Implementing a Course-based Undergraduate Research Experiences (CURE) Course

Matthew R. Hartings[1], Kathryn A. Bracht[2], Madeline E. Caballero[3], Alyssa D. Cordero[3], Monessha Jayabalan[3], John R. Bracht[3]

Affiliations:

[1] Chemistry Department, American University, Washington DC, 20016
[2] Independent Scholar, Washington, DC, 20016
[3] Biology Department, American University, Washington DC, 20016

Correspondence:
Matthew R. Hartings (hartings@american.edu)
John R. Bracht (jbracht@american.edu)

Introduction

Studies have shown that students from groups underrepresented in STEM are greatly benefited by research experience (Hernandez et al., 2013, 2018), but opportunities fall far short of demand. Given the hidden curriculum, it can be even more difficult for underrepresented students to land coveted research positions among majority white faculty. Course-based Undergraduate Research Experience (CURE) classes allow students to develop independent research skills in a guided environment, collaborating with peers and receiving guidance from mentors. Unlike lab courses, CURE isn't about finding the 'right answer' in following a predetermined set of protocols, but encourages students to formulate and execute their own research plan under the mentorship of the class professor. This not only builds their skillset at the bench, it further teaches them to think like a scientist and ideally generates academic publications, turbocharging their careers.

CURE classes require significant reconceptualization of teaching, with the scientific process itself performing the teaching; the faculty become co-learners along with the students. We recommend implementing a consistently offered CURE series that focuses on in-class experimentation, discovery, analysis, and publication (Hartings et al., 2015; Hernandez et al., 2018).

The CURE course structure provides ample opportunities for faculty-to-student, student-to-student, student-to-faculty, and even faculty-to-faculty mentorship. This web of relationships may also allow faculty who are not research-active to perform laboratory research with students during their teaching, thereby building their research profiles and benefitting all participants. The CURE also enables psychosocial and instrumental mentoring among students, as they collaborate, share experiences, and develop soft skills as well as scientific expertise. A good CURE implementation can shift the ethos from competition to collaboration, and by building research experience and mentorship, ultimately the STEM web will retain more underrepresented scholars.

Since laboratory research may be on a volunteer basis, it can raise significant financial barriers for students from lower socioeconomic backgrounds who may need to work part- or full-time throughout college to finance their education. Therefore, not only will the CURE course address the gap between students seeking laboratory research experience and available laboratory positions, it will also address the financial barrier that disproportionately impacts students from underrepresented groups (assuming it is not an extra course beyond the normal curricular electives needed to obtain the degree).

Lab work conducted within a CURE class builds beyond mentorship. Working on focused research projects together promotes community and the building of friendships between people who wouldn't otherwise get to know each other. Not only does working in the lab (or in the research classroom) teach resilience in the face of failure, it fosters excellence; the shared pursuit of knowledge in the lab forms a friendship oriented around the good, ideally strengthening all involved. Further, through research collaborations, friendship can offer a model for buffering failure and inculcating excellence not only among students, but between faculty and even among schools. This could work against isolationism and careerism in the academy, fostering resilience at every level of the STEM web.

Additional recommendations for CURE:

- As a research-focused course, the CURE must be implemented by research-active faculty: do not saddle teaching-only faculty with the burden of CURE course design. Research has a hefty hidden curriculum that needs to be navigated in this course.
- For similar reasons, engagement by faculty familiar with the publication process is critical because scientific publication is another aspect of the hidden curriculum for faculty.
- However, co-teaching by research-oriented and teaching-only faculty is a good way to build faculty-to-faculty mentorship and give everyone research publications.
- To maximize the impact of the CURE for students from underrepresented groups, the publications produced by the CURE program should be submitted to high-quality research journals, rather than in undergraduate-only journals. They then need follow-up through the peer-review process; ideally this also involves mentoring of students at every stage.
- The CURE needs to be very high quality in terms of research implementation. Do not create a ‘second-tier’ of research (CURE) relative to the best (joining a research laboratory)--this will disadvantage students from underrepresented groups; furthering the disparity we are attempting to remedy.
- The CURE needs to be within the scope of electives students can take, not an extra course that they must pay extra for on top of normal curricular offerings. It should reduce financial burdens for underrepresented students, not impose them.

Citations

Hartings, M.R., Fox, D.M., Miller, A.E., and Muratore, K.E. (2015). A Hybrid Integrated Laboratory and Inquiry-Based Research Experience: Replacing Traditional Laboratory Instruction with a Sustainable Student-Led Research Project. J. Chem. Educ. *92*, 1016–1023.

Hernandez, P.R., Schultz, P.W., Estrada, M., Woodcock, A., and Chance, R.C. (2013). Sustaining optimal motivation: A longitudinal analysis of interventions to broaden participation of underrepresented students in STEM. J. Educ. Psychol. *105*, 89–107.

Hernandez, P.R., Woodcock, A., Estrada, M., and Schultz, P.W. (2018). Undergraduate Research Experiences Broaden Diversity in the Scientific Workforce. Bioscience *68*, 204–211.

Next pages: Example syllabi and course assignments from CURE course implemented in the AU Chemistry department by Dr. Matthew R. Hartings.

American University Chemistry Advanced Laboratory Sequence

We offer a student-run research laboratory in the chemistry department at American University that is slightly different from other CUREs. This laboratory sequence runs two semesters. During the first semester, students perform experiments from previous research projects. This gives them a chance to engage with a research project and learn about its goals and about some advanced techniques and analyses. In the second semester, students design their own experiments, based on what they've done and learned in the first semester. The students are responsible for selecting materials and supplies, managing day-to-day laboratory activities, and making changes in their planning based on results. These second-semester projects are meant to break new ground in the scientific world. In the following academic year, the first semester projects are based off of the student-designed projects from the previous semester. In this way, we have designed a self-sustaining, continuing, student-run research program. The great benefit of this work is that we do publish experimental results, giving students academic authorship along with training.

To give an idea of how this process works, this document contains several syllabi and some second-semester student work from previous years.

First Semester Syllabus (CHEM 571)
Second Semester Syllabus (CHEM 572)
Sample Student Project Proposal
Sample Student Project Final Report

Syllabus
CHEM 571 Experimental Biological Chemistry
Fall 2014

Lab Sessions: Beeghly 102 [12:15-3:50 pm Tuesday and Wednesday]. The scheduled lab time is 11:45-3:50. But, we're budgeting in some time for you to grab lunch.

Laboratory sessions will be held primarily in 207. We may also be using equipment in 203B, 205, and 302. During some sessions we may meet for discussions in Beeghly 102.

The beginning of each session will include a brief introduction to the day's topic by me. Please do NOT be late.

Instructor: Dr. Matthew Hartings
Office: Beeghly 308
Lab: Beeghly 203B
Phone: (202) 885-1778
Email: hartings@american.edu

Office Hours: Tuesday and Friday 9 am until noon. (Note: you may have to track me down in my lab during this time)

Textbooks: There will be no textbooks used in this course.

Online notebooks: All of our lab protocols, data, safety sheets and other information will be kept online at: http://openwetware.org/wiki/AU_Biomaterials_Design_Lab Students are expected to keep an account with an active notebook on this site. Students are also expected to update and add to the content of this site. It is our goal that this is a living document that future classes can learn from, edit, and update as we learn more about the systems we are studying.

Attire: You are required to wear pants, closed-toe shoes, and a lab coat over your clothing.

Introduction to Experimental Biological Chemistry:

Traditional upper-level chemistry courses require students to follow some set of directions in order to learn the techniques/concepts of a given sub-discipline. In the experience of the faculty here at American, this approach can lead to a situation where students try to get the "right answer" at the end of a lab. Beside the fact that trying to get the "right answer" can distract students from the lessons that are meant to be learned, science is almost NEVER about finding some known "right answer". The fact of the matter is that science is about discovery, understanding, and certainty and not about trying to repeat someone else's work to prove your worth as a scientist.

Also of note is the fact that chemistry holds few professionals who can definitively classify themselves as being of only one sub-discipline (i.e. Biochemist, Physical Chemist, Organic Chemist, Inorganic Chemist, Analytical Chemist, etc). The lines

separating these disciplines have certainly blurred. To be a successful organic chemist, you certainly must be able to come up with new synthetic methodologies – and many times these methodologies involve catalyst development (often the realm of inorganic chemistry). You also have to be able to master analytical separations and purifications as well as perform physical measurements. To wit: chemistry done well means being a master of multiple disciplines at once and not individually mastering a single discipline. Our instruction at the university level should reflect this fact.

This laboratory course, CHEM 571 – Experimental Biological Chemistry, is more along the lines of what the chemistry faculty at American University believes chemistry instruction should be. CHEM 571 employs a problem-based learning approach whereby we expect our students to draw upon techniques from the multiple disciplines of chemistry. This course Labs will meet twice a week for four hours (eight hours total) and count for three academic credits. The increased amount of time (over classical lab courses) is meant to compensate for the fact that the students will be covering multiple techniques in a single day and is also meant to reflect the time-intensive nature of true chemistry research.

CHEM 571 is the first semester of a two semester laboratory sequence. In the overall sequence we will be relying heavily on techniques from biochemistry, analytical chemistry, quantitative analysis, and biophysical chemistry with forays into the worlds of inorganic and physical chemistry – as will be readily apparent farther along in the syllabus. The first semester will be mostly designed by the faculty members and will take a known set of experiments and tweak them in order to produce some new result. In terms of the course, this is our control experiment.

The second semester, from the faculty/department/school's point of view, is the most important semester. We are going to ask the students to design and carry out their own novel experiments based on the experiments that we performed during the first semester. We are, in essence, turning over the keys of the lab to the students. We want this lab to be driven by the curiosity and innovative thinking of our students. In turn, the first semester of the following year will begin where these students finish. This course will never be the same from year to year. But it will thrive and grow and develop and morph into something completely different as a result of our exceptional students.

Learning outcomes:

1) Use chemistry concepts to solve unknown problems
2) Obtain, evaluate, and present reference materials
3) Effectively evaluate, quantify, and present experimental data
4) Operate sophisticated scientific instrumentation and present analyzed data obtained from them

Specifics:

The lab in general will be centered on efforts to create novel biocompatible materials: i.e. new materials (nanoparticles/plastics/etc) that can easily interface with biological systems. The research could lead to any number of future uses for

these materials: plastics for band-aids, disease sensors, activated drug-release materials, etc. As stated earlier, the faculty are starting the students down this highway, they will choose the exit and byways to take from here.

In the first year, we focused on the production of biocompatible nanoparticles with gold salts and bovine serum albumin (Fall 2011). This synthesis proceeds by the protein unfolding, reducing the Au^{3+} to Au^{0}, and wrapping itself around the growing nanoparticle. During this first semester, the students discovered a novel material. We have submitted this research for publication. The work from the first semester led to the study of their stability and efforts to functionalize these nanoparticles (Spring 2012 – student led). In the second year we took off from the stability research and began the fall semester by studying the stability of nanoparticles produced with gold salts and multiple other proteins (horseradish peroxidase, lysozyme, and adenosine deaminase). In the second semester, students were interested in studying the enzymatic activity of adenosine deaminase on its own.

The goal for our semester is to determine whether or not the proteins in the protein-nanoparticle material are still functional. That is, when wrapped around the nanoparticle, do the proteins still behave as they are supposed to? To look into this, we will be studying several enzymes (horseradish peroxidase, pepsin, and adenosine deaminase) on their own. We will compare the results from these studies with experiments that look at the proteins after they are used to make nanoparticles and in the instance when they are just in the presence of other nanoparticles. I expect this research, if carried out well, will lead to a publication.

In terms of standard courses, this lab course will touch on the following:

Analytical	Biochemistry	Biophysical	Inorganic
UV-Vis	Enzyme analysis	Kinetics	Nanoparticles
Fluorescence	Protein stability	Thermodynamics	Electrochemistry
Atomic Absorption	Enzyme inhibition	Protein unfolding	
Data analysis	Electorphoresis	Stopped-Flow mixing	
HPLC			

All of our work will be recorded in an online notebook (http://openwetware.org/wiki/AU_Biomaterials_Design_Lab) as opposed to the standard paper notebook. This will allow the class to track each other's data and work collaboratively with one another on different projects. We expect the students to not only update their own entries, but to update and add entries for protocols, materials, and equipment. These entries will be used by future classes and are the bedrock foundation for the development of this lab. The entries, written and collaboratively refined, will display our students' abilities to fully understand and communicate the scientific concepts that are covered in the labs.

We have initiated a new mode of learning here at American. We want chemistry (and science in general) to be seen not as a set of techniques in search of a value, but as a mind-set one must take when searching for something unknown. Because this is truly student-run and student-driven research, the students will be rewarded not just simply with grades but, importantly, with publications, presentation experience,

and the ability to show industrial and graduate school recruiters that they have successfully led real-life problem solving exercises. It is our hope that this course becomes a beacon showing the talent and passion of the chemistry students here at American.

Academic Integrity Policy: The Academic Integrity Code (http://www1.american.edu/academics/integrity/code.htm) describes the standards of the code of conduct. Violations of this code will be met with the proper disciplinary action. Plagiarism and cheating are serious offenses in the academic world. All allegations will be referred to the Undergraduate Dean of the College of Arts and Sciences.

Tue., Aug. 27		Intro and Rachel Borchard (Lit searches/EndNote)
Wed., Aug. 28		AuNP synthesis w BSA and citrate
Tue., Sep. 3		UV-Vis of an unknown
Wed., Sep. 4		Fluorescence of an unknown
Tue., Sep. 10		UV-Vis of protein
Wed., Sep. 11		Atomic Absorbance with AuNP
Tue., Sep. 17		UV-Vis reduced/oxidized HRP
Wed., Sep. 18		Redox titration HRP
Tue., Sep. 24		Pepsin/Hb electrophoresis
Wed., Sep. 25		Pepsin/Hb electrophoresis
Tue., Oct. 1		HRP fluorescence turnover
Wed., Oct. 2		HRP fluorescence turnover
Tue., Oct. 8		ADA turnover UV-Vis
Wed., Oct. 9		ADA turnover HPLC
Tue., Oct. 15		ADA inhibition UV-Vis
Wed., Oct. 16		HRP AuNP synthesis **REPORTS DUE!!**
Tue., Oct. 22		HRP-AuNP fluorescence turnover
Wed., Oct. 23		HRP-AuNP fluorescence turnover
Tue., Oct. 29		HRP-AuNP redox titration
Wed., Oct. 30		HRP-AuNP redox titration/Pepsin-AuNP synthesis
Tue., Nov. 5		Pepsin-AuNP/Hb electrophoresis
Wed., Nov. 6		Pepsin-AuNP/Hb electrophoresis/ADA-AuNP synthesis
Tue., Nov. 12		ADA-AuNP turnover UV-Vis
Wed., Nov. 13		ADA-AuNP turnover UV-Vis
Tue., Nov. 19		ADA-AuNP turnover HPLC
Wed., Nov. 20		ADA-AuNP turnover HPLC
Tue., Nov. 26		Data Meeting
Wed., Nov. 27	**THANKSGIVING**	**No Classes**
Tue., Dec. 3		Data Redo
Wed., Dec. 4		Data Redo
Tue., Dec. 10		**Final Report Due**

First Report (Individual): **10%**
Are the references fitting?
Is the chemistry adequately described?
Are current and potential uses adequately described?
Are there any questions that remain?

In Class: (Individual) **70%**
Are safety procedures followed (being mindful of wearing goggles and jackets)? **4%**
Is appropriate effort being expended toward research goal? **4%**
Do the group members manage their time wisely? **4%**
Is proper technique being used? **4%**
Does the student arrive prepared for labwork? **4%**
Are experiments performed and analyzed correctly? **15%**
Are lab notebooks updated in a timely fashion? **15%**
Do lab notebooks contain proper information? **20%**

Final Written Project (Group) **20%**
Are the data and information clearly presented?
Is there anything omitted?
Was future work suggested?
Is it thorough?

Syllabus
CHEM 572 Advanced Chemistry Lab
Spring 2015

Course Meeting Times: Beeghly 207 [12:15am-3:50 pm Tuesdays and Wednesdays]

Instructor: Dr. Matthew Hartings
Office: Beeghly 308
Lab: Beeghly 203B
Phone: (202) 885-1778
Email: hartings@american.edu

Open WetWare: As in the previous semester, your lab notebooks are to be kept at openwetware.org

Grading: see attached rubric

Academic Integrity Policy: The Academic Integrity Code (http://www1.american.edu/academics/integrity/code.htm) describes the standards of the code of conduct. Violations of this code will be met with the proper disciplinary action. Plagiarism and cheating are serious offenses in the academic world. All allegations will be referred to the Undergraduate Dean of the College of Arts and Sciences.

This course (CHEM 572) is the natural extension of the course you took in the previous semester (CHEM 571). In the fall semester, you were initiated into a research project and learned some new techniques and syntheses as well as some general properties about gold nanoparticles. In the current semester, you will be asked to push this research project forward. Your interests and vision will ultimately craft where this research goes in the remainder of the semester as well as in future iterations of this class. The research project is yours. The department and your faculty are placing great trust and capital in your abilities to develop novel research and projects out of the foundation you have been given at AU.

During the semester you will be required to develop and write a research proposal and plan, perform your proposed research, and present your research in both a written and oral form. (For those of you who have taken the 581/582 laboratory series, this process should be familiar to you.) You will be graded on your ability to carry out these activities. The assessment of your performance is more clearly described later in this syllabus.

The first semester of this course centered around the synthesis of gold nanoparticles using enzymes as the reducing agent and stabilizer. You studied enzyme kinetics and observed how changes in gold:protein ratio affected the identity of the product. During the current semester, you must push this project forward. You will be graded on how well you make strides into reaching this objective: Are you making enough samples to test?; Are you using appropriate measures to test your samples?; Do you make reasonable adjustments to your procedures when your initial results come back poorly? These are all things that I will be looking for and working with you on as we go through the semester. My primary responsibility as faculty coordinator of this course is to enable and facilitate your research and to be a voice of guidance as you try new things and are forced to think differently about science.

The success of this course, and this department, is in many ways pegged on the research that you choose and perform. All of the faculty here, me in particular, are very excited to see what you come up with!

Tuesday Jan 13	Proposals
Wednesday Jan 14	Proposals
Tuesday Jan 20	Proposals
Wednesday Jan 21	Proposals
Tuesday Jan 27	**Full proposals due** Laboratory
Wednesday Jan 28	Laboratory
Tuesday Feb 3	Laboratory
Wednesday Feb 4	Laboratory
Tuesday Feb 10	Laboratory
Wednesday Feb 11	Laboratory
Tuesday Feb 17	Laboratory
Wednesday Feb 18	Laboratory
Tuesday Feb 24	Laboratory
Wednesday Feb 25	Laboratory
Tuesday Mar 3	Laboratory
Wednesday Mar 4	Laboratory
Tuesday Mar 10	**Spring Break**
Wednesday Mar 11	**Spring Break**
Tuesday Mar 17	Laboratory
Wednesday Mar 18	Laboratory
Tuesday Mar 24	**Individual Intro and Results due** Laboratory
Wednesday Mar 25	Laboratory
Tuesday Apr 31	Laboratory
Wednesday Apr 1	Laboratory
Tuesday Apr 7	Laboratory
Wednesday Apr 8	Laboratory
Tuesday Apr 14	Laboratory
Wednesday Apr 15	Laboratory
Tuesday Apr 21	Laboratory
Wednesday Apr 22	**Presentations and Final Paper due**

Proposal (Done as a team): **10%**
Is there a stated goal?
Is the goal a worthwhile target?
Is the goal attainable within a semester?
Is there a well-drawn plan to reach the goal?
Was there appropriate consultation with the faculty member in preparation of the proposal?

In Class: (Individual) **50%**
Are safety procedures followed (being mindful of wearing goggles and jackets)? **4%**
Is appropriate effort being expended toward research goal? **4%**
Do the group members manage their time wisely? **4%**
Is proper technique being used? **4%**
Are lab notebooks updated in a timely fashion? **10%**
Do lab notebooks contain proper information? **24%**

Writing Assignments: **20%**
Independent Writing: Results and Discussion (At half-way point) **8%**
- Are results described completely and in detail?
- Does the discussion use adequate and proper data analysis?
- Does the data analysis correspond to the actual data?
- Does the discussion make proper use of the literature?

Team Writing: Final Paper **12%**
- Introduction
  - Is the topic introduced well? Is scientific reasoning for the process developed? Is general interest in the project's importance developed? Is there proper use of the literature?
- Materials and Methods
  - Is this section written such that a novice could (or would know how to) repeat the experiments? Are the instruments and protocols described in appropriate detail (Note: this description should be as concise/detailed as in similar papers from literature)?
- Results and Discussion
  - See above description in independent writing.
- Conclusion
  - Are the experiments placed in their proper context and given broader interest? Is the future of the project used to deepen the interest of the reader?

Presentation (Group) **15%**
All of the points from the final paper must be presented orally in a manner that is engaging and informative to those in the audience.

Semester-Long **5%**
Do students expand upon their work from last semester? Do they use new techniques? Do they use new syntheses? Do the use new physical interpretations? How well do results match expectations?

Investigation of Protein-Mediated Gold Nanoparticles and Their Roles as Catalysts

Introduction

Colloidal metal nanoparticles have sparked interest as potential catalysts that offer a greener alternative to current inorganic catalysts used in industry. Gold nanoparticles (AuNPs) are of high interest because they have electrical and thermal conductivities, strong optical absorption, and good biocompatibility (1). Gold nanoparticles are easy to synthesize, surface modify, and characterize. AuNPs also have a low level of toxicity, are inert, and readily attach to biological molecules (2). Additionally, their ease of synthesis is rather advantageous. The many characteristics that colloidal gold embodies can be applied to electron microscopy, nanotechnology, electrons, and medicine (2). The catalytic activity of AuNPs is governed by the size, shape, and structure. The surface of the gold nanoparticle is modified by the addition of protein support. The protein is used as a reducing agent to gold and a capping ligand to prevent self-aggregation (1,3).

Alcohols are easily synthesized and easily transformed into other compounds, serving as important intermediates in the synthesis of organic compounds. The oxidation of an alcohol is one of many ways to synthesize carbonyl compounds that have various uses. Specifically, secondary alcohols can be oxidized to form ketones. This functional group transformation is an important organic synthesis reaction because ketones are used to synthesize various medications, perfumes, and other biochemicals used in industry (3). The oxidation reaction stops at the ketone synthesis because ketones are resistant to further oxidation without breaking carbon-carbon bonds.

In this study, we aim to investigate the catalytic properties of protein assisted gold nanoparticles in the oxidation of a secondary alcohol to a ketone. Previous studies have shown that the non-protein supported AuNPs are highly active and recyclable catalysts for the oxidation of an alcohol using hydrogen peroxide as an oxidizing agent (4). Though our aim is not focused on the recyclability of the catalyst, we are focusing on how the protein support can be advantageous in ketone synthesis and if the state of the AuNPs has an effect of the synthesis.

Polymerization reactions are vital in industry to produce plastics, fibers, elastomers and biosensors, among other applications. Some polymerizations can be carried out in the absence of catalysts but certain chemicals require the use of catalysts and strong oxidants which are harmful to the environment (5). One such reaction is the polymerization of polyaniline; this conductive organic polymer is generally synthesized employing strong oxidants, such as $(NH4)_2S_2O_8$, $K_2Cr_2O_7$, KIO_3 and metals in high oxidation states. A new method has been reported that is more environmentally friendly and makes use of gold nanoparticles as catalysts (5). This study would like to focus on how lysozyme-supported gold nanoparticles can affect the yield for this reaction.

This investigation also aims to explore the use of protein stabilized AuNPs as a catalyst for the oxidation of double bonds. The use of gold catalysts, and specifically AuNPs, have been studied in the past for their catalytic abilities when oxidizing styrene. This organic reaction has been used as a model for the assembly of many pharmaceuticals, high-value fine chemicals, and agrochemicals (6,7). AuNPs are found to be a very active catalyst, however if they are too small they become unstable and cannot maintain catalytic efficiency during long reactions, such as the oxidation of styrene (7). Many organic oxidations can take hours to complete and require a catalyst that can maintain proficiency (8). This

investigation will explore the effect the protein-assisted AuNPs have on the oxidation process of the double bond in styrene. AuNPs of varying size and shape as well as gold nanofibers will be tested. The investigation hopes to determine the most efficient catalytic conditions to produce the highest yield which could then be developed for industrial purposes.

Oxidation-reduction (Redox) and polymerization reactions play a vital role in biological and chemical processes. Most importantly, redox reactions are used in energy production and are utilized in living systems, such as the human body. This investigation examines how the presence of protein-assisted gold nanoparticles affect the synthesis of polyaniline, the oxidation of secondary alcohols into ketones, and the oxidation of styrene into styrene oxide. The reactivity of protein supported AuNPs in solution will be compared to the reactivity of protein supported gold nano-fibers. Monitoring the catalytic activity of the nanofibers and nanoparticles within the aforementioned reactions can assist in the research of the biocompatibility of AuNPs and their biomedical applications (9).

Statement of Work

This project aims to investigate the following reactions:

- Polyaniline synthesis
- Oxidation of secondary alcohols to ketones
- Oxidation of Styrene to Styrene Oxide

Within each reaction under study, there are some topics of interest.

- Yield of the reaction, in the presence of AuNPs and Au nanofiber catalysts.
- For a given yield, whether reaction conditions such as catalyst concentration, temperature and duration can be manipulated without harming the efficiency.
- Stability and durability of the product.

Hazards & Toxicity

Our research will utilize some chemicals, in particular organic solvents, which can be harmful to the human health. In addition to using appropriate laboratory equipment (lab coats, goggles, gloves), it is important to carry out the reactions under a ventilated hood.

Find below a comprehensive list of the chemicals and products that will be used and formed, respectively, along with their hazard level:

Formula	**Molecular weight (g/mol)**	**Melting point (ºC)**	**Boiling point (ºC)**	**Density at 25 ºC (g/cm^3)**	**Health**	**Flammability**	**Reactivity**

1-Methyl-2-pyrrolidone (C_5H_9NO)	93.13	-24	202 to 204	1.028	2	2	1
Acetone (C_3H_6O)	58.08	-95 to -93	56-57	0.791	1	3	0
Chloroauric Acid ($HAuCl_4$)	339.785	254	N/A	2.89	3	0	1
Lysozyme	14,307	N/A	N/A	N/A	0	1	1
Horseradish Peroxidase	44,000	N/A	N/A	N/A	2	1	0
Aniline ($C_6H_5NH_2$)	93.13	-6.3	184.13	1.02	3	2	0
Hydrochloric Acid (HCl)	36.46	-26	48	1.18	3	0	1
Hydrogen Peroxide (H_2O_2)	34.0147	-0.43	150.2	1.135	3	0	2
Diphenlymethanol ($C_{13}H_{12}O$)	184.23	69	298	1.103	2	1	0
Benzophenone ($C_{13}H_{10}O$)	182.22	48.5	305.4	1.11	1	1	1
Fluorenol ($C_{13}H_{10}O$)	182.22	152-155	367.5	1.151	0	0	0
Fluorenone ($C_{13}H_8O$)	180.20	83.5	342	1.13	1	1	0
Ethyl Acetate ($C_4H_8O_2$)	88.11	-83.6	77.1	0.897	1	3	0
Ethanol (C_2H_6O)	46.07	-114	78.4	0.789	2	3	0
Hexane (C_6H_{14})	86.18	-96 to -94	68.5-69.1	0.6548	2	3	0
Sodium Sulfate (Na_2SO_4)	142.04	884	1,429	2.664	1	0	0

Chloroform ($CHCl_3$)	119.38	-63.5	61.15	1.489	2	0	0
Diethyl Ether ($C_4H_{10}O$)	74.12	-116.3	34.6	0.7134	2	4	1
Styrene (C_8H_8)	104.152	-30.6	145.2	0.9015	2	3	2
Toluene (C_7H_8)	92.14	-95	111	0.8622	2	3	0
Dodecane ($C_{12}H_{26}$)	170.33	-10	214	0.7495	1	2	0
Styrene Epoxide (C_8H_8O)	120.15	-37	194	1.052			
Benzaldehyde (C_8H_8O)	106.12	-26	178.1	1.0415	3	2	1
Acetophenone (C_8H_8O)	120.15	20	202	1.028	1	2	0

Reactivity 1: Normally stable, but may become unstable at elevated temperatures.
Reactivity 2: Undergoes violent chemical change at elevated temperatures and pressures, reacts violently with water, or may form explosive mixtures with water.

Health 3: Short exposure could cause serious temporary or residual injury.

Personnel, Facilities & Experimental Overview

This experiment will be conducted by [redacted] junior and senior undergraduates and master students of chemistry at American University. Matthew Hartings, Assistant Professor of Chemistry at American University, will monitor the progress of our research. The protein supported gold nanoparticles will be quantified using UV/Vis spectroscopy and ICP-MS spectroscopy. The method for determining size and concentration was replicated from a previous study (9). The reactions to be studied are to be carried out in a nitrogen atmosphere under a well-ventilated hood, and the products will be purified and quantified using FT-IR,column chromatography, gas chromatography, and hydrogen and carbon NMR.

References

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Investigation of Gold Nanoparticles and Their Roles as Catalysts

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I. INTRODUCTION

Gold is considered a precious metal. It has many uses and great value in art, jewelry, and electronics, but more recently in chemistry as well. On its own, gold is chemically inert. However when formed into a nanoparticle, it takes on useful optical and electronic properties and is very biocompatible (1). These gold nanoparticles (AuNPs) have been produced in a colloidal solution for centuries due to their vibrant colors. More recently however, they have found many applications in drug delivery in biological and medical applications, electronic conductors, and therapeutic agents. Gold nanoparticles have also been showing promise as potential catalysts that would be a greener alternative to current industry standards (2). The optical and electrical properties are easily modified by changing the size, shape, surface chemistry, and aggregation state of the particles (2).

AuNPs are readily made by a variety of methods, most of which utilize chloroauric acid ($HAuCl_4$) and a form of surface modification, often being produced in a liquid, creating a colloidal solution (3). Essentially the chloroauric acid is reduced in solution. After dissolving and adding a reducing agent added, the Au^{3+} ions are reduced to neutral gold atoms. As more and more ions are reduced, the solution becomes supersaturated and precipitates into a nanoparticle (4). In order to stop the nanoparticles from total aggregation, a stabilizing agent must be used. Well known procedures for the synthesis of AuNPs include the Turkevich, Brust, and Martin. The Turkevich method involves the citrate synthesis of gold nanoparticles. A layer of adsorbed citrate anions on the surface of the nanoparticle keep the AuNPs separated, allowing for general size control (5). The less citrate anions present, the larger the nanoparticle that is formed. Depending on the size and shape of the particle, the solution can appear red, purple or even blue (4, 6). This reaction is easily confirmed by a drastic color change.

Another method of stabilization is the use of proteins. Common proteins used can include bovine serum albumin (BSA), lysozyme, and horseradish peroxidase. This synthesis utilizes heat and the protein, which acts as a reducing agent and a capping ligand that prevents self-aggregation. Using a protein allows for new applications in the fields of biotechnology and biomedicine. The protein can produce effects such as allowing easy transport across a cell membrane and easier binding with other biological molecules (7). Through the use of different proteins and methods, nanoparticles of various widths and shapes can be produced allowing for numerous applications in biological and organic chemical reactions. Lysozyme was chosen for its documented ability to form a colloidal solution as well as gold nanofibers.

The first type of reaction studied is ketone synthesis from a secondary alcohol. Alcohols are easily synthesized and modified within organic reactions. This ability to transform makes it an important intermediate in the synthesis of many different compounds. The oxidation of an alcohol can form many carbonyl compounds including a ketone from a secondary alcohol when the associated hydrogen atoms are removed.

Ketones have many applications in industry such as the synthesis of various medications, perfumes, and other biochemicals in industry (8). This study will focus on the oxidation of benzhydrol with hydrogen peroxide as the oxidizing agent to produce benzophenone. Previous studies have shown that AuNPs are very effective at catalyzing this reaction (9).

The second type of reaction studied is polymerization. Polymerization is the process of reacting a single unit monomer to form a chain or three-dimensional network of a repeating sequence. These types of reactions are vital in the production of plastics, fibers, biosensors, etc. (10). Some of these reactions have the ability to happen on their own in the presence of a strong oxidizer, but some require a catalyst to lower the oxidation energy. Typical oxidizing agents can include $(NH4)_2S_2O_8$, $K_2Cr_2O_7$, KIO_3 and metals in high oxidation states (10). Using the AuNPs, the study will explore the polymerization of aniline into polyaniline in the presence of H_2O_2 and HCl.

This investigation also aimed to explore the use of protein stabilized AuNPs as a catalyst for the oxidation of double bonds. The use of gold catalysts, and specifically AuNPs, have been studied in the past for their catalytic abilities when oxidizing styrene. This organic reaction has been used as a model for the assembly of many pharmaceuticals, high-value fine chemicals, and agrochemicals (11, 12). AuNP catalysts however are size sensitive. As the size of the nanoparticle increases, the catalytic efficiency decreases. Smaller AuNPs have a very high efficiency. Though useful, the small AuNPs are not stable enough to maintain efficiency during longer reactions. Many organic oxidations can take hours to complete and require a catalyst that can maintain proficiency (12). This investigation explored the effect the protein-assisted gold nanofibers have on the oxidation process of the double bond in styrene. The goal of the investigation was to determine the most efficient catalytic conditions to produce the highest yield which could then be developed for industrial purposes.

II. MATERIALS AND METHODS

Reagents

All chemicals were reagent grade and were used as received from different commercial sources without further purification. Benzhydrol (99% purity), aniline (99+% purity) and styrene (99.5% stab. with 4-tert-butylcatechol) were purchased from Alpha Aesar. Toluene (99+%) and chloroform-d were purchased from Sigma-Aldrich. Acetone, hexane and ethyl acetate were received from BDH. Methanol used was purchased from J.T.Baker.

Instrumentation and Methods

Absorption spectra were accomplished using Shimadzu UV-2550 spectrophotometer at wavelengths between 400 nm and 800 nm. NMR spectra were obtained using Brunker 400MHz NMR. Total ion chromatograms and mass spectra data were obtained using an Agilent 6890 N Network Gas Chromatograph equipped with a 5973 mass selective detector. Compounds were separated using HP-5MS 5% Phenyl Methyl Silox Capillary 15.0 m x 250 μm x 0.25 μm nominal Column.

The GCMS parameters for the analysis of benzhydrol and benzophenone are as follows:

The flow of He through the GC column was constant and set at 1.2ml/min. The

injector was operated in the splitless mode at 250°C and a split flow of 50 mL/ min. The oven was set at 80°C for 1 minute and ramped 30°C/min to 280°C and held for 10 minutes. The temperature of the transfer line was set at 280°C and the source temperature at 250°C.

For the analysis of aniline and its by-products the GC program was as follows. The initial temperature was 50□ and was held for 3 min and then ramped at 5□/min to 185□. Helium was used as the carrier gas at a flow rate of 2 mL/min and the temperatures of the injector, transfer line were set at 250□ while the ion source was set at 270□ (13).

For the analysis of Styrene Oxide, the method was as follows. The oven temperature was 130□, injector temperature of 175□, detector temperature of 200□. The carrier gas (helium) had a flow rate of 20mL/min. The detector gases were hydrogen (23 mL/min) and air (250 mL/min). The injection size was 1.0μL (14).

Citrate Synthesis of Gold Nanoparticles

90mL of water was placed in a 100mL volumetric flask with rapid stirring. 1mL of HAuCl4 (1% weight) solution was added and stirred for 1 minute, causing the solution to become yellow. 2mL of sodium citrate (38.8mM) was added and stirred for another minute. Finally 1mL of NaBH4 (0.075% weight) was added. As soon as the NaBH4 was added, the solution became a deep red or purple (15).

Protein-Assisted Synthesis of Colloid Solution

Solutions were prepared in a 10mL test tube. A ratio of 30:1 $HAuCl_4$ to lysozyme was maintained. For each 5mL sample, 2.095mL of Lysozyme (19.81μM), 0.5mL of $HAuCl_4$ (2.5mM), and 2.405mL H_2O were added. The samples were placed in an oven and heated at 80˚C for 4 hours. The colloid solution was a pink color. The samples were time sensitive and would begin aggregation after a week.

Protein-Assisted Synthesis of Gold Nanofibers

Solutions were prepared in a 10mL test tube. A ratio of 45:1 $HAuCl_4$ to lysozyme was maintained. Each sample contained 1.4mL Lysozyme (19.81μM), 0.5mL $HAuCl_4$ (2.5mM), and 3.1mL H_2O. The samples were placed in an oven and heated at 80˚C for 4 hours. The nanofibers were dark purple and the solution was clear. The samples were very stable and suitable for long term storage with no degradation.

Oxidation of Benzhydrol to Benzophenone

Benzhydrol (1 mmol), 30% hydrogen peroxide (2 mmol), 3 mL pure ethanol were transferred to a 50 mL double-neck, pear-bottom flask. Gold nanoparticle fibers (0.051 g) were added to the reaction flask and the reaction flask was attached to a reflux condenser. The apparatus was connected to a Schlenk line to remove oxygen, allowing the reaction to proceed under a nitrogen atmosphere. The reaction was heated and stirred at 75°C in an oil bath for 20 hours. Following, it was removed from heat, and excess water was added, while stirring continuously for an additional 2 hours. A white solid formed and was filtered then re-dissolved in ethyl acetate to remove the gold nanoparticles from the solution. Column chromatography was used to purify the product using hexane and

chloroform (80:20) as an eluent. A sample was prepared for GC-MS analysis using methanol as a solvent and another sample was prepared for NMR analysis using deuterated chloroform as the solvent.

Polymerization of Aniline

The experiment was performed in a round bottom flask. 0.511mL of aniline, 89 mL of distilled water, 3 mL of 1 M HCl, 7 mL of hydrogen peroxide and 21 mL of Au-Lys NPs, in that order, were added. Gold-lysozyme or gold-citrate nanoparticles, depending on the reaction, were added in a ratio of An/Au =1000 mmol/mmol. The reaction contents were gently stirred at room temperature for 24 hours under nitrogen atmosphere.

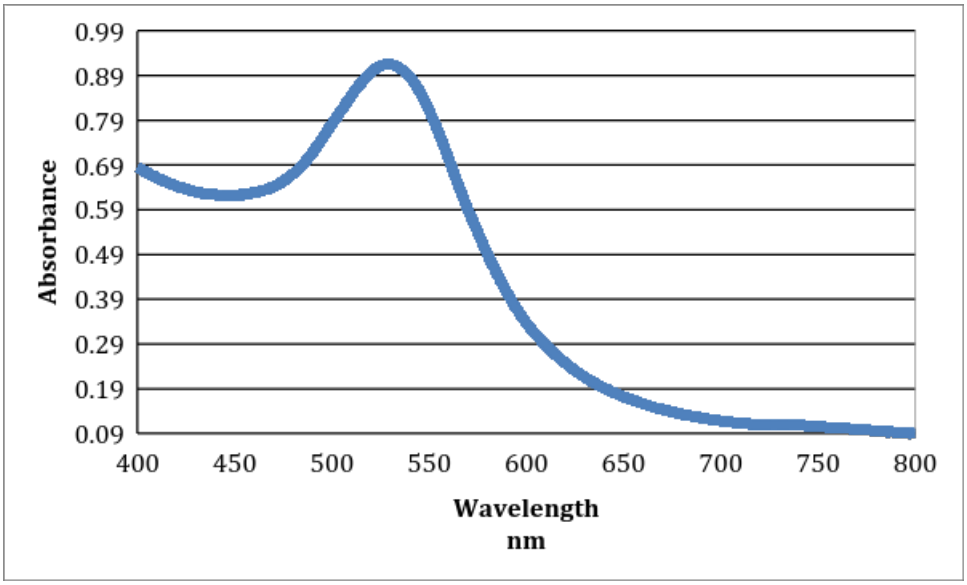
After 24 hours, the round bottom contents were filtered and washed with distilled water, followed by methanol. Each wash was saved for further analysis.

Oxidation of Styrene

This experiment was performed in a 25mL round bottom flask. 20mL of toluene and 0.6g of Au nanofibers were added to the flask. The flask was capped and placed in a mineral bath to be heated to 80˚C and kept for the duration of the reaction. 1.38mL of Styrene was injected into the flask and the reaction was gently stirred for 15 hours. The product was then analyzed with the GC-MS.

III. RESULTS

Characterization of Nanoparticles



UV Absorption Spectrum of Lysozyme Stabilized Gold Nanoparticles

Figure 1. The absorption spectrum above identifies the surface plasmon resonance peak of the Gold-Lysozyme nanoparticles at 529 nanometers having an absorbance of 0.915 (A_{529}).

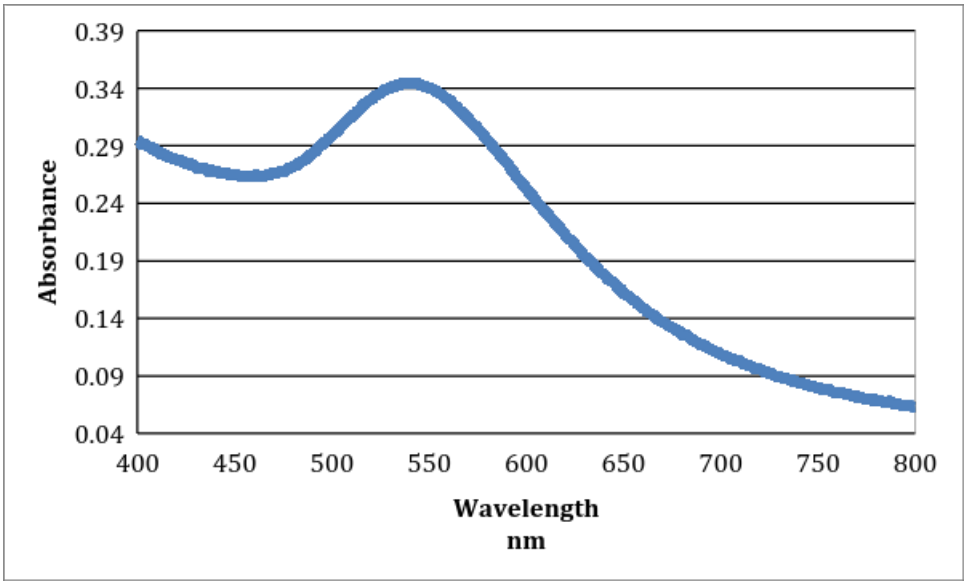


Figure 2. UV Absorption Spectrum of Citrate Stabilized Gold Nanoparticles
The absorbance spectrum for the gold citrate nanoparticles shows the surface plasmon resonance peak at 539 nanometers, where the absorbance is equal to 0.343 (A_{539}). The concentration of the nanoparticles was determined using Table 1 and Table 2, and was found to be 2.08×10^{-8} M.

Table1. The size of the uncoated gold nanoparticles in solution was determined using this table (Haiss, Thanh, Aveyard, and Fernig, 2007) from the UV/Vis spectra in Figures 1 and 2. The ratio of A_{spr} : A_{450} is used to determine the diameter.

A_{spr}/A_{450}	d / nm	A_{spr}/A_{450}	d / nm
1.10	3	1.56	12
1.19	4	1.61	14
1.27	5	1.65	16
1.33	6	1.69	18
1.38	7	1.73	20
1.42	8	1.80	25
1.46	9	1.86	30
1.50	10	1.92	35

Table 2. Using the size of the uncoated gold nanoparticles in solution and the molar absorptivity, the concentration was determined.

d / nm	ε_{450} / M^{-1} cm^{-1}	d / nm	ε_{450} / M^{-1} cm^{-1}
3	1.49E+06	7	2.03E+07
4	3.62E+06	8	3.07E+07
5	7.20E+06	9	4.43E+07
6	1.26E+07	10	6.15E+07

Table 3. Summary of the values used to determine the concentration of the nanoparticles.

Type of AuNPs	A_{spr}	A_{450}	Diameter	ε_{450}	Concentration
Citrate	0.343	0.262	6	1.26E+07	20.8 nM
Lysozyme	0.915	.622	9	4.43E+07	14.04 nM

IV. DISCUSSION

Oxidation of Benzhydrol to Benzophenone

The oxidation of benzhydrol was initially carried out using equivalent moles of benzhydrol and hydrogen peroxide, and 2 mL of lysozyme stabilized gold nanoparticles to catalyze the reaction. After 20 hours, excess water was added to the reaction flask and a white precipitate began to form instantly. After removal of the nanoparticles and purification, the reaction product was analyzed using NMR spectroscopy. In literature it was found that the proton NMR of benzhydrol has a quartet around 7.3 ppm, a two singlets at approximately 2.4 and 5.9 ppm. The ^{1}HNMR in Figure 3A shows a spectrum similar to benzhydrol where there is a multiplet between 7.3-7.6 ppm. The multiplet and other random chemical shifts at 0.9,1.6, and 2.2 ppm indicate that benzophenone did not form as a product and the sample is impure.

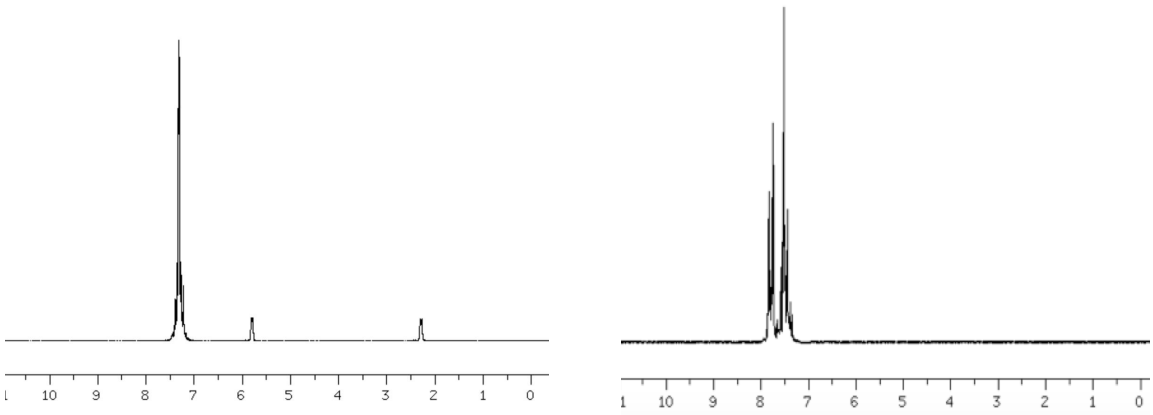


Figure 3. Actual NMR spectrum of benzhydrol in A and benzophenone spectrum expected after oxidation in B.

A second oxidation reaction was assembled using citrate stabilized gold nanoparticles and was analyzed using ^{1}HNMR and GCMS. The ^{1}HNMR spectrum in Figure 3B shows signals similar to benzophenone near 7.8 ppm but still display a multiplet near 7.3 ppm, comparable to benzhydrol. Based on the spectrum, benzhydrol started to convert into benzophenone, but the reaction did not complete. The mass spectrum of the purified "benzophenone" product confirmed that there was only benzhydrol in the sample.

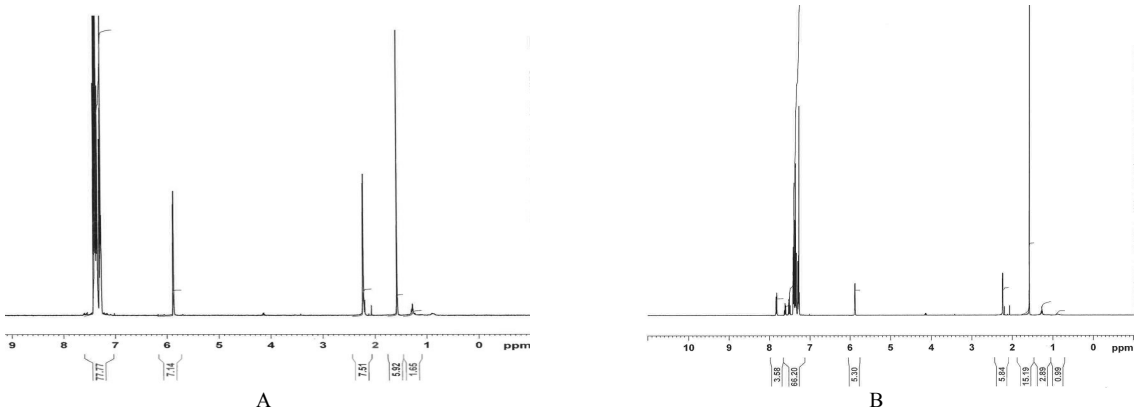
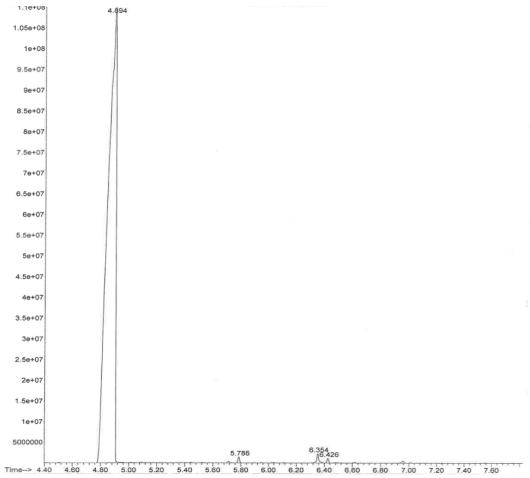


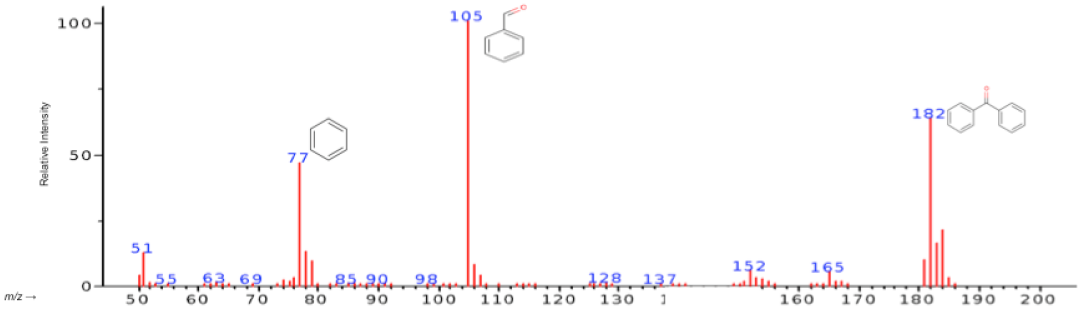
Figure 4. Proton NMR Spectrum of "Suspected Benzophenone" formed in oxidation of benzhydrol catalyzed by lysozyme stabilized gold nanoparticles in A and citrate stabilized gold nanoparticles in B.

The third benzhydrol oxidation reaction was carried out by increasing the molar concentration of hydrogen peroxide from 1 mmol to 2 mmol and using lysozyme stabilized gold nanofibers to catalyze the reaction. The mass spectrum of the purified sample indicated that benzophenone formed as a product but was masked by unreacted benzhydrol.



Total Ion Chromatogram of "Suspected Benzophenone"

Figure 5. The TIC of the sample shows a peak for benzhydrol at 4.894 minutes. There was not an integrated peak for benzophenone.



Mass Spectrum "Benzophenone"

Figure 6. The mass spectrum of the "suspected benzophenone" sample revealed that benzophenone formed as a product and eluted at 4.879 minutes. The ratio of unreacted benzhydrol to benzophenone was extremely high and it masked benzophenone.

Overall, the lysozyme and the citrate AuNPs are not efficient catalysts for the oxidation of benzhydrol. The gold concentration may potentially be too low to produce the catalytic effect needed for the reaction. Using the lysozyme stabilized AuNPs the reaction did not proceed, and while using the citrate AuNPs benzhydrol began to transform, but did not fully convert into the desired benzophenone product. The lysozyme and citrate AuNPS used are aqueous and may have a negative effect because the reaction is in an organic medium. Of the three experiments, the use of protein-gold nanofibers yielded a benzophenone. The nanofibers were dried making them more advantageous producing a product that has a 97% match to benzophenone.

Role of gold nanoparticle catalysts in the polymerization reaction of aniline

The polymerization of aniline was first carried out using gold-lysozyme nanoparticles with concentration of 11.59 nM. 21 mL of said nanoparticles were added to the reaction vessel, at which point the contents were pink/purple. After 24h, the solution had an orange/red colour and was filtered under vacuum to obtain the reaction's yield. The round bottom flask and filter paper were washed with distilled water, and this filtrate was kept for further study. The first few times the reaction was run, yield was so low (0.26%), that it was hypothesized that either gold-lysozyme nanoparticles are not conducive to the reaction, or most of the product is dissolved in solution. Following the wash with distilled water, a methanol wash was applied and the filtrate was saved for further study. The aim of this step was to recover the polyaniline and to remove any gold catalyst nanoparticles. The filtrates were allowed to air dry to remove any methanol solvent.

The dried filtrates were redissolved in methanol, and along with concentrated 99+ % aniline, they were spotted on a fluorescent reverse phase plate. The solvent used was methanol. Note that Figure 7 displays the spotted TLC plate for the polymerization of aniline using gold-lysozyme nanoparticles. The spot on the left corresponds to the first filtrate, while aniline is the right circle that travelled further up the plate when thin layer chromatography (TLC) was performed. Inspection of the TLC plates under UV light revealed no aniline present in the filtrate side. The left circle moved slightly upwards due to capillary action, suggesting the presence of a small amount of oligomers.

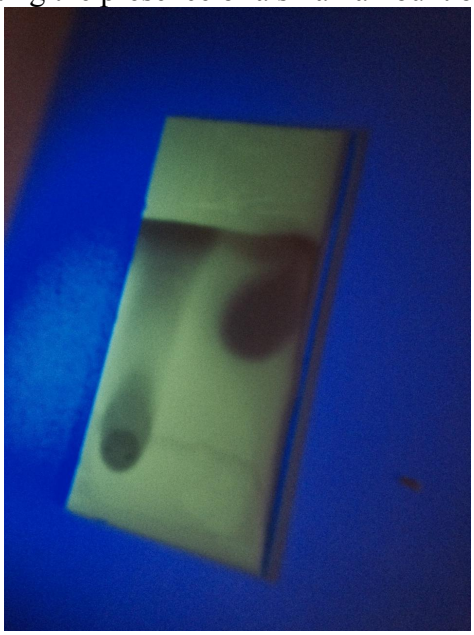


Figure 7. TLC plate of concentrated aniline (right) and first filtrate (left) for the reaction carried out with AU-Lys NPs

Another TLC plate was run with aniline and the second filtrate (allowed to dry and redissolved in methanol). Similarly to the first filtrate, no aniline was found, suggesting that oligomers form.

The polymerization of aniline was also studied using Gold-citrate nanoparticles. The same reaction conditions as for the gold-lysozyme reaction were followed, except that 14.28 mL of catalyst was added in order to respect the An:Au ratio of 1000:1. TLC was

run on the filtrate products washed with water and methanol. Similarly to the gold-lysozyme system, no aniline was observed in the products. A small presence of oligomers was seen as discrete bands.

GC-MS was used in an attempt to analyze the products of the reaction. Diluted samples of reconstituted filtrates redissolved in methanol, in addition to the starting material aniline, were prepared for analysis on the Agilent gas chromatograph. Figure 7 displays the mass spectrum for the dilute aniline sample. A 95% match to the NIST library was found, confirming that the starting reactant was aniline. Subsequent runs of the filtrate products were not as successful however. Small traces of aniline were found across the filtrates from the reactions carried out using citrate and lysozyme-supported gold nanoparticles, most importantly however, there were no identifiable fragment patterns that could be attributed to polyaniline.

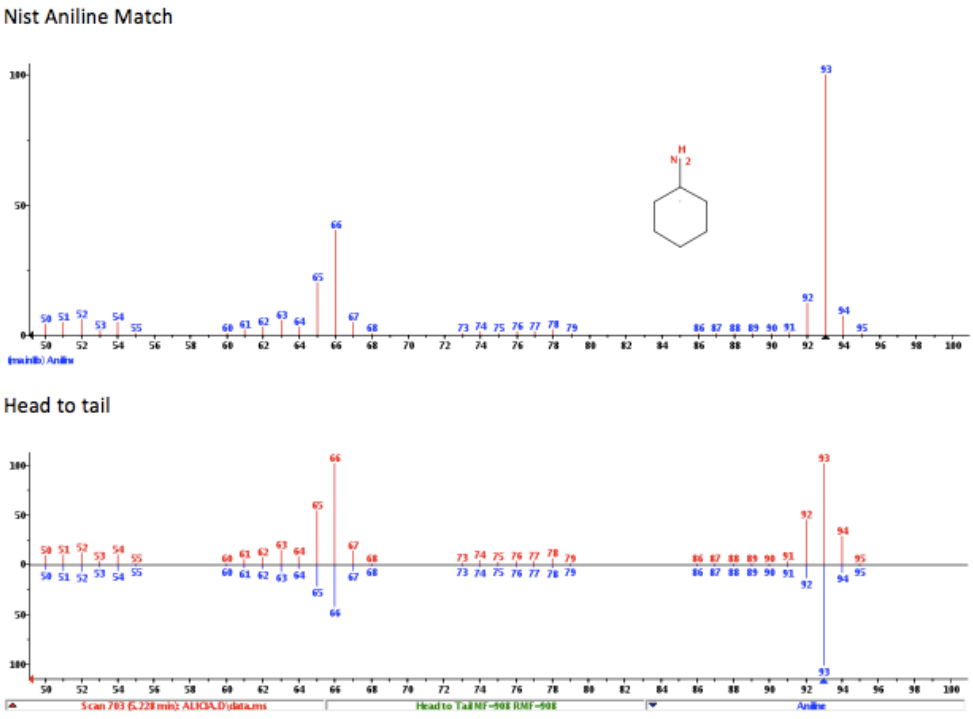


Figure 8. Mass spectrum of aniline

Oxidation of Styrene to Styrene Oxide

The oxidation of styrene has been a reaction of interest for many different fields of study. Studies have shown that there is a sharp size threshold on gold nanoparticles when

oxidizing styrene. When using 55-atom gold clusters supported on inert materials, Turner et. al found that if the nanoparticle exceeded a diameter of 2nm the nanoparticle would become completely inactive (12). However when using a catalyst that was stabilized by amino-modified porous polydiviylbenzene (PN), Zhang et. al reported catalysts of 20-150nm diameter and were extremely stable (11). The use of a protein modification should be feasible then.

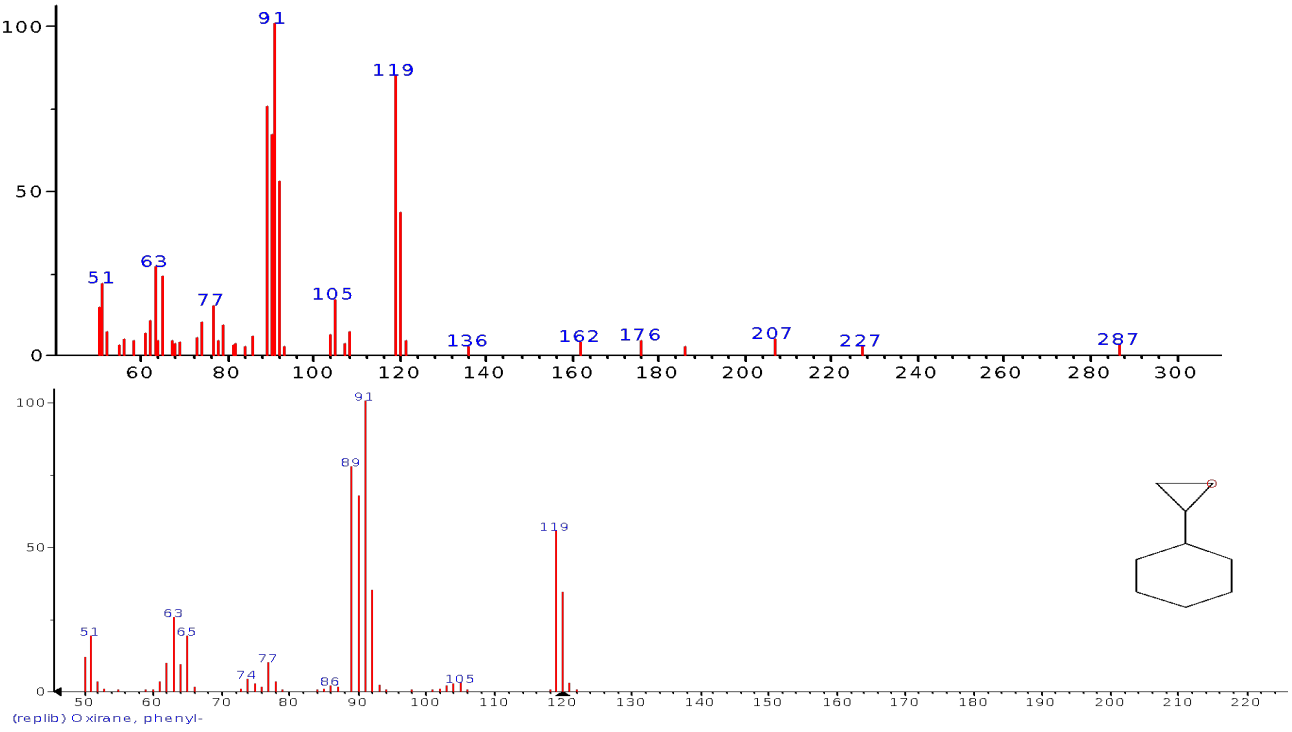


Figure 9: Mass Spectrum of Styrene Oxide

The reaction was tested using the GC-MS. The scan was able to confirm the presence of styrene oxide, which confirms a successful oxidation as seen in Figure 9. The peak for Styrene is at 104m/z and Styrene Oxide is at 119 m/z. Due to limited availability of the instrument, only qualitative testing was able to be conducted. Further testing using dodecane as an internal standard is required for accurate percent conversion (12). However when comparing the peak heights, the yield of the reaction appears to be good. Zhang et. al reported around a 30% conversion (11).

V. CONCLUSION

Protein stabilized gold nanofibers had the most success in catalyzing the reaction of benzhydrol to benzophenone. The nanofibers do not contain any water, which stops the reaction. Further experimentation of this specific reaction should include determining the optimum concentration of reactants to produce the highest yield, increasing the catalytic effect of the nanoparticles by increasing their concentration and to quantify the amount of benzophenone formed. Lastly, addition purification techniques should be added to ensure the quality of the sample.

In the reaction involving the oxidative polymerization of aniline, it was determined that GC-MS is not an adequate technique to identify or quantify the presence of polyaniline. More adequate techniques would be FTIR, UV-Vis spectroscopy or X-Ray diffraction. In previous studies Chen *et al.* have identified that supported gold catalyst, 0.5% Au/C and 1% Au/TiO2, provides better yields than unsupported gold nanoparticles, which have a short catalytic life (16). This was the premise for our investigations with protein-supported nanoparticles. It is unclear at this point in time whether proteins are adequate supports for the oxidative polymerization of aniline. It may be possible that the strong oxidizing environment degrades the protein surface, rendering the catalyst ineffective. This is however speculation, and further research would be necessary in order to analyze the reaction products. Additionally, it would be worthwhile to carry out the oxidative polymerization of aniline using a control catalyst, such as 1% Au/TiO2 which has been shown to provide good yields of 70% (16). Being able to reproduce such yield would give an insight as to whether the reaction setup is correct and give further insight into the problems encountered when using protein-supported nanoparticles.

For the oxidation of styrene, it has been confirmed through this study and previous ones that gold catalysts are successful in this reaction. The synthesis and end form of the gold greatly affects the yield and efficiency of the catalyst however. Based off of this study the styrene was oxidized, however it is unclear how much of that may be attributed to the gold nanofibers. Future work would include quantitative studies as to the effect and ideal dosage of gold nanofibers for the reaction.

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