13 is slightly more up-field with the withdrawing effects of the thiocarbonyl slightly mitigated by the alkyl group. These ^{15}N assignments are confirmed by ^{1}H-^{15}N HMBC in Figure 7, and nitrogen atoms 1, 4, and 8 are as-signed as well. Each of the nitrogen atoms not attached to a proton does not appear in the HSQC experiment. With the low natural abundance and sensitivity of ^{15}N it is often difficult to observe ^{15}N chemical shifts making this HMBC spectra a very useful method of observing nitrogen atoms 1, 4 and 8.

This characterization with the assignment of all peaks in the spectra is the first step in determining the identity and purity of this potential medication. This process also confirms there is no other structural equilibrium or that the structures interchange faster than the observation time of the NMR experiment. These experiments can be compared to future works to see how the ligand changes when complexed with the metals.

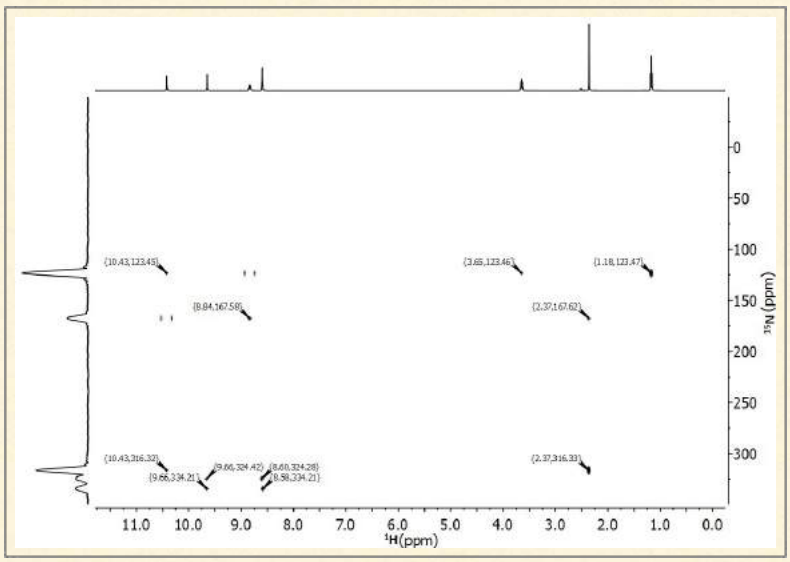

Figure 7. ^{1}H-^{15}N HMBC of APZ-ETSC in DMSO-d6.

Future Works

This ligand will be complexed with transition metals such as platinum, palladium, and copper. Such metal compounds usually adopt a square planar geometry, and are hypothesized to inhibit human topoisomerase. These will be tested in vitro using enzyme assays and in vivo using breast cancer cells.

Acknowledgements

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References

(1) Beraldo, Heloisa; Gambino, Dinorah. 2004. “The Wide Pharmacological Versatility of Semicarbazones, Thiosemicarbazones and Their Metal Complexes.” *Mini-Rev. Med. Chem*., 4, 31-39.

(2) S. Padhye, and G.B. Kauffman. *Coord. Chem. Rev.*, 1985, 63, 127 -160.

(3) J.S. Casas, M.S. Garcia-Tasende, and J. Sordo. *Coord. Chem. Rev.*, 2000, 209, 197-261.

(4) Ratner E.S., *British Journal of Cancer*, 2016, 114, 777-786

(5) A.C. Sartorelli, K.C. Agrawal, and E.C. Moore. Biochemical *Pharmacology*. 1971, 20(11), 3119-23.

(6) F.A. French, E.J. Blanz, S.C. Shaddix, and R.W. Brockman. J. Med. Chem., 1974, 17(2), 172-81.

(7) J. W. Carter, R. Mayes, K.A. Pierce, R. Lawson, and E.C. Lisic. *J. Und. Chem. Res*., 2003, 2, 73-77

(8) T. Bell, R. Mayes, R. Lawson, E.C. Lisic. *J. Und. Chem. Res*., 2004, 1, 39-45

(9) E. Lisic, R. R. Nareddy, R. Huxford, E. C. Lisic. *J. Und. Chem. Res*., 2006, 2, 61-66.

(10) Matesanz, Ana; Souza, Pilar. 2009. “ α-N-Heterocyclic Thiosemicarbazone Derivatives as Potential Antitumor Agents: A Structure-Activity Relationship Approach." *Mini-Rev. Med. Chem*., 9, 1389-1396.

5 Poems

Geoffrey Pippin

Heated

Lizard

No Time

The Ordered Web

Where to Look

Heated

The weather here ticks forward with the broken thermo-
static clips of mood, its scourge of air hard-boiling you
or me or anyone else who turns through the wrong side
of a word, the temperate season's balance dynamic only
the span it would take a drop of sweat to hit the floor.

Stuck out, and wrestling with the microscopic trembling
of the world, we're pinned against clothes swollen thick
closer than any cries of uncle could relieve, skin turned
adhesive gripped like a nose a crafty uncle seized. Funny
how more energy → the sluggish languor seen with freeze.

Different, though, isn't it? I thought so too before I knew
its sticky ministries so hard to shake, heat so soon to haze
one's thoughts like rhetoric, the pride of being right, until
its blunt rolling smoke-screens the sharp identity of things,
and what is hot and cold, or which of us is banking on the til-

ted side of being wrong is suddenly unclear; but when it cools,
and we find out the truth of it, I bet both of us will feel like fools.

Lizard

Sitting on his rock

without a tail,

having just escaped,

perhaps, some cat's

flawed clawed attack,

he rests like worry's

not for him, knowing

no tail goes for good.

Soon, he's off, his loss

all but forgotten,

as I still wait and watch

for the parts of me

gone missing to

grow back.

No Time

The jig is up

across the world

grandma has gotten sick

projects are almost due

we’re late for what

we now cannot

undo

and now we find

near deadline

lacking time

so needing more

to focus us for what

we had no time

before.

The Ordered Web

The staffroom's mostly keyboard clicks and shifts

Toward the printer's nauseous gasp and heave.

Outside, the rain punctuates its ground intent,

Its tight, encasing greying-down to green

Tomorrow with the urgency of now.

Inside, the whole school wraps its arid rooms

In walls that leave the ways for leaving one

For all, and stretch their planned experience

To dot the battered blueprint of our lives.

The kids come hushed and shuffling down the halls

A whispered roar, tip-toeing tiny steps

In time with every day they're here,

Caught like bugs in longing for the web,

Confined within each instituted year.

Where to Look

Last day, third grade, report cards handed out,

Kids shoulder backpacks, shuffle out at noon,

Giggling to where their parents pick them up.

One girl, living close, splits off toward home

Alone, and soon is running in the house

Clutching a fistful of columned A's.

But coming in, she finds no angling light

From other rooms, no whispering mom and dad

Too busy to listen—just the ache

Of want, of having to ask from home

Where she can look to find what isn't found,

Where to look for home when home is not around.

Sound of Home

Samia Rachel Anderson

Sound of Home

The sneering snake hisses at its

Invisible, intimidating enemy.

A dog barks at

Nothing seeming to

See what we cannot

Fathom. Inconceivable,

Unincorporated hate rallies

At the cool blue crater of

A pool of crass exclamations screaming

“Boo! You crude, corrupt, corroded animal of

The sad system out of which you were birthed.”

A fish filters water through its gills yelling about

The ills that are plaguing our

Insufficient hours of sifting through media like

Instagram is a grain of sand and

Facebook is the filter. Filtered out and

About. Society rounds the astounding

Mounds. Piles of shit

We animals are climbing like

Our menial lives depend on it. The

Denial we are all in that

Life is a lie. But God IS

Real. What we feel comes out of instinct, and

We make up a precinct of flat Stanleys’ trying to

Find our purpose, but

We never will. Will

Is power and

Power is knowledge and

You don’t ever know what

You’re talking about. A tide rising on a

Shore falls back lacking the

Wit it needs to stay put. The door is

Wide shut. Eyes filled with grit. And,

Crawling out of your gut is

A sprawling, silky spider only wanting to

Weave a dome of web that we can

All call home.

.

The Tree of Life

Roberta D. Hamm and Josephine Ann McQuail

ABSTRACT

Charles Oldfield was a late 19th century resident in Historic Rugby, a village still preserved on Tennessee's Cumberland Plateau. His ghost reputedly haunts the Newbury House, one of the surviving original structures in the village. Oldfield came from London to conduct business at the settlement in the Plateau. Copies of letters from Mr. Oldfield to his wife in London (she remained in London even after his death) are held in the Rugby Archives to this day. No letters from Mrs. Oldfield survived. Using the mannerisms, language, and the over the top rhetorical style from a Victorian's perspective, Mrs. Oldfield's identity and story have been recreated on the basis of factual evidence and are presented here as a conjectured record of the untold story of a typical Victorian woman of the time.

27 Jun, 82 London

My family,

I am sorry for the disappointment that I have brought upon you. It saddens me to the core, knowing that Charles left a burden on me when he left this earth. Not having him here for a proper burial breaks my heart. I am sorry Mamma for not listening to you about Charles. I was young and I fell in love. Love is blinding for the eyes, even when they can see disaster right in front of them. Charles was a loving man and he cared for me. Even when Papa took to the liquor, he wanted to save me from my own pain. Not knowing that he himself would bring much more pain than Papa's addiction to liquor. I cannot stand in front of my poor children any longer. I cannot stand the sight of their cries of hunger, when I alone can barely feed myself. Don't be mad at me Mamma, be mad at Charles. For when he died, he dragged the last piece of my soul down with him. I love you, my family.

Your dearest daughter

Ana Oldfield

The Tree

There was no possibility of taking a walk that morning. The fog was thick as cream and the morning dew was surely going to ruin the hems of her dress, but Ana did not care. She wanted freedom, she wanted to be one with nature, and mostly she craved air. Even though her mamma did not approve of such notions, Papa wanted her to be free.

'One day Ana you will be free like a bird. When that day comes, run! Run so far and so fast that no one can catch you.' And that she did.

'I'll be back!' Ana yelled while running out the door.

'Ana! Come back-' Mamma started, 'Oh leave the girl alone. Let her have fun, she is always cooped up like an animal. She is about to come of age,' hushed Papa.

'She should have taken a chaperone, Victor! Just think of what Mrs. Betty would say if she saw our Ana running down the streets! God forbid if she comes back looking like an animal that rolled around in mud,' replied Mamma very harshly.

'Speak to her then. I cannot stop you, you ungodly woman,' said Papa as he grabbed for another bottle of liquor. 'You make my head hurt. Speak softly like a woman should.'

'Be quiet and go drink away your problems. You can't take your anger out on me anymore.'

'Oh how I wish I could, woman, but I would fear my life would be beaten out of me. I am not ready to see the ground yet!' yelled Papa as he walked away.

'Oh Ana. Please come back,' whispered her Mamma while looking out of the window.

Ana flew that day. She flew so hard that Mrs. Betty fainted on her front porch. The old woman was very much intolerable and had a knack for things that were not in their proper places. As Ana rounded the corner, she skidded to a halt and looked around her. It was the same four walls that she had been living in for the past seventeen years. The same people that looked at her from under their noses.

'Proper,' she muttered while straightening her bodice and hat. She slowed her walk and rounded about the town square. Ana did not like the town square, because it had too many eyes; there was so much hunger.

'Come, come! Buy a loaf of bread-'

'Fresh fish, come see for yourself!'

'Ana! What in heaven's name are you doing here?' asked Victoria, one of Ana's close friends.

'I am free at last!' Ana replied back, twirling her tattered dress that was mixed in with the dirt from running.

'Oh Ana, I wish you truly were. Run along now, I need to sell this fruit before Papa gets back from the farm. Run!' giggled Victoria.

Ana turned and started to pick up her pace while she clenched her book. As she was heading towards the park, all she could think of was one word, 'Proper-'

'Sir, Charles has run off again. Should I bring him back?' asked Mr. Smith, Charles's butler.

'No Smith, let him be. Let him breathe, he has a lot of responsibilities as it is,' replied Lord Oldfield. 'One day he will be overlooking all of this-' gesturing out of the window that looked over acres and acres of land. 'He will need all the free time he can get.'

'Yes sir.' replied Mr. Smith while pouring Lord Oldfield's afternoon tea-

'Perfect,' she said while sitting under a willow tree. The sun was beaming just right, and a few people were taking a stroll around the park. She opened her book and started to act like she was reading, when she was truly gazing at the people strolling. 'So elegant, so graceful,' she whispered as a woman in lavender strolled not too close to a gentleman.

'What are you doing?' asked a manly voice.

Ana looked around to come upon eyes that were staring straight at her.

'Oh heavens! You startled me,' she gasped while clutching her chest.

'I am sorry. I did not mean to. Are you alright?' the young man asked Ana with a nervous look in his eyes. Eyes that were blue as the ocean, but warm as a fire place.

'Yes… yes sir I am quite alright,' she replied while rearranging her hat and tucking in her tattered hem of her dress.

'Wonderful. May I ask, what are you reading?' he asked curiously while holding out his hand.

‘Uh… a book-’ she said.

‘Yes, what kind of book, though?’ he persisted.

She was taken aback with his demanding hand and said quite harshly,

‘Why sir... please let a lady finish speaking.’

‘You are not a lady,’ he muttered while turning to gaze across the park.

‘Why! What a critic you are! How insolent, barbarous-’ snatching the book out of her hand, he opened the cover to snicker and hand the book back to her.

‘Romance. That book will bring you nothing but nonsense to your head,’ he replied back with a cold tone.

‘I was not reading it,’ she muttered.

‘What was that?’ He asked while looking directly at her.

‘I was not reading it, I was watching people strolling in the park,’ she turned away and stared back at the engaged people.

‘Not reading, huh? What is your name?’ he asked.

‘What is your name?’ Ana bit back while still looking away.

‘Charles. Charles Oldfield,’ he replied back to her.

‘Nice meeting you Mr. Oldfield.’ she said while gathering up her book in her lap.

‘What is your name?’ he asked earnestly.

‘My name is Ana. Ana Lewis.’

‘Ana… it is nice to meet you, Miss Lewis,’ said Charles as he stared off toward the distance.

As Ana turned, she really looked at Charles. She noticed that he was not just an ordinary man. He looked well dressed, with hair brown as the tree that he was laying against. Shoulders broad as a valley, and legs long as branches. Elegance that radiated from him as one would imagine from an angel.

‘Are you alright, Miss Lewis?’ Charles asked with concern in his eyes.

‘Yes! Yes I am, thank you,’ she replied back hastily. ‘Oh no. Proper,’ she thought to herself while blushing.

‘Pink suits your skin tone,’ Charles said while getting up and dusting off his pants. ‘My lady?’ He held out his hand to help her up.

Blushing, she laid her tiny hands in his large ones as he pulled her up with utmost grace.

‘Thank you,’ she whispered while blushing even more.

‘Where do you live?’ Charles asked while gazing at her book.

‘Uh-,’ she stuttered. ‘It is not proper to ask a lady where she lives.’

‘You are not no lady,’ he said while smiling a little. ‘But that is OK. Would you care to meet me here again in two weeks at the same time and day? You can bring a chaperone if it will make you feel at ease,’ Charles said with a glint in his eyes.

‘Depends on the weather. Good day sir,’ replied Ana while turning around to walk slowly away.

She did not dare look back at the barbarous man with blue eyes, but she could not help it. She turned just a little to see that her barbarous man was already walking away with his head held high like he just won at a gambling game. ‘What a barbarous man,’ Ana whispered while turning around and walking back home. Mamma would have scolded her for looking back towards a man, but she did not mind. After all, he was quite lovely to look at.

When the Big Fish Gets Caught

'Charles! Where have you been? Your father has been worried sick about your whereabouts,' said Charles's mother.

'Mother, I am here. Where is Fa-'

'Oh leave the poor lad alone, Ava. He needs to explore a little more in life. Come Charles! We have much to discuss,' said Lord Oldfield as he walked back into his study room.

'Yes sir,' Charles replied while giving his mother a quick peck on the cheek and running upstairs.

'Charles, my son. Mr. Smith told me you left your studies today? I see I saved you from your worried mother, so do you mind telling me where you went?' asked Lord Oldfield.

'I went for some air Father, nothing more,' Charles replied while sitting down and looking around his Father's study room. Those four walls, the same four walls that were suffocating him since he was a child.

'Charles, you cannot just run around like a child anymore. You have responsibilities, and duties that you have to focus on. If Mr. Smith did not see you leaving, what state would your mother be in right now? Hysteria, that's what she would be in right now!' said Lord Oldfield sternly.

'Yes sir,' Charles muttered while clenching his jaw.

'Go son. Your brother is in his studies, go fetch him. I need to talk to him,' stated Lord Oldfield.

'Yes sir,' Charles replied while walking towards the door.

Two weeks went by and the air became much drier than before. As Ana was getting dressed, all she could think of was Mr. Oldfield and his eyes. 'Are you awake my dear?' asked her Mamma while opening Ana's door.

'Mamma, oh how I have wonderful news!' She screeched while tying a faded pink ribbon in her hair.

'What is it, my love?' Mamma asked while sitting down on her bed.

'I think I found the one…' Ana said while gazing out of the window.

'Found what Ana?' replied Mamma with a confused look.

'I found him Mamma. Him,' she said with a glint in her eyes.

'Oh… him…' stuttered Mamma as she looked at the door.

'Yes him! Oh please come with me Mamma! Please? He said I could bring a chaperone and-'

'Ana! Did you meet this young lad without a chaperone? How many times do I have to tell you, it is not proper for a young woman to meet a man! Especially outside of her class,' she added the last part with a whisper.

'Forget class! He was really barbarous, I tell you, kind, and funny! He also has the bluest eyes I have ever seen. Oh Mamma, those eyes are real! Those eyes are in my book,' she said while grabbing her worn out book on her desk. She gazed at her book and wondered what he meant when he said that pink faired her skin.

'I am not feeling too well to go with you today, Ana,' said her mamma while straightening her apron and walking towards the door. 'Don't get in too much trouble Ana. Eyes are everywhere.'

'Yes Mamma,' she said while deflating on her bed. 'I am a young woman. I am proper,' she said while feeling empty from those words.

'Good girl. Run along now,' replied Mamma while closing the door behind her. Ana gathered up her book and pencil and walked out the door by herself.

'See you at dinner time Mamma!' Anna yelled while closing the front door-

'Miss Lewis. It is nice to see you again on this beautiful day,' Charles said while walking up to Ana. Giggling, she slowed down and smirked, 'Yes Mr. Oldfield, it is quite lovely.'

'Care to take a stroll with me?' asked Charles as he held out his arm.

'I would love to… but it is not proper to hold your arm in public, and I do not have a chaperone with me,' said Ana with a hurtful look in her eyes. Charles glanced around and saw a few people staring at them.

'Then I guess it is too bad,' he said while grabbing her arm and running to the willow tree that they sat at two weeks ago. All she could do was giggle and run.

'Charles! My arm will fall off if you keep pulling it,' Ana said while gasping for air.

‘My apologies, my lady,’ Charles said while lying down on the ground and looking up at the tree. ‘It has been very suffocating these last few days,’ he whispered while closing his eyes. She watched him from a distance, wanting so badly to stroke his brown hair and look up at the tree herself.

‘Ah!’ she screamed as he pulled her down next to him. ‘Lie with me,’ he said while lying back down. She could not help but blush even more.

‘This is not proper, you know,’ she whispered to him, while gazing around her and up at the tree.

‘And you are not a lady, so it really does not matter,’ he teased back at her.

‘I am too! I am a young woman of age,’ she stated proudly. ‘Congratulations,’ he muttered while dozing off. A few minutes went by when Ana heard a soft snore.

‘Are you sleeping!’ she gasped while sitting up right. Charles turned over on his side so that his back was facing towards her. ‘This barbarous man,’ Ana whispered with a smile. She sat up even more to look at his back side. Firm yet not firm, clothes that looked like silk, and an aura that with authority that surrounded him. ‘Goodnight Charles,’ she whispered as she got up to leave.

A few hours went by and Charles awoke from a slight shiver from the wind. ‘Ana?’ he whispered while turning over. She was not there, but a torn page from her book was there stating her address and her disappointment of his company. ‘Foolish girl,’ he smiled while clutching the torn paper.

A month passed and Ana did not hear from Charles, until one day she heard, ‘Ana, my dear. You received a letter from someone,’ yelled up Ana’s mamma.

‘A letter!’ stated Ana while running down the stairs.

‘You are a young woman, not a child anymore,’ stated her mamma while holding the letter away from Ana’s hands.

‘I am sorry, mamma, I won’t do it again,’ promised Ana.

‘Here, run along.’ said mamma as she walked away. The letter read:

23 May, 66 London

Miss Lewis,

My apologies for writing so late. I have been busy with family affairs, and the weather has been very gloomy for a walk in the park. With permission, I would like to meet you again. Meet me at the town square on the twenty-fifth of May after tea time. I will be waiting for you.

Yours truly.

Mr. Charles Oldfield

‘Who was that from?’ whispered her mother.

‘Mamma!’ Ana gasped in horror as she clutched the letter to her chest, ‘No one. I have to go,’ replied Ana as she ran up the stairs.

Two days went by and Ana was preparing herself for this day. ‘The town square has lots of eyes,’ she thought, and she was determined to avoid making a scene. ‘I’m off to the square,’ called Ana as she walked out of the door. ‘Today is the day,’ she said as she walked down the street. As she passed Mrs. Betty’s house and rounded the corner to pass the four walls, she found herself standing in the middle of the town square looking for Charles.

‘Miss Lewis!’ Ana turned to watch Charles push through the crowd of people.

‘You made it, Mr. Oldfield,’ Ana giggled.

‘Yes, yes I did. How are you on this lovely day?’ Charles asked with a gleam in his eyes.

‘I am alright,’ replied Ana while watching everyone stare at them. ‘Would you like to go somewhere quieter?’

‘Perfect. What do you have in mind?’ asked Charles.

‘Follow me,’ she said as she turned to head towards the outskirts of the town, next to an old barn with an open field of buttercups.

‘So beautiful,’ Charles said, awestruck.

‘I know. This is one of my many hideouts from everyone… and everything,’ she whispered as she sat down in the middle of the field while playing with a flower.

‘I am on a mission!’ Charles said while sitting beside her.

‘What mission is that?’

‘A request for permission to court a certain lady,’ replied Charles.

‘Oh…’ said Ana with a deflating heart.

‘What do you think?’ asked Charles.

‘I think the young lady would be happy that you are courting her,’ replied Ana.

‘Perfect,’ whispered Charles as her leaned in to kiss her. As they slowly wrapped each other in a long embrace, time seemed to have slowed down for Ana and she finally saw a way to fly.

The Journey of Early Livelihood

Two more months went by, and Ana became furiously sick. Letters ceased from both Ana and Charles, until one day Charles opened a letter not from Ana, but from her mother.

19 Jul, 66 London

Mr. Oldfield,

It is my deepest regards to inform you that Ana has taken a bug of some sort. A family doctor of ours will be visiting on the twenty-first of July. I request your presence on this day, for if I am correct, this bug is not curable.

Signed,

Mrs. Lewis

‘Not curable?’ Charles thought out loud.

‘What is not curable my son?’ asked Lord Oldfield while standing in front of the window in his studies.

‘Nothing sir. Some friends of mine are sick and are requesting my presence. I will be gone for a few days is all,’ replied Charles.

‘Don’t stay too long, because we need to discuss your marriage with the Robinsons,’ replied Lord Oldfield as he sat down and looked Charles in the eyes. ‘We need this alliance son, don’t disappoint me.’

‘Yes sir,’ said Charles-

‘She is with child,’ hushed the doctor with worried glances between both parents.

‘Wha… What did you say?’ asked Ana’s mother

‘She is carrying a child, and what she is experiencing is morning sickness,’ replied the doctor as he wiped the sweat from his forehead. ‘She will need to drink plenty of water and pray that this child lives.’

‘I knew it…’ replied her mother.

‘How much do we owe you, doctor?’ asked Ana’s father.

‘Nothing… I advise you keep her inside for now,’ said the doctor as he packed up his things to leave.

‘Yes doctor, thank you again,’ spoke her mother from across the room.

A knock came at the door as the doctor was heading towards the door.

‘I will get that… good day,’ the doctor said while opening the door.

‘Hello sir,’ said the doctor as he stepped around Charles.

‘Hello,’ replied Charles.

‘Oh… you must be Mr. Oldfield,’ said Ana’s mother with an absent look in her eyes.

‘Nice to meet you, how is Ana?’ replied Charles with concern in his voice.

‘Ana… is sick,’ said Ana’s mother.

‘Yes, she is sick, sick with child… did you know anything about this?’ replied Ana’s father with a cold tone to his voice.

‘Wi… With child,’ Charles said with a look of disbelief.

‘Yes, with child,’ replied Ana’s father with a more menace look in his eyes. ‘How will you fix this lad?’

‘I will fix this,’ muttered Charles with a shocked look on his face. ‘I will fix this.’

‘We know you will, son. We know you will,’ said her mother.

The marriage took place within a fortnight, and both families were present, but no other family members attended. Lord Oldfield did not want a big wedding for his son, so he requested a small one in the backyard of his estates. There was no catering present, no decorations for the bride, just a priest and a few chairs. After the wedding was finished, both parties went inside to eat a light dinner before departing their separate ways.

‘Lord Oldfield, you have a beautiful home,’ said Ana after she finished her small bowl of soup.

‘Thank you,’ replied Lord Oldfield with a stiff tone. ‘I worked very hard, as you can tell.’ ‘I see,’ said Ana quietly.

‘Actually, I am needing to speak with my son for a few moments, do you mind?’ asked Lord Oldfield to everyone that sat at the dinner table.

‘No, of course not-,’ ‘Absolutely not-,’ chorused everyone.

‘See me in my study in a few moments,’ replied Lord Oldfield as he stood up to leave the dining room.

‘Yes sir-’

‘Shut the door, lad,’ said Lord Oldfield from his chair. ‘Have a seat.’

Charles sat as he waited for his father to speak to him.

‘Do you understand what you have done?’ said Lord Oldfield icily.

‘Yes sir, I do.’

‘No lad, you do not! If you did, you would not have married that trollop-’

‘She is not a trollop, father,’ said Charles. ‘She is the mother of my child. The woman that understands me. That woman sees me, father! Not just a bag of money, but me.’

‘Does she? I see a woman with a child, that broke our alliance with the Robinsons. They were going to help us excel your career Charles, did you not think for once-’

‘I did think! Just not how you wanted me to.’ spoke Charles with a look of hurt in his eyes.

‘You did not think, son, and I am sorry for that. Your mother erased you from the family tree as I ordered her to do so. I cannot have this woman and you together in my house. I will give you some money, but you are to never show your face here again,’ stated Lord Oldfield.

‘As you wish,’ replied Charles with tears in his eyes as he accepted the money.

28 Nov, 81 London

Dear Mr. Oldfield,

It is with regards to say that your process to the colony was successful. The Board of Directors has decided to send you to Rugby Tennessee, and upon your request you will be given free boarding on the next ship to United States. As mentioned before, your responsibilities are to write back your experience in Rugby and give full detail of the colonization. Your wife and children will be well taken care of by Mr. Kimber himself. We ask that you prepare your leave in a few months.

Best wishes,

Rugby Board of Directors

'Ana, this is a wonderful opportunity for us,' said Charles while holding up the letter. 'Besides, your mother said she would check up on you every now and then. I also requested for Mr. Kimber to look after you. Ana please-'

'Fine, Charles, go to Rugby,' replied Ana as she mentally started to build up walls around herself.

'Ana, you know this is for you and the family. I will be back home soon, and I promise it will be just a few months. You know we have not been doing well financially and the children add on to our cost-'

'Why can you not take me? You promised you would protect me ever since your father-'

'And I have,' said Charles. 'You will be fine. That I promise.'

'Alright, if you must go then go. I am not stopping you,' replied Ana.

The Letters

Rugby, Tenn.

12 Jan, 82

Dearest Ana,

I have made it to Rugby, and it is different from London. The air is much more breathable and the colony is more spacious than I thought. Young men are here to learn about farming, they are from families like mine. The people are nice here and have been accepting of me. I wish you and the children were here. I am currently looking for a place to stay, and I asked Mr. Kimber to give you a monthly allowance. I do not know if he will or not and if needed he can pay you of what they owe me for coming here.

Believe me ever,

Charles Oldfield

'Ana, my dear, it is from Charles,' said Mamma as she handed Ana the letter.

'I cannot, Mamma,' Ana whispered with tears in her eyes and loathing in her heart. 'Why am I here and he is there? I do not understand-'

Rugby, Tenn.

14 Feb, 82

My dearest Ana,

I am still looking for a place to live at. It is quiet at night, and the views are magnificent. How are you my love? I have not heard from you in a month, the children must be curious on where I am at. I hope you told them the news by now and I am even considering of moving you down here. I am sorry that it is taking me so long, but I promise just a few more months.

Your dearest beloved,

Charles Oldfield

'A few more months,' whispered Ana as she tore up the letter. 'So much for coming home soon.'

7 Mar, 82 London

Dearest Charles,

Word has come from Mamma that you have wrote another letter again. I dare not read it, for my emotions are tangled up with the idea of your newly founded freedom. The children are growing so much without you here. I dare ask when will you be coming back home to London? Mama is not doing too well, for she is taking it very hard with the social withdrawal. Papa has taken the liquor bottle again, and the house is about to be up for sale soon if we don't fix our financial issues. Oh Charles why did you have to leave me with this situation? If only I knew what it meant to marry you-

'No I can't send this,' said Ana while crumbling up the letter. As she looked out the window into the dewy morning, she thought back on the brighter days when Charles and she were not married.

19 Apl, 82 London

Dear Charles,

I apologize for not writing to you sooner for I have become sick with erysipelas. The weather has become quite dreadful and the house was sold for a much lower price than expected. I am currently living next door to Mamma and Papa and the children are well. Henry misses you a lot. We received word from Mrs. Betty that your mother has passed away. Word was mentioned that a beautiful funeral was being prepared for her, but we are not invited to it. I am sorry Charles, this is my fault and we both know it is. That day… I was not thinking, I wanted away from all of this, but it seems that I just dragged us down even more. I miss you Charles, please come back home.

Your loving,

Ana Oldfield

'Are you really sorry Ana?' asked her mother.

'No I am not,' replied Ana with a distant tone to her voice.

Rugby, Tenn.

6 May, 82

Dear Kimber,

I have received by this mail a copy of your letter to your Brother as to my Boy. I feel I can never thank you as I wish, But one thing certain nothing that I can do to serve your interests shall be left undone. I will also write my thanks to Mr. Wilson for kindly helping me.

I am afraid my poor little wife is ill from erysipelas in a letter received last night Henry said her eyes were nearly closed. Poor little girl she has had her ___ of troubles since I left, but if I can only get them over here and see my way to enough to live upon for a few years I shouldn't mind. By this time you will have received my letter weekly how ___ I am and be able to judge what is best to do. Each day has also been of service to me in looking the future in the face.

Yours very truly,

Charles Oldfield

'Henry, go see who is at the door,' whispered Ana weakly.

'Young lad where is your mother?' asked Mr. Kimber at the door.

'She is in the drawing room, come in,' replied Henry.

'Ana… how do you fare?' asked Kimber while he sat down across from her.

'I am alright. Why do you ask?'

'I received word from Charles. He is struggling to find land and mentioned how you were sick from your letter you wrote to him,' said Mr. Kimber.

'As you can see, I am not well. I miss my husband terribly,' whispered Ana.

'Then let me book you a trip to the colonies. You will feel better once you see your husband again,' said Mr. Kimber in a confident voice.

'No let Henry go. I am to unfit to go right now, but Henry can go with no troubles. He needs to experience the world.' replied Ana tiredly, leaving no room for arguments.

Rugby Tenn

23 May, 82

My Dearest Ana,

I have fallen sick with diarrhea, and I am allowed to stay at the doctor's house until he comes back. I wish you would come sooner, and bring all the children with you. I miss you terribly and my loneliness has increased by far. Why do you not write to me anymore my love? Please write soon, how are you truly?

Believe in me,

Charles Oldfield

'My loneliness has increased too, Charles, but you cannot see that because you are across the world,' said Ana absently. She tossed the letter on the floor where the rest of his letters lay. 'I am tired,' she whispered as she crawled into bed when it was barely noon.

Rugby Morgan Co.

Tennessee

U.S.A.

Dear ____,

I have not received any answer to my last letter to you about the Boots and Trousers. I wish you had sent them to Shilton at once as I find my eldest boy is coming out to join me. He will arrive at New York in about a week if all's well. And I am sure if the rest of my family will come out before the Autumn. I am inclined to try and see what good can be done before they come. The "Board of Aid" in London has given me a farm here and freehold to entice me to remain. And I shall do so if they will give me a salary for the first 5 years while I am getting my farm clear and in order. I wish I had come out to this country ___ years ago, while I had some money to work with but what's the use of wishing.

Yours Truly.

Charles Oldfield

23 Jun 82 London

My dearest Charles,

I cannot fathom coming to the colonies and living with you. Rugby sounds so disorganized and not well kept for any class structure. I would feel so lost being in Rugby, and it seems to me you are happy there with your new freedom from London's laws and the eyes-

'No I will not write to him,' Ana whispered as she threw away the last piece of paper she owned. 'If he seeks me, then he can seek me in London.'

Rugby, Tenn.

27 June, 82

Dear Mrs. Oldfield,

It is sad for me to inform you that Mr. Oldfield has passed away on the twenty-fourth of June from a severe attack of rheumatic fever. His son was present for his death, and was present for his funeral. He will be buried at Rugby Tennessee if you would like to go make your condolence. I hope you are feeling well Mrs. Oldfield.

Sincerely sorry,

Mr. Kimber

'You barbarous man,' whispered Ana as she read the short letter that Mr. Kimber sent her. 'I should have gone to him, I should have been there,' she yelled.

As night became morning, morning became night, Ana lost track of time. Time ultimately stopped for her when she opened that door and received that unforgettable letter. As few days went by, Ana found herself at the entrance of the family doctor's shop.

'Mrs. Oldfield! What can I do for you today?' asked the family doctor.

'I am need of some Strychnine and paper, do you have any in stock?' replied Ana.

'Uh…' stuttered the doctor. 'I do have some left actually. It's the season again for pests, and the strychnine has been in high demand lately.'

'Oh, that's good,' muttered Ana as she took the strychnine and paper back home. When the evening turned into night, Ana sat at her desk rewriting her letter to her family until finally she wrote the perfect one.

1 Aug, 82 London

My family,

I am sorry for the disappointment that I have brought upon you. It saddens me to the core, knowing that Charles left a burden on me when he left this earth. Not having him here for a proper burial breaks my heart. I am sorry Mamma for not listening to you about Charles. I was young and I fell in love. Love is blinding for the eyes, even when they can see disaster right in front of them. Charles was a loving man and he cared for me. Even when Papa took to the liquor, he wanted to save me from my own pain. Not knowing that he himself would bring much more pain than Papa's addiction to liquor. I cannot stand in front of my poor children any longer. I cannot stand the sight of their cries of hunger, when I alone can barely feed myself. Don't be mad at me Mamma, be mad at Charles. For when he died, he dragged the last piece of my soul down with him. I love you, my family. Mamma look after my children and tell them every day how much I loved them.

Your dearest daughter,

Ana Oldfield

That night as Ana Oldfield lay in bed, she thought back on her life. She thought back on her memories of the buttercup field, and Charles's blue eyes.

'Oh how I miss you, my barbarous man,' Ana whispered as she closed her eyes and went into a deep sleep.

Acknowledgements

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References

Brontë, Charlotte. Jane Eyre. 4th ed., W.W. Norton, 2016.

Burstyn, Joan N. Victorian Education and the Ideal of Womanhood. Barnes & Noble, 1980.

Coontz, Stephanie. Marriage, a History: How Love Conquered Marriage. Penguin, 2005.

De Barenne, J.G Dusser. "The Mode and Site of Action of Strychnine in the Nervous System." Physiological Reviews, vol. 13, no. 3, 1933, pp. 325-335.

Draznin, Yaffa. Victorian London's Middle-class Housewife: What She Did All Day. Greenwood Press, 2001.

Marcus, Sharon. Between Women: Friendship, Desire, and Marriage in Victorian England. Princeton UP, 2007.

Oldfield, Charles. Stop 5 Charles Oldfield. Box 4, Papers of Rugby and Rugby Connected Families, folder 8. Historical Rugby Archives and Research Center, Rugby, TN. 6 March 2017.

Oldfield, Charles. Letters from Charles Oldfield. Box 4, Papers of Rugby and Rugby Connected Families, folder 9. Historic Rugby Archives and Research Center, Rugby, TN. 6 March 2017.

Thomas, Kate. Postal Pleasures: Sex, Scandal, and Victorian Letters. Oxford UP, 2012.

Walkowitz, Judith R. Prostitution and Victorian Society: Women, Class, and the State. U of Cambridge P, 1999.

Detection of Lead Contamination in Water using Fluorescence of Functionalized Gold Nanoparticles

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Introduction

With lead contamination in drinking water becoming an increasingly prevalent issue for residences and for human health, there is a growing concern that detection should be enabled for real-time analysis by the consumer. The lead water crisis of Flint, MI is one of the most recent incidents where lead contamination caused widespread health issues. In 2014, the city of Flint, MI changed its main water supply from Lake Huron to the Flint River. Due to changes in pH for the source, lead from the water lines began leaching into Flint's water system. Lead levels in some homes were as high as 397 ppb1, more than 25 times higher than the Environmental Protection Agency (EPA) limit of 15 ppb2. More timely analysis in the hands of residents could have averted some of the consequences of this excursion, including sick children throughout the community. The research presented here sought to establish a new method of lead detection, based on nanoparticle fluorescence. Currently, to have water reliably tested, a sample of the water would be sent to a lab to undergo atomic absorption spectroscopy or differential pulse anodic stripping voltammetry3. These methods are capital, labor, and expertise-intensive. Colorimetric methods exist, but the nuance of a color change can be misleading to the eye of the lay user. A large volume of work utilizing functionalized gold nanoparticles in colorimetric sensing does exist4 5 6. The novelty of the present approach was the use of excitation/emission matrix (EEM) fluorescent spectroscopy. Fluorescent spectroscopy features increased sensitivity and selectivity compared to colorimetry. The interactions between very small numbers of particles can be detected at length scales of nanometers or angstroms. In colorimetry, solutions which appear completely transparent before the introduction of a contaminant may change slightly afterward, but the human eye might have difficulty noting the difference. With fluorimetry, however, solutions which appear completely transparent to the human eye can still exhibit a detectable fluorometric response. Extension of this research would open the door to the creation of a device which is comprised of a membrane doped with fluorescent nanoparticles, the nanoparticles acting as the sensor. Testing to see if water is contaminated with lead would be as simple as turning on a UV flashlight (commonly sold even on eBay), or UV LED to see whether the membrane's fluorescent response has changed after exposure. One might conceive of a device in which the embedded membrane can be viewed through a window in a filter attached to the faucet, and half the membrane fluoresces in the presence of lead while the other half has control fluid and does not fluoresce. The human eye should be able to detect the comparative difference. Thus, this work will describe research in three parts: 1) identification through literature searches and experimentation of a nanoparticle/coating pair responsive to lead, 2) EEMs studies indicating a unique excitation-emission pair after proper corrections of the spectra, and 3) composition studies to verify sensitivity of the proposed method and quantification of the composition of lead ion in drinking water.

Background

Previous work using gold nanoparticles functionalized with 11-mercaptoundecanoic acid (MUA) has been performed suggesting its validity as a fluorimetric sensor for lead in water. First, Kim et al.4 showed an introduction to colorimetric detection by utilizing 2.4 nM suspension of 13.6 ± 4 nm gold nanoparticles capped with MUA, as well as an addition of a 1.0% poly(vinyl alcohol) stabilizer. Varying concentrations of lead were added to the suspension, and the resulting color change was analyzed using UV-visible spectroscopy. Kim et al.4 speculated that, in an aqueous solution, the –COOH groups found at the end of the MUA could create a chelate complex with the 2+ charge of a lead ion. This chelation between multiple nanoparticles would cause aggregates to form in the solution, which would therein change the color of the solution due to aggregation of the GNPs. A similar response was seen for Hg2+ and Cd2+ ions but not for Zn2+. One interesting aspect was the implementation of ethylenediaminetetraacetic acid (EDTA), where increasing amounts of EDTA were added to the lead-containing suspension of MUA-GNPs, and the lead chelation was reversible. They attained a limit of detection (LOD) as low as 400 µM. It should be noted that colorimetry has a relationship to fluorimetry in that the necessary (but not sufficient) condition for a molecule or nanoparticle to fluoresce is that it must first absorb light energy. Thus, the work by Kim et al.4 is a positive indication that this nanoparticle/coating pair is a) responsive to lead and b) has the potential to fluoresce as it satisfies the condition that it absorbs light energy.

Secondly, Huang et al.7 notes that gold nanoparticles coated with MUA can act as a fluorescent sensor for some heavy metal ion. GNPs of diameter 2.0 ± 0.1 nm capped with MUA were used to investigate the resulting fluorescent intensity response upon the addition of Hg2+. When Hg2+ was introduced to MUA-GNPs at a concentration of 10 nM, the fluorescent intensity was quenched. These GNPs also showed quenching of the fluorescent signal upon addition of lead. In order to drive the specificity towards Hg to prevent the occurrence of false positives from lead, pyrrole-2, 3-dicarboxylic acid (PDCA) was added to the solution. Using a 10 nM solution of MUA-GNPs in a buffer solution of 5 mM sodium tetraborate (pH = 9.2) with 1.0 mM PDCA, Huang et al.7 were able to achieve a remarkable tunable detector for Hg2+ ions in water at concentrations as low as 5.0 nM.

Materials and Methods

For the procedures described below, 5 nm unconjugated gold colloid was purchased from Ted Pella, Inc. (Redding, CA, USA). These nanoparticles were stabilized with citrate. HPLC grade water purchased from Fisher Scientific (Fair Lawn, NJ, USA) and was used for all dilutions and functionalizations. The as-purchased citrate coated gold nanoparticles were functionalized using 95% 11-mercaptoundecanoic acid purchased from Sigma Aldrich (Darmstadt, Germany) by a procedure described below. For the dialysis step described below, seamless cellulose dialysis tubing with a molecular weight cutoff of 12,000 D was used and purchased from Fisher Scientific (Fair Lawn, NJ, USA).

For the UV-Visible spectroscopy, a Varian Cary 3E UV-visible Spectrophotometer was used, made by Agilent (Santa Clara, CA, USA). A Varian Cary Eclipse Fluorescence Spectrofluorometer was used, also made by Agilent (Santa Cara, CA, USA).

In order to functionalize the as-purchased citrate coated gold nanoparticles with MUA, 1 mL of Cit-GNPs was combined with 1 mL of a 3.76 x 10-5 M solution of MUA, and 1 mL of an equimolar solution of NaOH. The resulting mixture was placed under sonication for 1 hr at 60 °C. After sonication, dialysis was used to purify the MUA-GNPs and remove any excess citrate and MUA.

Quartz cuvettes were used for all fluorimetric and UV-Visible testing. Prior to all testing, all cuvettes were cleaned using aqua regia. EEMs spectra were collected scanning from excitation wavelengths of 200-800 nm and emission wavelengths of 200-850 nm. UV-visible spectra were obtained scanning from 200-800 nm. For each data set, a sample of pure HPLC grade water (the solvent), MUA-GNPs, lead-contaminated water, and a sample of the MUA-GNPs with lead contamination were all separately tested.

Data Analysis

After the raw data from the EEMs were collected, corrections for primary and secondary inner filtering effects (IFEs), as well as for the Raman spectra of water were performed. A MatLab code was used on the data set in accordance with Tucker et al8. Eq. 3.6 gives the equation used to correct for primary inner filtering, and Eq. 3.7 gives the equation used to correct for secondary inner filtering.

$$f_{prim} = \frac{F_{corr}}{F_{obs}} = \frac{2.303A(y-x)}{10^{-Ax} - 10^{-Ay}} \quad 3.6$$

$$f_{sec} = \frac{F_{corr}}{F_{obs}} = \frac{(v-u)/\left(\frac{1}{b}\right)\ln(T)}{T_v - T_u} \quad 3.7$$

Here, A is the absorbance per centimeter at the excitation wavelength, T is the transmittance of the sample, b is the entire cell pathlength, and x, y, v, and u are the dimensions of the interrogation zone of the spectrofluorimeter (Figure 1).

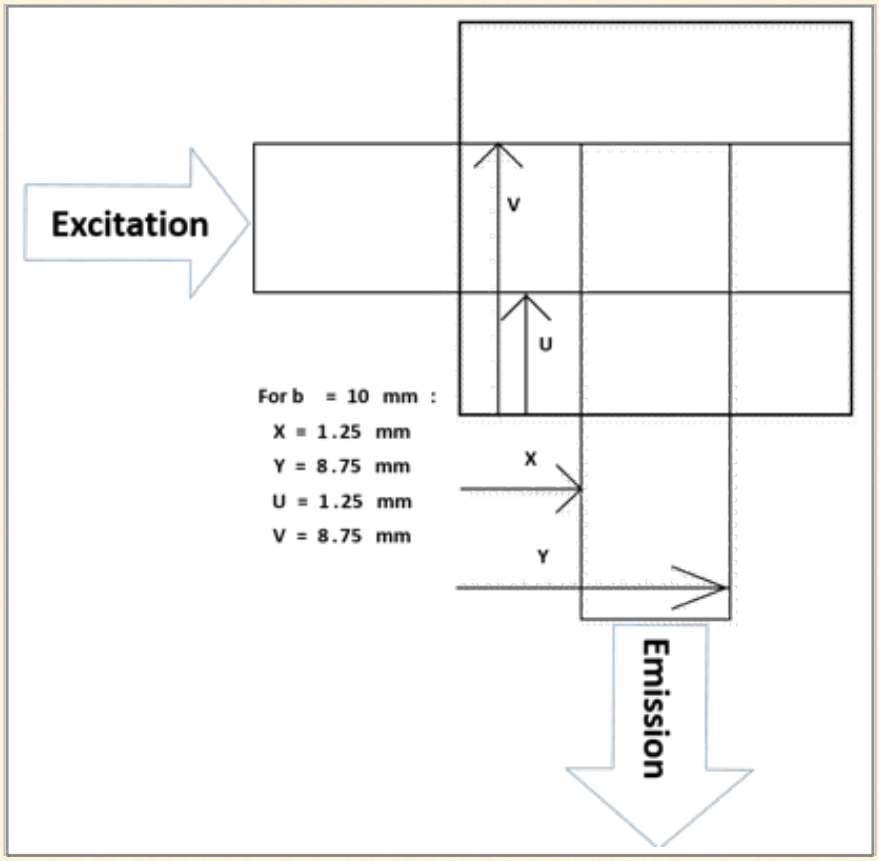

Figure 1. Dimensions of the spectrofluorimeter interrogation zone (not to scale).

The raw spectrum generated fluorescence is a three dimensional spectrum, excitation vs emission vs intensity. In order to determine whether quenching or enhancement occurs as a result of adding lead to the MUA-GNP solution, a 2 dimensional "predicted" curve was generated from the data. This predicted spectrum is taken of the intensity versus excitation wavelengths for a set emission wavelength, 342 nm. The predicted spectrum is the sum of the individual intensities of the lead contaminated water and the pure MUA-GNPs. If a comparison of the predicted spectrum and the intensity versus excitation wavelength for the MUA-GNP with Pb2+ samples are different, it can therefore be said that enhancement or quenching has occurred. Enhancement would be represented by the experimental intensity lying below the predicted, while quenching would be represented by the experiemental intensity appearing above the predicted.

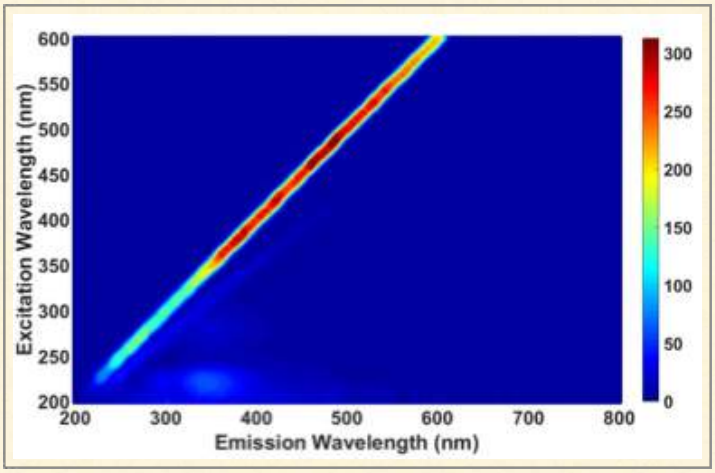

Figure 2. EEMs spectrum of HPLC grade water

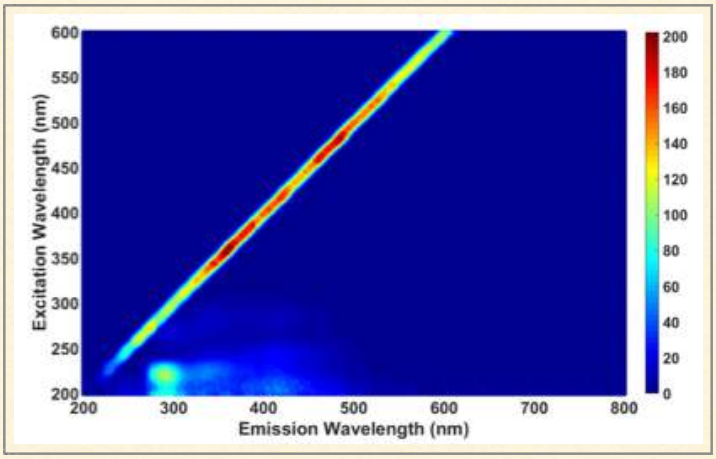

Figure 3. EEM spectrum of 100 µg/L Pb2+ in HPLC grade water.

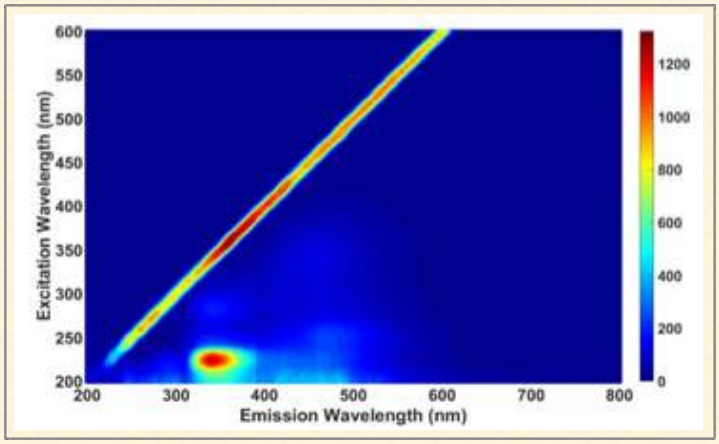

Figure 4. EEM spectrum of 5 nm MUA-GNPs at 11.375 µg/mL

Results and Discussion

Figure 2 shows an EEMs spectrum of HPLC grade water. The diagonal line shown in the spectrum below is representative of the Rayleigh scattering. For the purposes of this research, the Rayleigh scattering will effectively be ignored.

Figure 3 shows an EEMs spectrum of 100 100 µg/L Pb2+ contaminated water. This spectrum, along with that of pure MUA-GNPs, is used to generate theoretical predictions of mixtures where neither component interacted with the other.

Samples of 5 nm MUA-GNPs at 11.375, 9.6, and 5 µg/mL were prepared and tested. A representative EEMs spectrum of these different concentrations can be seen below in figure 4. For the pure MUA-GNP samples, the only difference between the concentrations lies in the intensity of the primary peak at EX220 EM 342.

Samples of the MUA-GNPs at the concentrations mentioned above were prepared with 100 µg/L Pb2+. The resulting 3D EEMs spectra was found to be difficult to determine whether quenching or enhancement had occurred. Therefore, the 2D curves are shown below for the different concentrations.

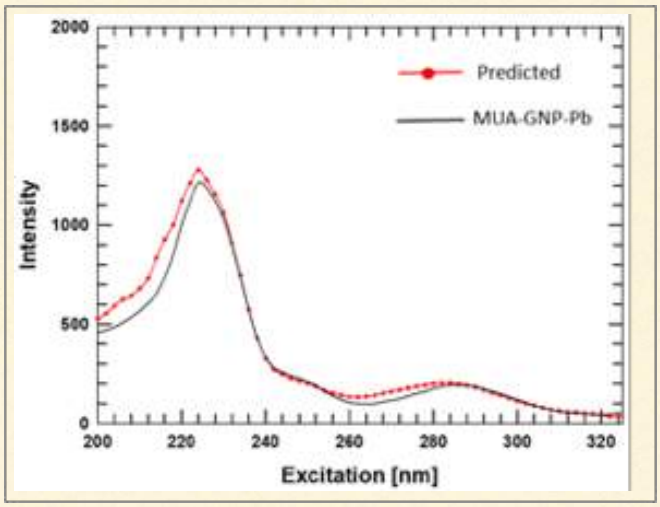

Figure 5. 2D spectrum of MUA-GNPs at 11.375 µg/mL with Pb2+ at 100 µg/L

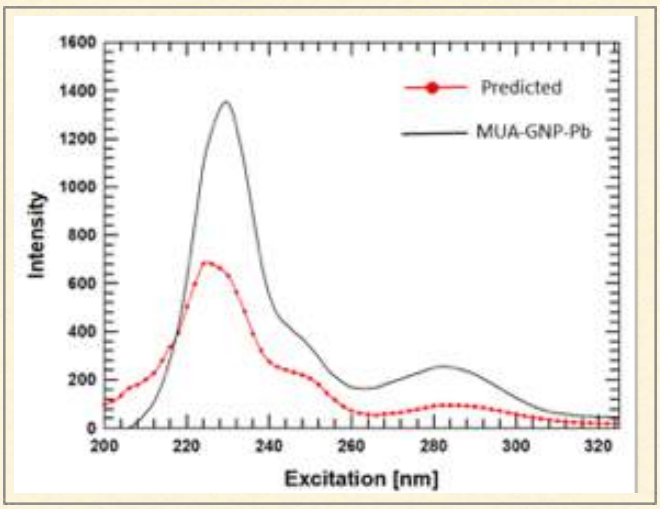

Figure 6. 2D spectrum of MUA-GNPs at 11.375 µg/mL with Pb2+ at 100 µg/L

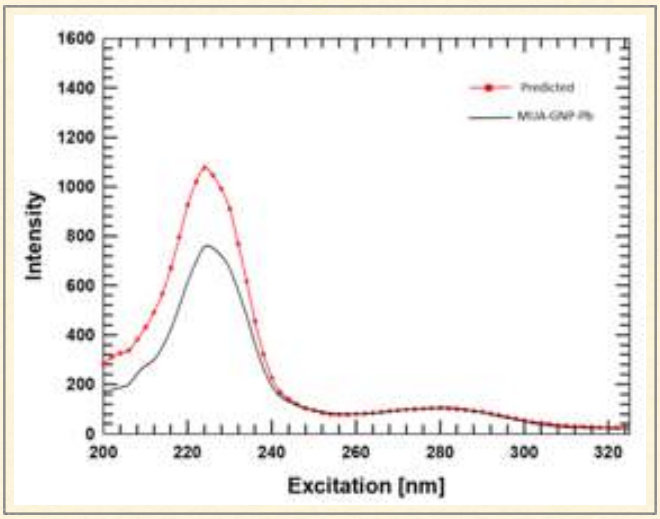

Figure 7. 2D spectrum of MUA-GNPs at 11.375 µg/mL with Pb2+ at 100 µg/L

From the above figures, it can be seen that all three possible responses are exhibited (no change, enhancing, quenching, respectively).

Conclusion

The goal of this research is to determine whether the fluorescence of gold nanoparticles functionalized with 11-mercaptoundecanoic acid can be used as a sensor for lead in water. The preliminary results of this study shown above are promising. The addition of lead to varying concentrations of gold nanoparticles does have an effect on their fluorescent intensity. The issue which now arises is in consistency of data. Future work will be to conduct these experiments again, at the same concentrations, to determine whether these results are reproducible. Once reproducability has been established, the research will focus on sensitivity and selectivity studies. This research is clearly still in the early phases, but the preliminary work is hopeful. We have successfully shown that the presence of a lead ion in a solution of gold nanoparticles functionalized with 11-mercaptoundecanoic acid can affect the fluorescent intensity of the nanoparticles. This result suggests that these MUA-GNPs can in fact be used as a form of detecting lead in water.

References

1. Morckel, V., Why the Flint, Michigan, USA water crisis is an urban planning failure. Cities 2017, 62, 23-27.

2. Code of Federal Regulations Title 40 - Protection of Environment Part 141 - National Primary Drinking Water Regulations. 2012, 24.

3. Standard Test Methods for Lead in Water; ASTM International: 2015, 2015.

4. Kim, Y.; Johnson, R. C.; Hupp, J. T., Gold Nanoparticle-Based Sensing of “Spectroscopically Silent” Heavy Metal Ions. Nano Letters 2001, 1 (4), 165-167.

5. Chai, F.; Wang, C.; Wang, T.; Li, L.; Su, Z., Colorimetric Detection of Pb2+ Using Glutathione Functionalized Gold Nanoparticles. ACS Applied Materials & Interfaces 2010, 2 (5), 1466-1470.

6. Beqa, L.; Singh, A. K.; Khan, S. A.; Senapati, D.; Arumugam, S. R.; Ray, P. C., Gold Nanoparticle-Based Simple Colorimetric and Ultrasensitive Dynamic Light Scattering Assay for the Selective Detection of Pb(II) from Paints, Plastics, and Water Samples. ACS Applied Materials & Interfaces 2011, 3 (3), 668-673.

7. Huang, C.-C.; Yang, Z.; Lee, K.-H.; Chang, H.-T., Synthesis of Highly Fluorescent Gold Nanoparticles for Sensing Mercury(II). Angewandte Chemie 2007, 119 (36), 6948-6952.

8. Tucker, S. A.; Amszi, V. L.; Acree, W. E., Primary and secondary inner filtering. Effect of K2Cr2O7 on fluorescence emission intensities of quinine sulfate. Journal of Chemical Education 1992, 69 (1), A8.