Implementation of a Research-Based Lab Module in a High School Chemistry Curriculum: A Study of Classroom Dynamics

Matthew Pilarz

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By Matthew Pilarz

Entitled IMPLEMENTATION OF A RESEARCH-BASED LAB MODULE IN A HIGH
SCHOOL CHEMISTRY CURRICULUM: A STUDY OF CLASSROOM DYNAMICS

For the degree of Doctor of Philosophy

Is approved by the final examining committee:

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David A. Sears

Brenda M. Capobianco

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Approved by Major Professor(s): Gabriela C. Weaver

Approved by: R. E. Wild 9/30/2013
Head of the Graduate Program Date
IMPLEMENTATION OF A RESEARCH-BASED LAB MODULE IN A HIGH SCHOOL CHEMISTRY CURRICULUM: A STUDY OF CLASSROOM DYNAMICS

A Dissertation
Submitted to the Faculty
of
Purdue University
by
Matthew Pilarz

In Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

December 2013
Purdue University
West Lafayette, Indiana
To my parents, John and Barbara Pilarz, and to Rob Drennan
ACKNOWLEDGMENTS

The process of earning my doctorate has not been – by any stretch of the imagination – a solo effort. There are too many people to mention by name and recount all contributions for each singular act that has helped me get to this point. Thus, I would like to begin by simply saying “thank you” to everyone who has done something for me – no matter how small – during my time at Purdue. There are, however, those that have impacted my academic career and personal life over the past five years so greatly that I must mention them by name and elaborate on their contributions.

I would first like to thank my advisor, Gabriela Weaver. She has been a mentor, critic, and support. I truly appreciate that she could assume those roles as she guided me through this process. It means a lot to me that she has been able to push me and support me academically, but also was able to show compassion and see me as a real person and not just a graduate student.

Next I would like to thank my committee – Trevor Anderson, Brenda Capobianco, and David Sears. Without their time and commitment, this dissertation would never have been completed. I have talked with each of them about my work as I prepared this dissertation, and their feedback always gave me something that I could use to improve upon what I had done. I must also give a special thanks to Brenda for those
times along the way when we met for lunch so she could talk me “down off the ledge” so
to speak. So much for my plans of going off the grid!

I would like to extend a sincere thank you to the members of the Weaver Group –
both past and present – who have encouraged me and have given me feedback for each
and every practice talk over the past five years. Of those group members, there are some
people that I must mention specifically. Laura Nikstad literally recruited me to the group
and became my first mentor at Purdue. My research project would not have been
possible without her guidance and wisdom. Nicole Cook has also been a wonderful
friend and colleague. Although it sometimes seemed like we talked way too long on
some days, it was never wasted time. There was always something that each of us took
away from our conversations. However, I think that we talked about frameworks so
much that we could actually develop and publish a framework for our conversations.
Also, I have to thank Rebecca (Pritchard) Brayfield. I could not have asked for a more
dedicated undergraduate researcher. Rebecca did so much in helping with transcribing,
data analysis, and writing that I can’t possibly mention it all. I must say that she knows
the data for this project so well that she could have given any presentation that I have
given during the last two years of my dissertation work. Rebecca was willing to work
even when she was no longer part of the group and helped me in the eleventh hour as I
prepared my final chapters for this dissertation. If it was not for her efforts, it probably
would have taken me another year to finish this.

Now on to Gabriela Szteinberg. For four years Gaby sat at the desk next to me.
Although I didn’t pick up even one word of Spanish, I can honestly say that I learned
more from Gaby than anyone else in the past five years. I thank Gaby for being my
mentor, my friend, my “work wife,” and my sounding board. Even now that she is away, not a day goes by where we don’t email, text, or talk. I am forever grateful for all that she has done for me. Believe me when I say that it is way too much to chronicle here.

My research project would not have been possible without some amazing people in the chemistry department. Without the help of Jeanne Meyer I would not have been able to get all the materials I needed. In addition, Jeanne was always there to help me out through troubled times with labs. I also have to thank Bill Bayley and Zach Grigsby for getting my teachers involved with Science Express and providing the necessary equipment to the schools for this project. It was not just a help to me, but it enhanced the experience for the students and teachers involved. My research also would not have been possible without the help of Debbie Steffen. I have to thank Debbie for having the patience to teach me all about analytical chemistry. Debbie took care of running my samples in the HPLC at the onset of my project, and then trained me to run the machine myself. She went beyond the call of duty for her work with CASPiE with her dedication to my project. I am truly grateful for all that Debbie has done for me. And one thing I must mention, beyond her professional help, is that I loved the secret pact we had to always keep each other “in the loop” about anything that concerned the chemistry department.

Completing this program involved much more than academics and research. There were also those people who did not directly affect my research, but still, in some way were important parts of this process. As a graduate student, I was a TA for several semesters. I also had the privilege of being a lecturer for two summers. I have to thank the tremendous prep lab staff for all their hard work. I must mention Jeanne Meyer
again, because none of the labs my students completed would have run as smoothly as they did without her hard work. I also must thank Kurt Keyes for running such a tight ship in this department. Kurt does a fantastic job and is the consummate professional – that’s why the labs are as good as they are. And finally, I must thank Sheila Niccum. Besides the wonderful way she has worked to help my students and me, I could always count on her sunny greeting and smile. That made my day on so many occasions.

As for Marybeth Miller, there is way too much that I could say about what she has meant to me and done for me over the past five years. I have known Marybeth as a course supervisor and coordinator, as a resource in the department, and as a friend and confidante. Besides helping me through all my duties as TA, supervisor, and lecturer, Marybeth has helped me through every facet of graduate life. I thank Marybeth for all the times I went to her office for casual conversation, but more importantly for all those times I went to her office and closed the door and said, “I need to talk.” It is my good fortune to have met Marybeth and that a friendship beyond the Purdue campus has grown out of our professional work together.

One of the best experiences I had as a graduate student was lecturing CHM116 in my final two summers at Purdue. Part of what made that experience so great was working with Chris Hrycyna. Not only was Chris there to show me the ropes for teaching a course here and act as a mentor, but she also became a dear friend, who was a much-needed emotional support many times over. I am forever grateful that she is my friend, but even more grateful that much of the support I got from her was over a few martinis!
To Lindsay Kelderhouse, I have to say that I am thankful for our friendship – a friendship that literally began on day one of graduate school. It is so tough to keep friendships alive in graduate school once everyone gets into their research, but somehow Lindsay and I kept it going. The fond memories of home football games and nights out at the bars together will never be forgotten. Also, it was truly an honor to work beside Lindsay as officers for PLU and co-chairs of GSAB. Perhaps the best part of all of our times together is that no matter how bad things were for either of us, the times we spent together always were kept positive.

The most surprising part of my five years at Purdue was that I began swimming competitively again and met three of the greatest teammates anyone could imagine. I affectionately refer to these three fine men – Steve Chambers, Nick Glowicki, and Walter Glowicki – as My Swim Boyz. The time we spent swimming together on Purdue Swim Club was mentally and physically the healthiest period of my days at Purdue. My Swim Boyz pushed me to be the best in the pool and supported me in my academic career outside of the pool. I am both thankful and resentful that they treated me as a peer and never let me use my age as an excuse for anything. With Steve, I gained a second friend with whom I could engage in a conversation that was purely quotes from The Simpsons strung together. (Tim Hassall, who will be recognized later, is the first.) I also will always remember the swims when Steve and I would plant songs in each others heads during long sets – the more annoying the song, the better! As for Nick and Walter, I still am overwhelmed that I was taken in as part of their family. (A tremendous thank you also goes out to their parents Nick and Shelly.) Nick and Walter are my little brothers. I will always cherish the times spent together in and out of the pool. I really cannot
express how much of an impact these three gentlemen – My Swim Boyz – have had on my life and all because one fateful night we ended up swimming in the same lane together.

Another huge surprise for me was meeting my upstairs neighbor Scout. Scout was born and raised in the Midwest and I am pretty much the quintessential East Coaster. With nothing in common, it must have been fate that made us best friends. Scout soon became Kramer to my Seinfeld – often bursting into my living room unannounced and without knocking. He was always there to watch TV, listen to my problems, and share in a drink or - well let me not say how many! Having a friend with absolutely no connection to my life as a graduate student was something that I didn’t realize I needed until it was thrust upon me. When people ask about what I learned in graduate school, I will always have to include my new knowledge of handguns, rifles, and crossbows – all thanks to Scout.

Another pleasant surprise that came my way at Purdue was becoming friends with Josh Schmidt. So many people have asked me how Josh and I became friends. I thought about it and the best response I could come up with was, “It just happened. And I’m really glad it did.” Josh came into my life during a really rough time and he was there for me through many meltdowns. (I offer a warning to everyone: one “bro-hug” can lead to a pretty serious friendship.) Josh had corrected me on several occasions when I would talk about times when I felt I was really lucky, so I won’t make that mistake here. Instead, I will say that I am truly blessed that Josh is my friend.

There are also many friends from back home that I must thank. One such person is my dear friend Candice Conn. Candice is a true fashionista and I have enjoyed every
single conversation about fashion that we have shared over the years. However, Candice is also someone who was there to help me through this entire experience. She has been there as a friend and as someone I could consult with for professional advice from the time I was researching graduate programs all the way through to the final stages of this dissertation.

Another person from home that has been a tremendous help to me is Vicki Sachetta. She has been a mentor, friend, psychologist, and perhaps my biggest fan. With every trip back home, I always looked forward to the time we would spend together, especially when Kathy Baldyga joined us. When I think of the many years Vicki and I taught together and became part of each others’ families, I have to say this: The perfect pairing for good wine is good friends!

Other visits home were made special by time spent with Michele, Tim, Danny, and Jack Hassall. Even when I was 700 miles away, I always felt like they were here with me. Their phone calls, texts, emails, and photos always encouraged me and put a smile on my face. I owe them a very special debt of gratitude for including me as part of their happy family and for their endless support and encouragement over these past five years.

One last friend from home that I need to thank is Sunitha Vege. Although we joke that we have a “friendship about nothing,” that couldn’t be further from the truth. Whether it was an hour-long phone conversation while apart or a walk around Philadelphia on my visits home, she has always been there when I needed her. Although I think it was spiteful on her part that she moved to Brooklyn, NY soon after I moved to West Lafayette, IN, I can’t be mad at someone who has been such an important part of
my life. Plus, spending time with Sunitha back home meant that I could also spend time with Hannah Kim. Hannah has also been a great friend and an inspiration, as our career paths have been very similar. (It is strange that she is a few years younger than me, but always a few steps ahead of me in leaving high school teaching and getting a Ph.D., but I can deal with that.) But the truth is that I have to be nice to Hannah Kim and thank her, because she still hasn’t forgiven me for stealing Sunitha away from her as my friend.

Now I must take this opportunity to thank my family for their unconditional love and support. I first would like to thank my extended Dallas family – Glenda and Jay Allen, Nancy Blakely, Mark and Cynthia Drennan, and Margie Drennan. Their hospitality on my visits and genuine interest in my studies has always made me feel loved. I also need to thank Margie for all the talks we have had (that no one knew about except the two of us) and the happy hours we spent together when I played the part of her personal bartender. Again I must say, I am truly blessed.

To my siblings – John, Mary-Lou, and Jennifer – I have to thank them from the bottom of my heart for every phone call and email along the way. Not every group of brothers and sisters has the relationship that the four of us share, and I am forever grateful for everything each of them has done for me throughout my time at Purdue. Of course, their spouses – Kathleen, Eric, and Michael – have been there for me as well. And, it should be obvious, but let me please mention, that I am thankful for the joy that all my nieces and nephews have brought to my life over the years – especially during these past five years. I wish we could have seen each other more often, but those holidays when we were all together were very special and gave me encouragement to continue working to reach my goal of getting my doctorate.
I would also like to thank Tosia Schmidt – the most fabulous Aunt in the world. For five years I have had to live vicariously through her as she spent time at the Jersey Shore. That has served as one of my main incentives for finishing my dissertation and making my way back home so I could go “down the shore” where I belong. My Aunt Tosia’s constant encouragement and love has been a guiding force my entire life, but even more so during my time at Purdue.

In addition, I need to thank my Grandmother, Helen Pilarz. Although she did not live to see me through to the end of my graduate career, she is still with me. With every trip home since I moved away, her welcoming smile warmed my heart and gave me a special feeling that only she could give. My Grandmother always told me how proud she was of me. That isn’t surprising to hear from a grandparent. However, after her funeral in 2012, I was stunned when so many distant relatives talked to me and told me how much my grandmother had told them about my work towards earning a doctoral degree. I heard the words, “she was so proud of you” from so many people it was overwhelming. My Grandmother was – and continues to be – an inspiration in my life.

To my parents, John and Barbara, I will be brief and simply thank them for their constant love and support. This dissertation is dedicated to them because they are two very special people who have always believed in me. I am truly blessed to have them as my parents.

Finally, I have to thank Rob Drennan. Rob shares the distinction with my parents of having this work dedicated to him. He is the love of my life and deserving of much more than a mere dedication. I find it impossible to compose a proper thank you to the person who told me that I should never sell myself short. When I was initially looking
into graduate programs he told me to find the best one possible and go if I got in, no matter where it was, because we could make it work. Not everyone is as fortunate as I am to have someone willing to live apart for so many years and make the sacrifices and adjustments along the way that he has made. Throughout this journey, Rob has been nothing but supportive and has told me how proud he is of me for each of my accomplishments, no matter how small. Rob has always wanted the best for me. It is obvious to me that since December of 1995, I’ve had the best. I would not have been able to complete this program without him, nor would I have wanted to do this without him. One final time, I must say this – I am truly blessed.
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ABSTRACT

Pilarz, Matthew. Ph.D., Purdue University, December 2013. Implementation of a Research-Based Lab Module in a High School Chemistry Curriculum: A Study of Classroom Dynamics. Major Professor: Gabriela C. Weaver.

For this study, a research-based lab module was implemented in two high school chemistry classes for the purpose of examining classroom dynamics throughout the process of students completing the module. A research-based lab module developed for use in undergraduate laboratories by the Center for Authentic Science Practice in Education (CASPiE) was modified and implemented in two high school settings. This module consisted of four phases: Skill Building, Experimental Design, Independent Research, and Results and Poster Presentation. Classroom dynamics were studied by considering the students’ and teachers’ perceptions of their experiences during the completion of the module and by examining the interactions between students and teachers that took place throughout the module. The results reveal that there are shifts in classroom dynamics throughout the four phases of the module. In the Skill Building phase there was a great deal of dependence on the teacher for help in completing tasks. However, there is a slight contrast to what the students and teachers reported about their experiences during this phase. The teachers describe the students as being very dependent on them and asking questions constantly during the Skill Building experiments. The students report that they tried to figure out their problems with their lab
partners and students in other lab groups before asking the teacher for help. The teachers perceived that students came to them immediately for help and did not realize that students were coming to them as sort of a last resort when they could not solve problems on their own. In the Experimental Design phase the students and teachers both report that the lab groups were working together as groups to design their experiments, and rarely had interactions with anyone outside of their lab group. For the Independent Research phase both students and teachers report that lab groups worked very independently of any outside assistance and that they began to use a division of labor strategy within their group to complete tasks. This also is the case for the Results and Poster Presentation phase of the module.

In examination of the student-student and student-teacher interactions, a comparison is made between the Skill Building and Independent Research phases of the module. During the Skill Building phase, students tend to be less confident in their work and their lab partners work as compared to the Independent Research phase. Lab groups also tended to be more dependent on seeking help from outside of their lab group when completing experiments in the Skill Building phase as compared to the Independent Research phase. One finding that contrasts these is that students are dependent on their teacher for help when completing data analysis calculations. The overall results show that classroom dynamics shift throughout the completion of a research-based lab module and that a community develops in the classroom that mirrors the scientific community.
CHAPTER ONE: INTRODUCTION

The call for students to have more authentic science experiences is not new. Perhaps the most notable source of the need for these reforms has come from the National Research Council (NRC) in their publication of the National Science Education Standards (NSES) in 1996. One major reform proposed in the NSES was the recommendation to provide students more authentic science experiences in their K-12 science classes (NRC, 1996). More recently, the NRC published A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas (2012), which addresses the ideas, skills, and practices that should be integral to every student’s K-12 science experience. A key feature of the framework is the list of the eight features that should be addressed in K-12 science curriculum. These features are listed in Table 1.1.

Table 1.1. Practices for K-12 Science Classrooms

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<td>Asking questions</td>
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(NRC, 2012, p. 42)
For the study presented in this dissertation, a research-based lab module that encompasses each of these eight features was implemented into a high school chemistry curriculum. The research-based lab module used in this study was developed by the Center for Authentic Science Practice in Education (CASPiE). CASPiE was developed through a grant from the National Science Foundation (NSF, CHE-0418902) to provide undergraduate students research-based lab experiences embedded in their first and second year chemistry courses (Weaver et al., 2006). CASPiE has developed several research-based lab modules and, although developed for use in undergraduate labs, each of the modules provides an opportunity for students to engage in the eight practices listed in Table 1 as essential for K-12 science classrooms. In each module, background is presented in the area of research that is the focus of the module and laboratory techniques and protocols are practiced as the first part of the module. Students then use that background information and lab experience to design their own experiments for their own research. This gives students the opportunity to formulate questions (Feature 1) and develop models (Feature 2) as they plan out their research experiments. Students then write a complete procedure and carry out the experiments for their project (Feature 3). Their experiments must involve the collection of data which is then to be analyzed and interpreted (Feature 4) using the appropriate mathematical and computational techniques (Feature 5). To complete their research project, they must present their result in the form of a poster that would be acceptable for a scientific meeting. This involves constructing explanations for their results and conclusions (Feature 6), engaging in argument from the evidence of their experiments (Feature 7) – which may be within their own lab group as they prepare their final poster or with peers when presenting their poster – and then
ultimately communicating their results (Feature 8) to their peers by presenting posters about their independent research projects. CASPiE modules therefore provide the opportunity for students to engage in all eight key features described by the NRC (2012), making it a viable option for high school science curriculum. The description of the particular module implemented for this study and how it was made accessible for high school students will be presented in Chapter Three.

Although this is an original CASPiE study, this study is not the first research conducted using CASPiE in high schools. Previously, two thesis projects have been completed using a modified CASPiE module in high school settings. Nikstad (2009) explored the effects of CASPiE on students’ mental models of school science and professional science. Kingery (2012) examined students’ level of confidence in completing the CASPiE module and gender differences during the CASPiE experience. Both of these studies looked at some type of effect of CASPiE making comparisons of the students before and after they complete the CASPiE module. With this study, I have examined the classroom as a whole, the groups within that classroom, as well as individuals as the module is being completed. I consider the reality that students do not have their classroom experiences in a vacuum. In every classroom, a community develops through interactions that occur among students and teachers and the roles they play during daily class activities. The relationships that develop within the classroom community play an important part in students’ learning experiences. For this study, a research-based lab module was implemented in two high school chemistry classes to study the effects on the classroom dynamics during the completion of the module. The two settings are different from each other in that one setting is a large urban high school,
with large class sizes, and a teacher with very little science background and experience, whereas the other is a small, rural school with small class sizes, and a teacher with an extensive chemistry background and several years of teaching experience. Thus the two settings and groups of participants for this study, which will be described in greater detail in Chapter Three, are quite different. What I present in this volume are the effects of CASPiE on classroom dynamics and the classroom environment as students and teachers progress through the various stages of the module.

Purpose of the Study

CASPiE has been implemented in seventeen undergraduate programs in universities and two-year colleges in the United States and Australia. An extensive external evaluation of CASPiE at these participating undergraduate institutions has been published and contains the evaluation of the CASPiE implementations as well as the results of the impact of CASPiE on participating students (Scantlebury, Li, & Woodruff, 2011). Individual studies on undergraduate experiences in CASPiE include focusing on content, attitudes, and understanding of the nature of science (NOS) (Russell, 2008), comparing the student experiences of students who completed traditional versus research-based lab experiments (Green, 2010) and the longitudinal effects of CASPiE on participating students (Szteinberg, 2012). There have also been two implementations of the modified lab module used in this study in two previous high school studies. These studies explored students’ perceptions of school science and professional science (Nikstad, 2009) and gender and efficacy effects of CASPiE (Kingery, 2012). The purpose of this study – and what sets it apart from previous research – is that the goal is
to find out what is happening in terms of classroom dynamics and students’ and teachers’ perceptions of their experience in an authentic science context. The research conducted for this study takes a more sociological point of view as to what is happening in the classroom during a CASPiE experience than previous studies.

Significance of the Study

The significance of this study goes beyond just providing students with the opportunity to experience authentic science, in that it strives to understand how they experience it. By examining classroom dynamics, the way in which students work within their lab group and within the community of the classroom will be revealed. Unlike papers that have been published with tips on how to shift traditional classrooms into inquiry-based classrooms (Corder & Slykhuis, 2011) or strategies and guidelines for implementation of inquiry activities (Jarrett, 1997; Bruck & Towns, 2009), this study will provide a first-hand account of students’ and teachers’ experiences in completing a research-based lab module. This will provide insight into the types of resources students rely on as they work through the module and why they use those resources. Finding out the experience from the perspectives of both students and teachers will provide a clear picture of the classroom during an authentic science experience. Gaining this understanding of what is actually going on in the classroom to complete the module can prove useful to understanding how to provide appropriate resources and classroom environments that would be most conducive to this type of learning experience. The results presented from this study could prove helpful for educators who are interested in implementing authentic science experiences into their curriculum because they can gain
insight into how other students have experienced authentic science practice, as well as how teachers have prepared themselves for the authentic science experience in their classroom. This information should not be specific to a CASPiE implementation, but could apply to any type of inquiry activity that provides some or all of the key features of the science classroom as described by the NRC (2012) K-12 Framework.

Research Questions

The overarching theme of the research questions that guided this study is the impact on the classroom environment of carrying out the CASPiE module on the classroom environment. The research questions were designed to explore the classroom dynamics throughout the module and elucidate both students’ and teachers’ perceptions of the module experience. The research questions are divided into two subsets that each have a different focus. The first set of questions seeks to gain insight into students’ and teachers’ perceptions of their experiences throughout the module:

1. What are students’ perceptions of their experiences completing a research-based lab module?
2. What are teachers’ perceptions of the student experiences during the completion of a research-based lab module?
3. What are teachers’ perceptions of their experiences in the classroom during the completion of a research-based lab module?

The second set of research questions seek to explore and compare classroom dynamics during lab phases of the module:
4. What, if any, differences are there in how a group of students performs an independent research-based lab as compared to a traditional lab experiment?

5. What, if any, differences are there in student-student and student-teacher interactions among students from different lab groups in a research-based lab as compared to a traditional lab experiment?

6. What, if any, differences are there in student-student and student-teacher interactions in a research-based lab as compared to a traditional lab experiment?

Role of the Researcher

To conduct the research presented in this dissertation, I worked very closely with the cooperating teachers. This included facilitating the professional development workshops to prepare them for their implementation of the CASPiE module, providing reagents and certain pieces of necessary lab equipment, planning out a calendar for the module implementation, and serving as a resource throughout the implementation.

I also was present in classroom taking field notes. This process put me, at times, in direct contact with the students. Like the classroom teacher, I was available as a resource for the students as they completed the module. Thus, I became part of the classroom dynamics studied in this research.
Perceptivity and Bias

The perceptivity that I have in this research stems from two distinct areas of experience. I have the experience of being a high school chemistry teacher for over ten years. I have served as a teaching assistant for an undergraduate chemistry course that completed the CASPiE module, which was modified and used for this research. These combine to give me certain perceptions about the high school chemistry lab environment and experience and well as perception about the completion of the module. The aim of this research is to explore and report the classroom dynamics throughout the module and to report the students’ and teachers’ perceptions of their experiences. The purpose of this is to find out what is going on within the classroom to gain an understanding of how the module is completed by the students. This information can help to make for better implementations of modules such as this in the future. I therefore offer that my perceptions have not acted as a bias towards the collection and analysis of data since I am as interested in the negative effects and perceptions as I am the positive ones. In addition, I have worked with other researchers throughout the process of data analysis and presentation of results including interrater reliability checks with coded data.
CHAPTER TWO: LITERATURE BACKGROUND

In this chapter I will present the most relevant background literature to establish how this study of classroom dynamics situates itself in the established published research. I will first discuss the significance of the laboratory in science education and describe the importance of providing more authentic experiences in the science classroom. This will lead into a discussion of cognitive apprenticeship and cooperative learning environments in the laboratory classroom. I will then discuss the research that has been done with respect to the culture that develops in the classroom and how the interactions that occur within the classroom community contribute to the students' learning environment. This will lead into presenting previous findings from CASPiE studies and a discussion of how the CASPiE model promotes situated cognition and a collaborative learning environment. This chapter will culminate with a summary of the background literature that will support how this study of classroom dynamics will be a relevant addition to science education research.

The Laboratory in Science Education

It has long been recognized that the laboratory plays a vital role in the chemistry classroom (Hofstein & Lunetta, 1982). However, research has shown that there are many shortcomings to the actual role the laboratory plays in the chemistry classroom.
One major shortcoming that has been identified and researched is that school science does not provide an accurate depiction of the actual scientific process. This shortcoming can be viewed in comparing the context of school and actual scientific research as well as the types of experiments that are performed in each setting. In consideration of context, Gaskell (1992) points out that there is a clear distinction between authentic science and school science. He describes the difference between school science that is taught in the context of the class curriculum and school environment and the practice of real science as it applies to everyday life and is practiced in the context of social and political influences. Thus he concludes that school science needs to become more authentic to reflect actual science practices so that students will gain a perspective of actual future careers in science (Gaskell, 1992). The other facet distinguishing school science from actual science practices is the contrast in the types of experiments performed. Most science classes use traditional “cookbook” labs in which students are given a set of procedures and work towards a known outcome. Research scientists perform experiments they create based on prior knowledge and experience as well as consideration of the most current research in that area. Thus, science classes that use the “cookbook” labs are misrepresenting the scientific enterprise and students are not given a true picture or experience of real scientific work (Hodson, 1996; Hodson, 1998).

In an effort to improve the lack of authentic scientific experiences in the classroom, the NRC published the NSES (NRC, 1996) and, more recently, a K-12 framework for science education practices (NRC, 2012). The NSES standards include the use of more inquiry activities in an effort to model the scientific enterprise so that students could gain a better understanding of the nature of science (NOS) (NRC, 1996).
The K-12 framework describes the best practices that should be integrated into all K-12 science classrooms to provide a more authentic and engaging learning environment in science classes.

Although improved understanding of NOS may have been the major goal, research has shown that there have been other benefits with the implementation of inquiry activities. For example, in the studies by Hofstein, Shore, and Kipnis (2004) and Hofstein, Navon, Kipnis, and Mamlok-Naaman (2005), inquiry-type experiments were implemented into high school classrooms with eleventh and twelfth graders. Teachers who participated in this were given extensive professional development to prepare them for this implementation. Students performed pre-inquiry activities in which they learned about various lab equipment and techniques before designing and executing their own inquiry activities. The results showed that students’ abilities in inquiry activities improved significantly over time. These students engaged in more peer discussions throughout the inquiry process and developed more quantitative questions and more scientifically in-depth questions as they progressed through the inquiry experiments (Hofstein et al., 2004; Hofstein et al., 2005).

Although inquiry labs allow students to design and perform experiments, they do not reflect the scientific process as well as research-based, or authentic, labs. The distinction between the two is that inquiry labs have the purpose of simulating the discovery process for the student, though the experiment itself may have a known outcome. On the other hand, research-based labs involve actual scientific research and engage students in designing and executing novel experiments (Weaver, Russell, & Wink, 2008). The CASPiE model was designed to scaffold these types of research-based
labs. A section that contains a more in-depth discussion of CASPiE research will be presented further on in this chapter. However, in considering the research-based laboratory setting, I would like to mention briefly that noteworthy findings in the implementation of research-based labs (specifically CASPiE) include: increased interest in the subject, better understanding of the connections between research and real-life applications, and improved perceptions of the understanding of the content (Scantlebury, Li, & Woodruff, 2011).

Research-based and authentic science context studies other than CASPiE have reported positive findings as well. Roth and Roychoudhury (1993) reported improvement in process skills that did not need to be explicitly taught separately from the lab. This study was done in an open-inquiry setting and reports that the key aspect to the development of the laboratory process skills is that they were practiced in an authentic and meaningful context (Roth & Roychowdhury, 1993). A study by O’Neill and Polman (2004) involved students that worked in an authentic scientific environment in which they were involved in the process of formulating research questions, data collection techniques, and analysis strategies for scientific investigations for their class. They report two major findings: 1. students gained agency over their project by having an active role in the development of the investigations; and 2. students developed practice-based science literacy skills that include the ability to write researchable questions, manipulate data, construct scientific arguments, and understand the process of the development of scientific knowledge.

Another positive effect observed in the research-based laboratory classroom is more student engagement in the lab and a better understanding of the goals and content of
the labs as compared to students in traditional lab settings (Cacciatore & Sevian, 2009). In the quantitative results of their study, students completing research-based laboratories scored statistically significantly higher than the control group students in a traditional laboratory in the areas of lab skills and stoichiometry problem solving skills (Cacciatore & Sevian, 2009). The qualitative results of this study will be discussed later in this chapter.

In review of the literature related to the significance of the laboratory in science education, there seems to be a consensus that the laboratory is an important and necessary part of science education, but is not always put into practice such that it provides the most meaningful experience students. Recommendations and frameworks have been published by the NRC to encourage and facilitate reforms in science classrooms. These reforms were written to guide science educators towards creating science curricula that could provide more authentic context for science experiments and a more accurate depiction of the scientific enterprise. Research has shown many positive effects when students engage in authentic science practices that are more representative of actual research practices and the scientific enterprise. Since the CASPiE model is based on providing the tools to create a research-based laboratory experience, it is a valid setting for this research project conducted in high school chemistry classrooms.

The Cognitive Apprenticeship Environment

As discussed above, the laboratory does hold an important place in the science classroom, but the types of labs that are being done by students vary greatly. The type of lab and the manner in which the labs are presented to students (i.e. procedures,
background information, connection to chemical concepts) also varies. For this project, the module implementation is meant to establish an environment known as cognitive apprenticeship. The cognitive apprenticeship environment is one in which students are given meaningful tasks to complete in an authentic context (Charney et al., 2007). This environment includes an expert to assist in the process, the necessary physical and conceptual tools to complete the task, and also a social context that mimics that of the scientific community (Collins, Brown, & Newman, 1989). It is important that the authentic context include all the facets of the real scientific enterprise: developing research questions; designing and executing labs; collecting, analyzing, and interpreting data; and communicating results (Chinn & Malhotra, 2002). Studies in which students performed research-based labs in authentic contexts do not seem to have completely positive results when students do not experience all facets as described by Chinn and Malhotra (2002). In one study, high school students worked in apprenticeship with university researchers to complete very specific tasks. Although an apprenticeship was established in an authentic context, students did not have the opportunity to formulate research questions. This missing piece to the authentic experience is what was discussed as a possibility for students not changing their view of NOS (Bell, Blair, Crawford, & Lederman, 2003). In another study, by Barab and Hay (2001), middle school students participated in a two week “Apprenticeship Camp” with university researchers. Although they felt it was meaningful for students to not separate doing science and learning science, they did report that there were limitations to the students’ experiences because they did not have the opportunity for a true apprenticeship experience, which would have included all aspects of an authentic experience as described above and the experience of
becoming part of the core scientists on a project. (Barab & Hay, 2001). In a study by Moss, Abrams, and Kull (1998), high school students participated in a partnership with a university that did provide the necessary parts of authentic science. This project, however, did not establish an apprenticeship. Although students did complete projects from formulating researchable questions through presenting their results, the students did not report being motivated or excited about their projects; in fact, for one student the experience was described as “uneventful.” This seems to be a result of not having any direct contact with the researchers involved and establishing any type of apprenticeship (Moss, Abrams, and Kull, 1998).

Thus it is important that in providing a cognitive apprentice environment it is properly designed to represent the scientific enterprise. This means that students need to have the proper skill set and background to formulate research questions, design experiments, perform the experiments, collect and analyze their data and report their findings. It has been reported that students need time and practice to develop lab skills and that these skills precede the conceptual understanding of the tasks; when students experience failure, they often get frustrated and become dissatisfied with the activity; and that lab group camaraderie and independence from the expert naturally develops throughout the process during cognitive apprenticeship (Ritchie & Rigano, 1996).

The lab module used for this research provides a cognitive apprenticeship environment with authentic context. I will describe in further detail in Chapter Three how the CASPiE model works as a scaffold for cognitive apprenticeship.
The Cooperative Learning Environment

Even though my research is not a study of cooperative learning, it should be mentioned that cooperative learning is a part of the CASPiE model. Although the five tenets of cooperative learning – positive interdependence, face-to-face promotive, individual accountability, social skills, and group processing (Johnson, Johnson, & Smith, 1991) – are not explicitly addresses in the CASPiE module, they are implicit in completing the module. As students work in their lab groups to complete the tasks of the module, there are opportunities for students to engage in the behaviors described by the tenets of cooperative learning. It is also important to mention that fostering an environment where students collaborate in small groups develops skills that are often necessary for successful entry into the workforce (Barron, 2000). These behaviors are a part of the development of the classroom community, which is implicit in the CASPiE module used for this study.

Classroom Community and Culture

When I talk about the classroom community, I am referring to the group of students and the teacher (and at times myself as part of that group) in a classroom. The community consists of each individual and their role in the classroom. For example, the teacher may be the director or facilitator of activities; the students may be the learners in the classroom. When I talk about the classroom dynamics or culture, I am talking about the behaviors within the classroom. This refers to how the members of the community interact with each other and behave in the classroom.
Little work has been carried out to study how the positive effects of inquiry and research-based labs emerge and how they are linked to classroom dynamics. To do so, it would be necessary to look at the laboratory classroom from a sociological perspective. Throughout a semester or school year, interactions occur within a classroom between students and instructors and a social structure forms that can directly impact student learning. The emergence of this social structure to build the community within the classroom, influenced by things such as the activities and goals in that classroom, can be considered the “classroom sociology” (Hirschy & Wilson, 2002). The sociology of the classroom can thus be looked at broadly through the lens of how the classroom community behaves as a whole or, more narrowly, how the individuals interact to develop not only the community as a whole, but also their place within the community.

In this section I will present the literature related to the overall development of community in the science laboratory classroom; the next section will look specifically at types of interactions that influence the development of the classroom community and culture.

One study that presents results from the sociological point of view in the development of a laboratory community was conducted by Del Carlo and Bodner (2009). In their study, they categorized and examined the conversations that took place in various levels of undergraduate labs. Although there were some small variations in comparing upper level and lower level chemistry lab courses, they concluded that social conversation among students – not just about chemistry – is important in a laboratory class because these conversations help students form the community in that classroom (Del Carlo & Bodner, 2009). Thus, it seems that the frequency and the types of
conversations that take place during the completion of experiments in the lab helps to develop a community in the entire lab and also contribute to individual students establishing a place within the community as it develops. This examination of interaction frequency and content will be part of the data analysis for this study.

In contrast to the Del Carlo and Bodner (2009) study where the only general guideline for the labs was that students worked in small lab groups, other studies have been done that examine the classroom community where there are other factors that contribute to the development of that community. One example of implementing an educational strategy that impacts the development of community in the classroom is the use of whole-class inquiry (WCI) assessments (Gallagher-Bolos & Smithenry, 2008). For WCI, students are each given their own paper with the problem to be solved. However, the entire class is instructed – with minimal guidance from the teacher – to work together to solve the problem. The results show that as the classes complete WCI assessments throughout the year, a community develops in which the class usually divides into two factions, each with a student leader. The two groups each work on separate parts of the problem, then reconvene to discuss the entire problem so that each individual in the class has the opportunity to contribute and arrive at a final answer for all parts of the assessment (Gallagher-Bolos & Smithenry, 2008).

Another study in which the development of a community was impacted by a specific assignment was conducted in a high school chemistry class that completed a project that integrated chemistry and a business model. The project had the class divided into small groups, each of which acted as a soap company. Each group had to work as a group to apply their knowledge of chemistry and use a business model to assume a role as
a member of their company’s team. It was reported that at the end of this project students gained an understanding of how real science and real business is conducted (Bolos & Smithenery, 1996). The following year, Smithenery and Bolos (1997), published suggestions and guidelines that would help educators establish the scientific community that they experienced with their initial study. These guidelines included taking time to establish an environment that fosters communication and trust among students, in addition to addressing issues of safety and journal writing. They also stressed the importance of evaluating class discussions during initial activities to ensure that students were working collaboratively to complete the tasks and facilitate where necessary to establish the desired type of environment for the WCI. These guidelines take into account both the community of the entire class as a model of the scientific community and the community each group, which is representative of a team of scientific researchers and business people (Smithenery & Bolos, 1997). Although, this study will not include WCI, the type of scientific community established in those studies are similar to that established by the CASPiE model, in which small groups work together as smaller communities within the larger community. The CASPiE model, however, does not explicitly give guidelines as given by Smithenery and Bolos.

Whether or not explicit factors are presented that contribute to the development of a community within the laboratory classroom, there is, indeed, a culture that develops. When students are given activities that are meaningful and framed in authentic contexts, the process by which scientific knowledge is constructed is more accurately portrayed (Cunningham & Helms, 1998). Creating such an environment for these activities should include opportunities for students to use communication skills and collaboration within
their lab group and within the classroom community as a whole to give them a better sense of the workings of the scientific community and understand that there is a social component in the generation of scientific knowledge (Cunningham & Helms, 1998). Thus the community of the classroom that develops – which may not need explicit instruction – will be similar to that of the scientific community.

Interactions Within the Classroom

In considering the community and culture of the classroom that develops, it is also useful to look more closely at the interactions that take place within the classroom, for it is the interactions among the individuals and groups of individuals that contribute to the formation of the overall community and culture. Students may interact with other students for both academic and social reasons within the classroom. This is also true of interactions between students and teachers. The purpose for the interactions, as well as the frequency and outcomes of those interactions may be a vital part of how the overall culture of the classroom develops. Examining the interactions within a classroom can reveal students’ and teachers’ perceptions of their roles within the classroom as well as give a description of the classroom dynamics.

Two studies that examined student-student and student-teacher interactions were done by Enyedy and Goldberg (2004) and Zion and Slezak (2005). Enyedy and Goldberg (2004) concluded in their study that the daily interactions during an inquiry activity shape and reshape the social structure within the classroom. The interactions that occur within the rules of the classroom are what develops the community and defines the roles of both teacher and student in that context (Enyedy & Goldberg, 2004). Similarly,
in examining student and teacher interactions in an inquiry activity, Zion and Slezak (2005) found that the teacher’s role shifts during different stages of inquiry. Dependence on the teacher was not a constant throughout the inquiry activity. These shifts in teacher dependence and student-teacher interactions created the community of the classroom (Zion & Slezak, 2005).

In a study by Crawford (2000), classroom interactions were examined in an inquiry-based classroom setting. In her findings, she reports that throughout the inquiry activity the roles of the student and teacher often shift. This shift seems to be related to the types of tasks that were being completed by the student. As students moved through the inquiry tasks, the role of the teacher changed, and, as a result, the types of interactions that occurred between the student and the teacher also changed. She also reports that as students progress through inquiry activities they develop a sense of ownership of the activity they are completing (Crawford, 2000).

Another study that reports on classroom interactions involved the use of the process-oriented guided-inquiry learning (POGIL) model for lab experiments (Gormally, Brickman, Hallar, & Armstrong, 2011). It was found that throughout the POGIL activities, the amount of discussions and interactions between lab partners in a group increased and resulted in improved group dynamics (Gormally et al., 2011). These findings are similar to the qualitative findings of the mixed methods study by Cacciatore and Sevian (2009) in which they compared students completing a traditional laboratory experiment to those completing an inquiry based lab experiment. Students completing the inquiry labs had much more interactions with their lab partners and asked each other many more questions about the lab and discussion of their data than the students who
completed the traditional experiment. In addition, the inquiry-based lab students’ interactions with the instructor involved asking more questions and also higher-order thinking questions than the students in the traditional lab setting who asked mainly questions about the procedures (Cacciatore & Sevian, 2009).

Other studies have looked more closely at the types of interactions that occur in the classroom. One study in a traditional laboratory environment examined both student-student interactions within a lab group and student-teacher interactions during the completion of a lab experiment. In this study by Högström, Ottander, and Benckert (2010), it was found that student-student interactions within a lab group occurred both verbally, as discussion or questions and answer, as well as non-verbally, as the demonstration of a technique or use of a piece of lab equipment. These student-student interactions were identified to be mainly as a clarification of procedures or for help in using a piece of lab equipment (Högström et al., 2010). The student-teacher interactions did share some similarities with student-student interactions, but the authors also believed that those interactions occurred for other reasons. Students did interact with their teacher regarding issues of procedure or use of equipment, but they also interacted with their teacher for concerns about lab safety and chemical concepts. It was ultimately concluded that students’ concerns over chemical concepts were related to their perceptions of what their teacher deemed to be the important concepts to learn from the experiments (Högström et al., 2010). This suggests that as these students were completing their experiments that they wanted to make sure they were learning – or focused on learning – what the teacher wanted them to learn by completing the experiment.
Another related study compared student-student and student-instructor interactions in non-inquiry and open-inquiry lab environments. Krystyniak and Heikkinen (2007) examined the student-student and student-instructor interactions in undergraduate laboratories as they completed non-inquiry and open-inquiry laboratory activities throughout a semester. They report that the lab groups worked more independently of the instructor during the open-inquiry activities as compared to the non-inquiry activities and that they also used higher order scientific reasoning in the open-inquiry activities (Krystyniak & Heikkinen, 2007).

In consideration of the literature that examines student-student and student-teacher interactions within a science laboratory classroom, it is clear that the frequency and types of interactions that occur reveal a great deal about the culture and community that develops in that setting. It also seems that regardless of the type of lab activity – non-inquiry, guided-inquiry, open-inquiry, research-based – these interactions exist and are important in understanding the overall classroom dynamics as well as both students’ and teachers’ perceptions of their environment and their role within that community.

The Center for Authentic Science Practice in Education

Thus far I have discussed the role of the laboratory in science education, the components of cognitive apprenticeship and cooperative learning environments, the development of the culture of the classroom in the science laboratory, and the significance of the interactions that occur within the science classroom. For my study, I have considered those factors as they apply in a very specific setting – high school chemistry labs completing a CASPiE module. The CASPiE model has been described
previously in Chapter One and the specifics of the module will be presented in Chapter Three. Here, I will discuss CASPiE research findings to date.

Several articles have been published and dissertations written that describe the effectiveness of CASPiE. The seminal paper on CASPiE reported the earliest results of the initial study, namely that students found completion of the research-based CASPiE module to be a valuable experience and they liked that their lab work was part of something that extended beyond their classroom (Weaver et al., 2006). Russell (2006) reported positive effects on students’ attitudes, content knowledge, understanding of NOS, and self efficacy for students who completed a CASPiE module. The dissertation research by Green (2010) revealed that students found the CASPiE experience to be more enjoyable, meaningful, and challenging than the traditional lab experience. Students in that study also were found to have a better understanding of how scientific research is conducted as compared to those students who did not have a research-based lab experience (Green, 2010). More recently, Szteinberg (2012) completed a dissertation on the longitudinal effects of CASPiE. It was suggested by the results of this study that CASPiE may have a positive effect on students’ performance in upper level chemistry classes, it may promote faster graduation rates, and that the perceived benefits of the CASPiE experience are similar to those who participate in undergraduate research (Szteinberg, 2012).

In addition, the final external evaluation of CASPiE was published in 2011. This report was prepared from data collected at 15 undergraduate institutions over a five year period, and showed several positive effects of CASPiE (Scantlebury et al., 2011). Some major summative findings reported include that CASPiE maintained or increased
students’ interest in science, CASPiE students reported a better understanding of scientific research than non-CASPiE student participants, and participation in CASPiE positively impacted students perceptions of their understanding of chemistry (Scantlebury et al., 2011).

For this study, a research-based lab module – developed by CASPiE – was implemented into high school chemistry laboratories to explore the classroom dynamics and the development of the classroom community. The CASPiE module provides the appropriate scaffolding to create a cooperative learning environment that includes cognitive apprenticeship. This study aims to gain insight into how the culture of the classroom develops through the completion of the CASPiE module.

Summary of the Background Literature

The studies described in this chapter exemplify how science laboratory classroom communities have been examined. Many of the studies have considered student-student and student-teacher interactions that occur throughout the completion of laboratory experiments in both high school and college undergraduate settings. The studies in high schools tended to be in a traditional or inquiry-based laboratory environment. One exception presented here did report on high school students’ authentic experiences in a cognitive apprenticeship environment, but that study was done at a summer intensive institute at a university (Charney, 2007). This was not a typical high school laboratory setting. Other studies that did take place in a research-based lab environment were done in undergraduate laboratories. Most CASPiE research studies were done with undergraduate student populations and those that were done in high schools did not focus
on the classroom dynamics. Thus, in review of the literature, the gap filled by my work is in the need for research that explores the development of the classroom community in a high school laboratory setting during the completion of a research-based lab module.

Considering students’ and teachers’ perceptions of their experiences throughout the module, will show how students’ and teachers’ perceptions compare to each other as they move through the phases of the module. This will also result in information on how students’ and teachers’ roles develop throughout the module and reveal if there are shifts that occur as the tasks in the module vary. In addition, the student-student and student-teacher interactions between the two lab phases of the module will be compared. This will show how students interact and complete experiments with given procedures versus how they interact when they complete experiments with procedures they prepared by themselves. Examining students’ and teachers’ perceptions and comparing the classroom interactions of two different types of experiments can prove to be useful in the development of other authentic science activities and how the development of a classroom community that reflects authentic science practice can be implicitly embedded into those activities.
CHAPTER THREE: METHODOLOGY

The overarching theme of the research questions that guided this study is the impact on the classroom environment using a CASPiE module. The research questions were designed to explore both students’ and teachers’ perceptions of the module experience in the classroom and to compare the classroom dynamics throughout the various phases of module. The first three questions look to explore students’ and teachers’ perceptions of the entire module experience. These questions were designed so that the story of how the module was completed could be heard from both student and teacher perspectives. This was done to give a voice to all participants as they progressed through the module. The second three questions specifically look at a comparison of classroom dynamics in the completion of the two lab-based phases of the module. To clarify, “traditional lab experiment” refers to an experiment performed in the Skill Building phase of the module; “research-based lab experiment” refers to an experiment performed in the Independent Research phase of the module. A comparison of “traditional” and “research-based” labs can be found in Table 3.1. These phases, as well as the other two phases will be described in detail later in this chapter.
Table 3.1. A Comparison of Traditional and Research-based Labs.

<table>
<thead>
<tr>
<th>Traditional</th>
<th>Research-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step-by step procedures provided</td>
<td>Procedures written by students performing the labs</td>
</tr>
<tr>
<td>Working to find the correct, known outcome</td>
<td>No documented known outcome</td>
</tr>
<tr>
<td>Generally used to show concepts that are curriculum-based</td>
<td>Used to collect and interpret data for novel experiments with real world context and applications that may integrate several curriculum-based concepts</td>
</tr>
<tr>
<td>Explicit instructions given for data collection and analysis</td>
<td>Data collection, analysis, and interpretation is open to those who designed and executed the experiments</td>
</tr>
</tbody>
</table>

Research Questions

This first set of questions seeks to gain insight into students’ and teachers’ perceptions of their experiences throughout the module:

1. What are students’ perceptions of their experiences completing a research-based lab module?

2. What are teachers’ perceptions of the student experiences during the completion of a research-based lab module?

3. What are teachers’ perceptions of their experiences in the classroom during the completion of a research-based lab module?

The second set of questions seek to explore and compare classroom dynamics during lab phases of the module:

4. What, if any, differences are there in how a group of students performs an independent research-based lab as compared to a traditional lab experiment?
5. What, if any, differences are there in student-student and student-teacher interactions among students from different lab groups in a research-based lab as compared to a traditional lab experiment?

6. What, if any, differences are there in student-student and student-teacher interactions in a research-based lab as compared to a traditional lab experiment?

Theoretical Frameworks

The theoretical frameworks applied in this project are ethnography (Patton, 2002; Bhattacharyya, 2007), activity systems (Engeström, 1987), and cognitive apprenticeship (Collins, Brown, & Newman, 1989). The methodological framework used for data analysis is phenomenography (Marton, 1981; Orgill, 2007).

Ethnography

Ethnography was chosen because the guiding research questions seek to examine the culture and dynamics of the laboratory classroom. The research questions were designed to explore the way in which lab groups, and individuals within lab groups, function as part of the society within the classroom of peers and teachers. As a former high school chemistry teacher, I have first-hand experience in both observing and being a part of classroom culture. It was therefore my assumption that in this study, there would be a classroom culture that would have already been established before the onset of this study, but also that this culture would undergo dynamic changes throughout the completion of the CASPiE module. This on-going development of the culture of this very specific group of individuals lies at the very core of ethnography (Patton, 2002;
Another tenet of ethnography is the orientation of the researcher as *emic* or *etic* (Bhattacharyya, 2007). The *emic* has the researcher as an insider who is part of the culture, whereas the *etic* places the researcher as an outside observer (Bhattacharyya, 2007). For this study, I have not chosen to adopt either singular orientation. I have allowed that either the *emic* or *etic* orientation would be appropriate at different times during this study. For classroom observations and interviews it was appropriate to be the outsider to view and inquire about the classroom dynamics. However, as the researcher who has experience with the design and implementation of the lab modules, I allowed myself to be open to student and teacher interactions that involved the labs and data calculations, thus drawing me in to be part of the culture. Thus, ethnography is a fitting framework for this study as it provides the cultural prospective of the classroom, with consideration for the role of the researcher as the culture develops through the completion of the CASPiE module.

*Activity Systems*

Activity systems theory is sometimes referred to as a “second generation activity theory” (Smidt, 2009). This framework was developed by expanding upon Vygotsky’s original activity theory (Vygotsky, 1976; Vygotsky 1987). Vygotsky’s activity theory considers the relationship between subject (learner), object, and artifact in completing tasks. It classifies actions of the learner to be artifact-mediated, in which the learner uses cultural tools such as language and communication, and object-oriented, in which the learner experiences an activity using material tools. Activity systems theory also considers the relationships addressed by activity theory, but draws distinctions between
individual and collective activities. Activity systems theory expands upon the basic relationship between learner, object, and mediating artifacts to include the rules, community, division of labor, and the ultimate outcome (Engeström, 1999). These additional relationships are important in this study as they reflect how the culture of a high school laboratory course setting develops. A modified version of the relationships in activity systems theory (Engeström, 1999) can be seen in Figure 3.1.

![Figure 3.1](image)

Figure 3.1. Activity systems theory relationships as per Engeström (1999) with specifications shown in parentheses for application in the setting of this study.

The importance of the theoretical perspective of activity systems as it applies to this research study lies within both the artifact-mediated central idea of its predecessor and the connections between the social activities that are also an integral part of lab work in the classroom. For this study, students were given a research-based lab module and
the classroom resources – mainly materials, instructions, and examples – to complete the module and present their results. However, in completing this module, students do not work alone, nor do the lab groups necessarily work entirely without any outside assistance or interaction. Each individual and each lab group functions as part of the classroom community. This may involve lab groups helping each other, lab groups and individuals asking the teacher questions, or even just general socializing within the classroom. Due to the complexity of the module completed for this study, there was also opportunity for groups to adopt a “divide and conquer” type of mentality and employ a strategy of division of labor within lab groups. It also should be noted that although students were given a large range of autonomy for designing and executing their independent research for the module, they were still required to work within some standard set of rules of the classroom. Activity systems theory captures all of these relationships and interactions, providing a very fitting lens for this study.

**Cognitive Apprenticeship**

Cognitive apprenticeship posits that desired skills to be learned can be taught by providing an authentic context in which those skills are employed and relevant (Collins et al., 1989). Thus, the learning of these skills is embedded in a task as opposed to being explicitly taught without context. Furthermore, cognitive apprenticeship involves an expert who provides the tools, which could be both conceptual and physical, and necessary information for the learner to complete the tasks which have in some way been modeled by the expert (Collins et al., 1989). In addition, cognitive apprenticeship includes the social context as part of the authentic context in which the tasks are
performed (Collins et al., 1989). This social context can include not only the social relationship between the expert and the student, but also the social context of working as part of a lab group and part of a larger scientific community that is modeled in the classroom. The CASPiE module used in this study was created by an expert in the field of food science and modified by the teacher participants in this study and me. The students who completed the module in this study utilized the research module, but also were “in apprenticeship” with the classroom teachers and me. (This also supports the idea that, for part of this research, I assumed the *emic* orientation of ethnography as part of the culture of the classroom.) The students learned skills in a real context that were embedded within the module, they employed the tools – both cultural and material, as described by activity systems theory – that were provided to them in the classroom, and thus worked to achieve their goal of reporting the findings of their independent research.

The way in which the CASPiE module was designed and implemented establishes a setting properly described by the framework of cognitive apprenticeship.

*Phenomenography*

The methodological framework chosen for this study is phenomenography. As described by Marton (1981), the way in which individuals understand their experiences can be put into “categories of descriptions.” In addition to categorizing individual’s reports of their experiences, phenomenography can be used to gain an understanding about how individuals experience a phenomenon and can be open to interpretation of those reported experiences (Orgill, 2007). The research questions for this study seek to uncover students’ and teachers’ perspectives of the experience of completing a research-
based lab module. The research questions also aim towards students’ and teachers’
descriptions of their interactions with other members of their classroom community.
Phenomenography was chosen because this research seeks to report the experiences of
those within the classroom community as they describe it. It cannot be assumed that
there is one interpretation and understanding of the experience in completing the CASPiE
module in the classroom, thus I have chosen to use phenomenography to seek out
patterns or categories for participants’ experiences throughout the completion of the
CASPiE module.

Settings and Participants

School 1 is a small, rural junior-senior high school in central Indiana. The
research-based laboratory module for this study was implemented into a second year high
school chemistry class at school 1. Classes met 45 minutes per day, five days a week.
The curriculum for this class would be considered a traditional high school curriculum
that consisted of lectures and “cookbook” labs. The class consisted of thirteen high
school juniors who had all completed a first year of chemistry the previous school year.
Eight students and the teacher agreed to be participants in this study. The teacher from
this school will be referred to as Teacher 1.

School 2 is an academy within a large, urban high school in central Indiana. The
curriculum of the academy follows a model of project-based learning. In addition, all
classes are two-subject integrated with two different content teachers in each classroom.
The research-based lab module for this study was implemented into the biochemistry
class at school 2. The chemistry taught is the equivalent of a first year high school
chemistry course in Indiana. Classes met for 80 minutes per day, five days a week. The class consisted of 32 students, most of whom were sophomores. Twenty-three students and the chemistry content teacher (who will be referred to as Teacher 2) agreed to be participants in this study. The two settings and groups of participants for this study are summarized in Table 3.2.

Table 3.2.  Settings and Participants.

<table>
<thead>
<tr>
<th>School 1</th>
<th>School 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small rural junior-senior high school</td>
<td>Academy within a large urban high school</td>
</tr>
<tr>
<td>Second year chemistry course</td>
<td>First year chemistry course</td>
</tr>
<tr>
<td>Small class size ( &lt; 15)</td>
<td>Large class size ( &gt; 30)</td>
</tr>
<tr>
<td>45 minute class periods</td>
<td>80 minute class periods</td>
</tr>
<tr>
<td>Traditional curriculum</td>
<td>Project-based learning</td>
</tr>
<tr>
<td>N = 8 (4M, 4F)</td>
<td>N = 23 (13M, 10F)</td>
</tr>
<tr>
<td>29% free and reduced lunch (130 free; 53 reduced; 634 total students)</td>
<td>83% free and reduced lunch (1783 free; 191 reduced; 2374 total students)</td>
</tr>
<tr>
<td>99% White; &lt;1% Asian, Hispanic,</td>
<td>20% White; 65% Black; 11% Hispanic; &lt;5% Multiracial, Asian, and Native American</td>
</tr>
</tbody>
</table>

The teacher-participants for this study were recruited through their school principals. After contacting the school principals during the summer before the pilot began, the principal of each respective school forwarded the information about my planned research project to the teachers and each contacted me and agreed to participate and incorporate the CASPiE module into their chemistry curriculum for the upcoming school year. Both teachers participated in professional development, which will be described later, and implemented the module in a pilot study the year prior to the study presented here.
Prior to this implementation, Teacher 1 had seven years of teaching experience. Her experience had been exclusively teaching chemistry. Being in a small district, she was the only chemistry teacher and had taught chemistry 1 and advanced placement (AP) chemistry in past years. During the pilot year of the study she had been assigned to teach chemistry 1, chemistry 2, and AP chemistry. The pilot year was the first year that chemistry 2 had been taught and she was the sole curriculum writer. She took the opportunity of being a part of this study to make the CASPiE module part of the curriculum. Teacher 1 holds a bachelor of science in chemistry and a master of science in education. As part of her college studies, she earned her Indiana state teaching certification for chemistry in the traditional way of taking the required course work, scoring above the state minimum required score on standardized tests, and completing practical field experience. In addition to her experience as a teacher, she also had interned in an analytical chemistry lab while in college.

Teacher 2 was a first year teacher during the pilot study for this research. She was in the Teach for America program and, as part of the provisions of the program, held a two year contract with the school district as part of her placement. During the pilot year she taught a class called Chem Analysis. This was an integrated curriculum of chemistry and statistical mathematics. In that class she was paired with a math teacher who was also in the Teach for America program. Although this was a co-teaching environment, she was responsible for the chemistry curriculum for the class and the other teacher was responsible for the statistical mathematics curriculum. During her second year of teaching, in which data were collected for this study, she had taught Biochemistry, which had her paired in the classroom with a veteran biology teacher. Similar to the previous
year, Teacher 2 was responsible only for the chemistry curriculum and the other teacher for the biology curriculum. Teacher 2 held a bachelor of science in Public Policy and was enrolled in a Master of Arts in Teaching program during the two years she was involved in this project. In contrast to Teacher 1, Teacher 2 had very limited chemistry content knowledge, science teaching experience, and practical lab experience. Table 3.3 summarizes the backgrounds of Teacher 1 and Teacher 2 at the start of this study.

Table 3.3. Teachers’ Backgrounds.

<table>
<thead>
<tr>
<th>Teacher 1</th>
<th>Teacher 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven years teaching experience</td>
<td>One year teaching experience</td>
</tr>
<tr>
<td>Bachelors degree in Chemistry</td>
<td>Bachelors degree in Public Policy</td>
</tr>
<tr>
<td>Masters degree in Education</td>
<td>Earning Master of Arts in Teaching</td>
</tr>
<tr>
<td>Interned in an analytical lab</td>
<td>Limited lab experience</td>
</tr>
<tr>
<td>Traditional teaching certification program</td>
<td>Teach for America</td>
</tr>
</tbody>
</table>

Description of the Module

The module used in this study was originally developed for the Center for Authentic Science Practice in Education, CASPiE (Weaver, Wink, Varma-Nelson, Lytle, Morris, Fornes, et al., 2006). CASPiE was developed by a National Science Foundation grant to give college students the opportunity to have research experience as part of their first or second year chemistry course. The module implemented in this study was a modified version of “Phytochemical Antioxidants with Potential Health Benefits in Foods” (Burgess, 2011). Modifications were made to the module for two reasons: 1. to make the module appropriate for the level of understanding of high school sophomores and juniors; and 2. to make the labs that were designed for three hour time blocks fit into
each high school’s schedule constraints. Revisions went through several iterations between me and both teachers who participated in the study. A pilot trial of the module was done in each school in the academic year prior to the implementation and data collection for this study. Upon completion of the pilot study, the module used in this study was finalized by me and both teachers. This module is divided into the following four phases: 1. Skill Building; 2. Experimental Design; 3. Independent Research; and 4. Results and Poster Presentation.

In the Skill Building phase of the module the students are first given an overview of the entire module. Lab groups (3 or 4 students each) then complete two experimental protocols, which they will later use to design and implement their own research project. One protocol is preparation of samples to be analyzed with high performance liquid chromatography (HPLC) to determine vitamin C concentration for a given food substance. In addition to the lab, students learn how to make the appropriate graphs and calculations needed to determine the vitamin C concentration. The second protocol is preparing samples for spectrophotometric analysis to determine antioxidant capacity via the Trolox equivalence antioxidant capacity (TEAC) method for the same food substance. This data analysis has a graphing component and calculations that apply Beer’s Law.

In the Experimental Design phase, lab groups apply the protocols they learned in Skill Building to design a novel experiment. They are given a list of materials and equipment available to them. They are instructed that they are to pick a food or beverage to study. Students have to determine a treatment to do to that food or beverage, make a hypothesis on how it will affect its vitamin C concentration and antioxidant capacity, then design a procedure for that treatment to perform in the laboratory.
For the Independent Research Phase, each lab group performs the labs that they have written in the Experimental Design phase of the module. This includes the appropriate calculations for data analysis.

In the final phase, Results and Poster Presentation, lab groups interpret their results and make a poster to present their Independent Research results. Students are given a template of what general sections should appear on a poster that is presented at a scientific conference. In addition they have access to seeing examples of actual posters that present research results. The culmination of the module is a poster session in which students must present and defend their findings to their peers. A brief summary of the module implementation can be found in table 3.4.

Table 3.4. Module Description. This table gives a description of each of the four phases of the module and the number of class periods spent in each school on each phase.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
<th>Days at School 1</th>
<th>Days at School 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skill Building</td>
<td>HPLC and TEAC protocols</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mathematical and graphical data calculations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Design</td>
<td>Development of Independent Research lab procedures</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Independent Research</td>
<td>Execution of student-generated lab procedures</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Results and Poster</td>
<td>Interpretation of analyzed data</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Presentation</td>
<td>Development and presentation of posters</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Professional Development

Professional development for the teachers in this study occurred during the summer prior to the pilot implementation. Each teacher completed the two skill building labs, including the appropriate calculations necessary for data analysis and interpretation. In addition to the labs, teachers were also given materials that included instructions and handouts for the module as well as tips on how to facilitate each phase. In addition to the professional development, I was also present in the classroom as a resource and support for the teachers throughout the module during both years of implementation.

Data Collection

The primary data sources for this study were interviews with students and teachers, carried out upon completion of each of the four phases of the module. Student interviews were conducted in a focus group format, which generally consisted of three students from three different lab groups for each interview. Each focus group completed four interviews at each school respectively. Teacher 1 and Teacher 2 each participated in four individual interviews. Student focus group and teacher individual interviews were conducted within two days of the completion of each phase of the module. All interviews were transcribed verbatim and entered into NVivo®, the software that was used for qualitative analysis. Secondary data sources were collected in the forms of journals kept by each teacher, reflection sheets completed by students throughout the module, and researcher field notes. These secondary data sources primarily serve for triangulation purposes during data analysis.
**Coding and Analysis**

Prior to coding all responses to interview questions, interview data were categorized in the following manner:

1.Responses describing interactions among students within the same lab group;
2. Responses describing interactions among students between two or more lab groups;
3. Responses describing interactions between a student or students and the teacher.

During the coding, each of the four phases of the module were coded separately within each of these three interaction categories. In this study, interactions include verbal interactions such as asking and answering question, explanations, and discussions, as well as non-verbal interactions such as watching a specific lab technique that is being performed. In the event an interview response described multiple categories, it was put into all that were appropriate.

Open coding was done for each of the three categories described above using the constant comparison method described by Glaser and Strauss (1967). The data were grouped based on similarities and patterns seen when comparing all open coded responses. A more structured coding scheme was developed as a result of the relationships seen after open coding. Definitions for these codes were developed and all data were recoded based on these definitions. The process just described was completed by me, but I also employed the help of another researcher in my group.

After the interview data were coded, interrater reliability tests were done with another researcher who had not previously seen the data. For interrater reliability testing,
the data sets for Skill Building and Independent Research were combined into one data set. This was done because those two phases of the module had the common component of the laboratory setting of the classroom. Interview questions for both phases were directed towards laboratory work and data calculations associated with those labs. Due to the similarities of both phases being centered on experimental tasks, the codes that emerged from the open coding were the same for both the Skill Building and Independent Research phases of the module. The other researcher who had not previously seen the data was given the definition of each code and an example of data for each code. This researcher was then given a portion of data from each of the three categories to code individually. Initial interrater reliability yielded 87% agreement. Each piece of data in disagreement was discussed until 100% agreement was reached. This process included referring back to the interview transcript to give the piece of data more context and discussing the definitions of the codes. Definitions were then refined and a second round of interrater reliability was completed with the researcher who had done the first round of interrater reliability. This was done in the same manner, with the researcher being given another set of coded data and the newly refined coding definitions. The second round of interrater reliability yielded a result of 91% agreement for the Skill Building and Independent Research coded data combined.

Interrater reliability was completed for sets of data from the Experimental Design phase and Results and Poster Presentation phases separately. The same researcher who had done the interrater reliability for the other two phases did these interrater reliability tests. The initial interrater reliability tests yielded the results of 95% and 93% agreement respectively for the Experimental Design phase and the Results and Poster Presentation
phase. Due to the high percent agreement of the interrater reliability tests in these phases, no additional adjustments were made to any defined codes and no further interrater reliability tests were performed. The final set of codes and their definitions can be found in Table 3.5.

Table 3.5. Coding Definitions.

<table>
<thead>
<tr>
<th>Code</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Procedures</td>
<td>An instance of asking a question about explaining or executing a lab procedure that is explicitly part of a written procedure</td>
</tr>
<tr>
<td>Problem Solving</td>
<td>An instance of asking a question about or working with other students to solve a problem that cannot be answered by reading the lab procedures</td>
</tr>
<tr>
<td>Checking Work</td>
<td>An instance when students ask for their work to be checked by another student or the teacher, or check the work of another student</td>
</tr>
<tr>
<td>Community Effort</td>
<td>An instance when students state that they work together with students in other lab groups or help students in other lab groups where the common goal of all involved is completing the lab</td>
</tr>
<tr>
<td>Division of Labor</td>
<td>An instance when lab groups split up work to complete tasks individually or in sub-groups. This includes descriptions of instances when groups come back together to complete the lab as a whole combining the results of their individually completed tasks</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>An instance of asking a question pertaining to calculations with and graphing of collected data</td>
</tr>
<tr>
<td>Opinion</td>
<td>An instance of asking an opinion of work that has been done or is being proposed to be done; this is not asking if something is right or wrong, but is done to seek some approval or judgment of the “goodness” of something</td>
</tr>
</tbody>
</table>
To address the first set of research questions, coded data from all four phases for all student and teacher interviews were analyzed. This was done by examining the strength and richness of the descriptions of students’ and teachers’ perceptions as they moved through each phase of the module. This included comparing and contrasting: 1. students’ descriptions of their own experiences; 2. teachers descriptions of their perception of students’ experiences; and 3. teachers’ descriptions of their own experiences. This will be presented in the next chapter as a description of trends seen in the perceptions of students and teachers throughout the module. The secondary data sources of teachers’ journals and researcher field notes were also utilized in triangulation of the presented results and discussion.

In analyzing the data for the second set of research questions, counts for each individual code were tabulated for the Skill Building and Independent Research coded data for student focus group interviews only. To formulate assertions to answer the research questions, three main factors were considered. The first was the distinct number of student participants who made a comment corresponding to each code. The second was the total number of occurrences of coded interactions. By occurrences, I mean that a student may have described more than one situation within a phase of a module that falls into the same code. Table 3.6 presents the counts for number of student participants and occurrences for codes in the category of interactions among students within a lab group, student-student interactions among students in different lab groups, and interactions between a student or students and the teacher.
Table 3.6. Coding Counts. The number of participants represented for each code (N) and the number of occurrences (O) of descriptions for each unique instance that was coded in that category. Interactions are listed as SSW (student-student interactions among students within their lab group), SSB (student-student interactions between lab groups), and ST (student-teacher interactions).

<table>
<thead>
<tr>
<th>Code</th>
<th>Interaction</th>
<th>Skill Building</th>
<th>Independent Research</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>O</td>
</tr>
<tr>
<td>Lab Procedures</td>
<td>SSB</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>Problem Solving</td>
<td>SSW</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Checking Work</td>
<td>SSW</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>SSB</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>Community Effort</td>
<td>SSB</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Division of Labor</td>
<td>SSW</td>
<td>22</td>
<td>34</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>ST</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>

The third factor considered in data analysis was the strength of the data. Once all interview data of the students were coded, the data were read for the richness of detail to support assertions. After assertions were made, the secondary data sources of teachers’ interviews, teachers’ journals, and research field notes were considered for triangulation and support. The assertions addressing the second set of research questions will be presented in the next chapter.
CHAPTER FOUR: RESULTS AND DISCUSSION

In presenting the results as they pertain to the research questions, I will first present the students’ perceptions of their experiences of the completion of the CASPiE module. I will then present the teachers’ perceptions of their students’ and their own module experiences, followed by a discussion that compares and contrasts the students’ and teachers’ perceptions. The chapter will conclude with a discussion of the findings presented as they pertain to the theoretical frameworks of this study. The findings addressing the second set of research questions will be presented as assertions in Chapter Five.

Students’ Perceptions Throughout the Module

During the student focus group interviews that were conducted within two days of completing each phase of the module, students were asked to describe how they worked through the tasks during each of the phases. Students were given the opportunity to talk about how they worked with their lab partners, students in other groups, and the teacher to complete the tasks within each phase of the module. Students were also given reflection sheets to complete throughout the module so that they could express how they thought they were doing on completing specific tasks throughout the module. At the completion of the module, students were also given a reflection sheet that asked them to
rank their opinion on comparing the difficulty of the four phases of the module as well as rank how independently their lab group worked throughout the four phases of the module. The semi-structured interview protocols and the reflection sheets can be found in the appendices.

Skill Building

Below are excerpts from interviews in which students were asked to describe how they worked to complete the lab experiments in the Skill Building phase of the module.

Student 23: We asked lots of questions [to our teacher]. I just wanted to make sure I was doing it right. I didn’t wanna make a mistake or anything. That was the reason. . . we wanna make sure we were doing it right.

Student 21: We kinda watched the people next to us to see if we was [sic] doing it right.

Student 10: We always had someone double checking it. I don’t want to screw something up. . . We were like, ‘I’m getting ready to do this, if you want to read over it and think I’m doing it right.’

Student 11: We always double check each other’s work.

One trend seen in students’ descriptions of their lab experiences in the Skill Building phase of the module is that students wanted reassurance that they and their lab partners were performing tasks correctly. Although neither their lab partners nor students in other lab groups had any additional experience in completing the labs, students still had a need for assurance from others to verify they were doing things the right way. As with Student 23, occasionally students did talk about getting verification from the teacher; however, students talked more often about having their work double checked by peers in their lab.
groups or from other lab groups. Student 21 described how he would watch the lab group next to his for validation of his lab group’s work. This use of other lab groups for validation and help with procedures was another trend seen during the Skill Building experiments.

Here are a few examples of students’ responses when they were asked if they had asked any other lab groups questions during the Skill Building experiments:

Student 5:  My group, we worked on everything together. We would look at it and go ‘OK’ and try to work through it, and then we’d ask another group. If they didn’t know, then we’d ask [Teacher 1].

Student 11:  We usually help each other out – the different groups. One group will ask the teacher then everyone else knows.

Student 15:  We usually discussed it among ourselves [lab group] and then we asked another group to see if they could help us and if they couldn’t help us we asked [Teacher2].

Student 23:  We would peak a little bit to see what they [next lab group] were doing, but not asking questions.

Student 26:  I pretty much watched over and see what the other groups did to see if we were going the same way they were going. If we weren’t then I asked the teacher.

Student 28:  [We were] comparing what we were doing to them [nearby lab group], to make sure.

These quotes reveal that the students perceived their classmates in other lab groups as valuable resources in completing the experiments. Students often described scenarios like those above where they talked about discussing something with their lab partners, then went to another group for help or to compare how they were performing a task to another group. Although students in both schools describe instances of interactions with other lab groups throughout the experiments, the students in School 1 describe a more
verbal and social interaction (Students 5, 11, and 15) than those in School 2 (Students 23, 26, and 28). This is not surprising since the students in School 1 were in chemistry 2 (a small class of 13) and had all been in chemistry 1 together the previous year. In a follow-up to the above response by Student 11, Student 10 added, “This is how it’s been for a long time. Like all of chem one.” This exemplifies that the students had developed a system by which they worked with other lab groups to complete tasks, then would ask the teacher for guidance when needed and share that information with the other lab groups. These types of responses were coded as “Community Effort” as described in Table 3.5. The students in School 2 were in a first year chemistry course with over 30 students. These students had not established the same close social relationships within the class as the School 1 students. There were students from School 2 who described instances of consulting with students in other lab groups for help during the Skill Building experiments in addition to visually checking with other groups. However, students from School 1 only described verbal interactions during this phase. Regardless of the interaction being verbal or visual, students still perceived students in other lab groups as a source for validation or verification in completing their Skill Building experiments.
Experimental Design

In the Experimental Design interviews, students were asked to describe how their lab groups worked to choose and design their research project. In the following responses, students describe how they worked with their lab partners during that process.

Student 4: We had thought about it before we even started CASPiE, so we already had an idea in mind. We wanted to do pomegranates. . . We discussed it, but I came up with all the ideas that we could have like cooking it, baking it, you know, different treatments we could have done. . . We discussed it and went over which ones they liked the best, then we, by process of elimination, eliminated it down to that one.

Student 6: We talked about different fruits and some might be a good idea. . . We just talked in our group.

Student 14: We all discussed it and came up with different things until eventually we agreed.

These represent typical responses from students as they designed their independent research project. This suggests that students perceived this as a negotiation process within their lab group. The trend seems to be that students offered suggestions for what food to use for their project in what might be considered a brain storming session, then students weighed out what would be considered good ideas before reaching an agreement as a group. Students were asked if they had gone to other lab groups for help during this process. Students responded that they did not go to other lab groups for help. The only mention of students from different lab groups having any type of interaction was after a few lab groups had decided on their project and were curious to find out what the other groups were doing.
Although there were not many instances of students going outside of their lab group for help, a few students did recall that they had used their teacher as a resource during the Experimental Design process.

Student 3: You came over and we bounced ideas off you.

Student 31: We basically just had like different kinds of fruits and we voted on which one we were gonna do. Then we asked [Teacher 2] to see if this was a good idea.

Student 11: We needed help on how to do dilutions. How to actually make them.

Student 3: Yeah. Same thing with the dilutions. We needed to figure out how many parts went into this one and how to get them.

Students generally described two scenarios for which they went to their teacher or to me. As defined as a code in Table 3.5, students went to the teacher or me to ask an opinion on an idea that they themselves already developed. As briefly stated above by Student 3, she “bounced ideas off” me and Student 31 asked the teacher if their group had “a good idea.” These students were looking for opinions on the quality of what they were proposing. This is different than in the Skill Building experiments where students were looking for verification of something being right or wrong.

The other reason students consulted a teacher was for help in writing the procedures for dilutions. Although students had been instructed on the process of making dilutions as part of the Skill Building and they had an example of an experimental procedure with dilutions that were different than what they had prepared in the Skill Building experiments, they still had a need to go to their teacher when writing procedures for calculating and preparing their own dilutions. In contrast to getting an opinion from the teacher, questions pertaining to dilution preparation were directed towards
verification that calculations and procedures were correct. This suggests that students perceived the concept of making dilutions to be one of the more difficult parts of the experimental design process and one that had an objectively “right” or “wrong” answer.

**Independent Research**

The following are some examples of how students describe their experiences during their Independent Research lab experiments:

Student 10: Since we had already gone through almost the same process with the tomato juice [Skill Building experiment], it was way easy.

Student 11: At the beginning [Skill Building] we used other groups to compare how to do it and stuff, but now [Independent Research] we all know how to do it so we don’t need other groups. [Student 5 and Student 6 nod in agreement.]

Student 25: It was easy because we already had an idea about it and the example [Skill Building] labs we did with the tomato juice. Basically at the beginning you had to learn it all and learn how to understand it, but once you got towards the [Independent Research] you already had your understanding, so it got really, really simple on and on throughout the process.

Students tend to discuss the process of doing the Independent Research labs as easier than the Skill Building labs. They describe the Skill Building experience as being a time when things were new and they were just learning how to do those types of labs. They describe their Independent Research labs as easy because they say that they are so similar to the Skill Building labs they had done previously. The general trend, therefore, is that students tend to see that performing a lab with a familiar protocol is easier than doing the experiment the first time. I describe this as “similar” because the principles of
the protocols from Skill Building had to be applied for the labs the students designed for the Independent Research experiments. The overall procedure was not exactly the same since different foods were used and a treatment had to be designed by the students and executed as part of the Independent Research.

Students were also asked to describe if they felt they were independent as lab groups or if they were dependent on help from outside of their lab group.

Student 8: I think that we were more independent during this. We weren’t asking as many questions as we were in the other two phases.

Student 12: I felt like we was [sic] more independent than before.

Student 16: We just talked it over with each other [lab partners]. We were more independent too ‘cause like the first lab [Skill Building] we were all confused and we didn’t know what to do so we kept asking questions and after each lab we get more independent. We didn’t ask the teacher any questions.

Student 18: We problem solved on our own. I got good group members.

Students express that they perceived their lab groups as being independent during the Independent Research Phase of the module. Student 16 even explains that they have become more independent in the Independent Research lab because they had asked questions and completed labs previously as part of Skill Building. The trend is that students talk about problem solving within their group as opposed to seeking help from outside their lab group. As stated previously, they say that they no longer needed the help of other lab groups. In addition, students also state that they did not need to seek the help of the teacher for the completion of these labs.
Another trend is the way in which students describe how they worked within their group to complete their labs.

Student 8: We split the tasks to make sure everything got done. We worked as a team and got everything done. . . We would kind of separate and do the things to get everything prepared and we’d come back as a team to put everything together.

Student 10: My quote is (reading from her reflection sheet), ‘We got a 9.5 because we worked so well together you’d think we were getting paid.’

Student 35: We split off into twos since we had four people in our group. We split up the work even though we didn’t have to.

What students described most often in how they completed their Independent Research experiments was employing division of labor tactics (see Table 3.5). Students describe how they divided into two smaller groups or that individuals took on specific tasks and how they would come together as a group so that they were able to complete their experiments. Students were not given instructions to do this, but it seems that the students realized that it would be beneficial to completing the labs. Although there is not one clear consensus, some students did mention that in dividing up tasks they could get done either faster or more efficiently.

**Results and Poster Presentation**

During the final phase of the module, students generally talk about dividing up the sections of the poster amongst their lab group members to complete the poster.

Student 3: We worked individually. We had one person looking for pictures, I was typing, and someone else was working on the graphs.
Student 5:  [One person] was designing the poster and getting pictures. I was actually looking up the other experiments that we could compare to, uh, the other experiments doing research. And then [Student 11] was, I think, doing some of the graphing stuff.

Student 21:  We divided up tasks. We had four people in our group, so two did our digital poster and two did the printed poster.

As was the case in the Independent Research phase of the module, students took the opportunity to use division of labor to complete their posters for their project. Students did not describe why they proceeded this way, but their responses suggests that they perceived that this was the best way to complete the task at hand.

When students were asked if they had consulted with students in other lab groups in preparing their final posters, most students simply answered “No,” that they did not go to other groups for any help or discussion. Students also report that they rarely had questions for the teacher during this phase. When asked directly if they asked their teachers any questions while they were preparing their posters, the majority of students just said things like “No” or “We just kept to our group.” One student (Student 9), when asked if she asked the teacher any questions during this phase, replied, “I didn’t because I pretty much knew how to make a poster.” There were a few instances that students cited on asking the teacher a question, most of which seemed to be like the following:

Student 13:  I asked the teacher I think one question, which was like the order of like what goes first as in the introduction, the hypothesis, the materials, the procedures, and all that. That’s probably the only question I asked.

Thus for the Results and Poster Presentation phase of the module, students saw themselves as independent lab groups working together to finish their project.
Phase Difficulty and Independence Ratings

After completing all phases of the module, students in School 2 only were given a final reflection sheet that had them rate the phases of the module based on the comparison of their perceived difficulty and independence as a lab group for the four phases of the module. Students were instructed to rank the four phases of the module as 1 through 4, where 1 represents the easiest phase and 4 represents the most difficult phase. Figure 4.1 shows the plot of the average for student responses in comparing their perceived difficulty for each phase of the module. As seen on the graph, there was not an overall consensus for the difficulty rankings for the four phases of the module. The Experimental Design phase had the highest average (most difficult) and the Results and Poster Presentation ranked as the easiest. Although they did not complete the survey, three of the eight students in School 1 said during their final interview that they ranked the Experimental Design phase as the most difficult phase. Of the remaining students, three commented on one of the other phases of the module being the hardest and the remaining two students did not express their perception of the hardest phase at all. In Figure 4.1 it can be seen that each phase has a very high standard deviation. Results of the ANOVA show that there is no statistical significance in the overall difference of the ratings between the groups (Table 4.1). A post-hoc Tukey was also performed and shows that there is no significance to the differences when comparing any two phases of the module in terms of difficulty rating (Table 4.2).
Figure 4.1. Students’ ratings for the comparative difficulty of each phase of the module. The bars at each phase represents standard deviation.

Table 4.1. Difficulty Rating ANOVA Results. In the table below module phase 1 = Skill Building, 2 = Experimental Design, 3 = Independent Research, and 4 = Results and Poster Presentation. For the ANOVA between groups, p = 0.087. (N = 21)

<table>
<thead>
<tr>
<th>Module Phase</th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.52</td>
<td>1.17</td>
</tr>
<tr>
<td>2</td>
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<tr>
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<tr>
<td>4</td>
<td>2.00</td>
<td>1.18</td>
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</tbody>
</table>
Table 4.2. Difficulty Rating Post-hoc Tukey Results. This shows the significance using a post-hoc Tukey test between each combination of the four phases of the module.

<table>
<thead>
<tr>
<th>Module Phase (I)</th>
<th>Module Phase (J)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.760</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.992</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
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</tr>
<tr>
<td>2</td>
<td>3</td>
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<tr>
<td>3</td>
<td>4</td>
<td>0.270</td>
</tr>
</tbody>
</table>

The same students from School 2 were also instructed to rank how independently they felt their lab group worked in completing each phases of the module. Students ranked each phase from 1 (least independent) through 4 (most independent). The results are presented in Figure 4.2 as the average for each phase with standard deviation bars shown. The averages show a tie for the Skill Building and Experimental Design phases as the least independent and a tie between Independent Research and Poster and Analysis. As was the case with the difficulty ratings, the standard deviations are also rather larger for these averages, as seen on Figure 4.2. The results of ANOVA (Table 4.3) and post-hoc Tukey tests (Table 4.4), like the difficulty rating, show that there is no significant differences in any comparisons.
Figure 4.2. Students’ ratings for the comparative independence of their lab group for each phase of the module. The bars at each phase represents standard deviation.

Table 4.3. Independence Rating ANOVA Results. In the table below module phase 1 = Skill Building, 2 = Experimental Design, 3 = Independent Research, and 4 = Results and Poster Presentation. For the ANOVA between groups, p = 0.208. (N = 21)

<table>
<thead>
<tr>
<th>Module Phase</th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>1.26</td>
</tr>
<tr>
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<td>1.14</td>
</tr>
<tr>
<td>3</td>
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<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>2.76</td>
<td>1.04</td>
</tr>
</tbody>
</table>
Table 4.4. Independence Rating Post-hoc Tukey Results. This shows the significance using a post-hoc Tukey test between each combination of the four phases of the module.

<table>
<thead>
<tr>
<th>Module Phase (I)</th>
<th>Module Phase (J)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
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</tr>
<tr>
<td>1</td>
<td>3</td>
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<tr>
<td>3</td>
<td>4</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Teachers’ Perceptions Throughout the Module

During the interviews conducted after each phase of the module, teachers were asked to describe their experiences as well as describe their perceptions of their students’ experiences throughout each phase of the module. The results presented here are the descriptions of how teachers’ perceived their students’ and their own roles and experiences during the CASPiE module.

*Skill Building*

During the Skill Building Phase, both teachers described situations in which students had a multitude of questions as they were completing their labs:

Teacher 1: I would say in that first phase, in that Skill Building Phase, it was very teacher dependent. The students just had a lot of questions for me and I felt really stretched.

Teacher 1: Almost before they would go to the next step in their process, they felt they needed to check in with me before they proceeded.

Teacher 2: I felt like the instructional leader of the classroom in phase one [Skill Building], like I was telling them exactly what to do and they just kind of took me at my word and we kind of walked through that process together.
Teacher 2: I’d say they weren’t very independent at all. Especially during the data analysis, they were very dependent on me.

Both teachers convey that they perceive the students as very dependent on completing the Skill Building phase of the module. This includes the lab experiments and the data calculations that accompanied both Skill Building experiments. During the calculations, students were given explicit instructions and sample calculations were performed by the teachers. The sample calculations were projected so the class could follow along before proceeding on their own with the remaining calculations. Even with those instructions and examples, Teacher 2 still felt that students were very dependent on her to complete the calculations. The second quote from Teacher 1 also suggests that students were asking for validation from her as they completed each step in the lab. This could suggest a lack of confidence on the students’ part. What seems to be clear is that both teachers experienced Skill Building as a time when the students were very dependent on them to complete the tasks during that phase of the module. They did not perceive that the students were acting as problem solvers. Instead, they saw their students as constantly asking them to give them answers or guide them through procedures. More detail about specific types of questions students asked will be provided in later sections of this dissertation.
Experimental Design

For the Experimental Design phase, the teachers describe a different environment than the Skill Building phase.

Teacher 1: They [the students] seemed more focused with the Experimental Design. They finally started brainstorming off of each other and talking to their own group members as to what they wanted to do. At that point, that’s when I think it became their own. Students had few questions and their questions were more double checking – I suppose because they were more math-based.

Teacher 2: I think I was more of a resource rather than an instructor. During the Skill Building I felt more like I was leading them to a specific goal and pretty much teaching them and pushing them. When we entered the experimental design phase I really felt a shift in terms of I’m now just a resource to answer questions but also to ask the right questions to help them figure out what to do.

Both teachers describe a shift in how each of their students worked and what their roles as teachers were in the classroom as compared to the previous phase of the module. The teachers present an environment where students are more focused and also dependent on their lab partners instead of just the teacher. This is the first time that either teacher describes a situation where it was observed that students were working together to complete the task and not immediately coming to her for help as described in the Skill Building Phase. Teacher 1 did mention that there were some math-based questions, so students are still somewhat dependent on teachers for checking calculations.
Teacher 2 also saw that students seemed to still have a dependence on her when it came to calculations.

Teacher 2: There were still several questions. Not in terms of what to test. In terms of what dilutions to choose, there were a lot of questions. At the very beginning of that Tuesday I showed them an example with different dilutions and how to write a procedure... Multiple groups didn’t know what the process of a dilution really was.

Teacher 2 continued on to discuss how she had walked several lab groups through the mathematical operations as well as the physical process of making a specific dilution. Similarly Teacher 1 had gone over dilution calculations and preparation with her class before the start of the module. Overall, the teachers describe their students working independently as lab groups and relying more on their lab partners to complete their task as compared to the Skill Building phase. Students are more independent from the teacher as compared to Skill Building, as well. However, students do feel that there is a certain amount of dependence on the teacher when it comes to math-based issues for their students.

Although she did describe students as gaining independence from her during this phase, Teacher 1 also said that she did not think students were in complete understanding of the independent research opportunity.
Teacher 1: Another set of questions was ‘What if we did this?’ or ‘Could we do this?’ I think they felt like they needed to ask permission. They’ve never been in a situation where it’s up to them and I think they thought they needed my permission when really it’s their group’s research. . . They don’t understand it like that yet. At least that’s the way I’m perceiving it. They are still thinking in the confines of the lab that I’m running. They aren’t looking at it as a lab they are designing and running. . . I think there is so much more and they’re not seeing the big picture right now. If they did see the big picture they would see, ‘Oh, it’s really mine.’

So although students seem to be gaining independence, they still are not taking complete ownership and grasping the concept of the freedom they have in designing their research project. Teacher 1 perceives that her students’ still do not understand that this project is truly theirs and that they have not taken full ownership yet.

*Independent Research*

The descriptions of the classroom dynamics by the teachers in the Independent Research are of a very student-centered environment with the teacher having a very minimal role.

Teacher 1: In the [Independent] research part, that was probably the part where I was more of an observer at that point and they knew exactly what they were doing. I felt like they had become even more independent and that was the best they were working together so far. They each kind of had roles to do and I could really take a step back and watch them all working at that point.
Teacher 2: Honestly, I felt like I was wandering around and didn’t have much to do. At some points, I mean, there are always students to redirect. You know, in terms of questions about procedures, there weren’t very many. . . I think they worked collaboratively and, you know, independently. . . my role was a lot less formal and more…I really didn’t feel like I was teaching them anything. I was there to help them do what they were doing. I was more of a facilitator rather than a teacher. I facilitated the environment they needed to do their research.

Both teachers describe the lab groups as not only being independent, but also that the lab group members had assumed roles and that they were working well together. This is a big change from their description of the work in the Skill Building phase. It is even a shift from the Experimental Design because in this phase the teachers describe themselves as “observer” and “facilitator” and an occasional resource as compared to the previous phase. Another difference as described by Teacher 2 is that she no longer has the pressure (due to the abundance of questions) to give students a direct answer (as described in her quote in the Skill Building phase previously) to help them, but now she can facilitate the students towards them finding a solution to a problem. When asked about how she responded to students’ questions, her response was:

Teacher 2: Honestly, I wasn’t very direct. I just said, ‘Well, you need to get the juice out, so what do you think is the best way to do that?’

Teacher 2 went on to say that responding by posing those questions resulted in the lab groups discussing what to do and eventually reaching a solution on their own.
There was however, an exception to the students’ independence.

Teacher 1: The data analysis of the HPLC, they had just almost completely forgotten from the Skill Building data analysis. They had forgotten about setting up the standard curve and it was helpful we all had the packets (previous instructions) and they were able to go back through. They had even forgotten how to enter in the data. I referred them back to the Skill Building HPLC. Once they saw the Skill Building, it jogged their memory.

Although students did have difficulty with their data analysis calculations, the teachers were able to refer them back to their previous Skill Building calculations, so they were able to facilitate how students could figure out their dilemma instead of directly solving their problem.

Results and Poster Presentation

In terms of the students working to present their results on their posters, the teachers describe their students’ lab groups as being independent and themselves as being more like observers of the students.

Teacher 1: In this last part with the poster design I was more just observing them, asking them questions and they really, kind of, at that point felt like there were on their own.

Teacher 2: Now that we’re in the poster phase, they are pretty much completely independent.

One teacher did mention helping a lab group with something on their poster and that was helping them to make a graph in Excel®, however, neither teacher described any other situations where students were dependent on them or students in other lab groups.
When each teacher was asked to briefly summarize their role throughout the module, here is how they responded:

Teacher 1: I would say that in the Skill Building phase it was very teacher dependent. The students just had a lot of questions and I felt really stretched. I was just trying to answer all their questions. Then we moved into the Experimental Design phase and students had few questions that were more double checking, I suppose because they were more math-based. In the [Independent] research part, that was probably the part where I was more of an observer and they knew exactly what they were doing. This last part with the poster design I was more just observing them, asking them questions and they really, at that point, felt like they were on their own.

Teacher 2: I kept using the word that I felt like the instructional leader of the classroom in phase 1 [Skill Building]. Like I was telling them exactly what to do and they just kind of took my word and we kind of walked through the process together. In phase 2 [Experimental Design] and phase 3 [Independent Research], I was more like a resource. I tried to help them, but I wasn’t leading them. They were taking ownership of it. And then in phase 4 [Results and Poster Presentation] they completely took ownership of what they were doing and how they were going to report their results and I felt like my role was just to monitor.

Comparison of Students’ and Teachers’ Perceptions

During the Skill Building phase of the module, both students and teachers perceive that students needed some type of assurance as they completed the experiments. Both the students and teachers described situations where the students were dependent upon asking teachers for help or validation that what they were planning on doing in the lab was correct. One contrast in the perceptions described by the students and teachers during the Skill Building experiments is that students discussed how they would try to
problem solve with their lab partners, then consult with students in other lab groups before asking the teacher for help. The students describe this system where lab groups constantly helped other lab groups to complete the Skill Building labs. The teachers, however, describe the Skill Building labs as a time when they were almost overwhelmed with the amount of questions they were receiving from students. They were not aware that students were trying work within their lab groups and with other lab groups to work their way through the experiments. Instead, teachers perceive that students were very dependent on them for help and were not trying to problem solve within their lab groups or with students in other lab groups. It is an interesting contrast that students describe a community that developed where students were helping each other through the experiments even though they were in different lab groups and the teacher described the students as coming to them with so many questions, not realizing that students were working together. Students even describe how they had worked with other groups, sent one person to ask the teacher a question, then disseminated that knowledge to other lab groups. In a sense, the students perceive asking the teacher for help as a sort of last resort and also as a way to help other lab groups, whereas the teachers perceive that students were coming to them immediately whenever a problem arose. Overall, however, it seems that students and teachers both agree that the students were not very independent of the teacher and that this was a relatively difficult phase for the students.

In the Experimental Design phase, students perceive that they worked well with their lab partners to negotiate and to reach a consensus in designing their independent research project. Students express that they did not consult with other lab groups during that process and that they utilized their teacher and me minimally as they designed their
experiments. Their interactions with the teacher and myself seemed to be simply to ask the opinion of either of us on any of their ideas for their project. One area in which students did describe needing help in Experimental Design was dilutions – this included both calculating what was needed to make dilutions as well as writing procedures on how to prepare the solutions for their proposed experiments.

Similarly, teachers did perceive that students were working well within their lab groups during the Experimental Design phase. They describe their students as being more focused on their tasks than in the Skill Building phase and working more as a group. Just as the students described, teachers still perceive the students as being dependent on them for the math-based questions that concerned dilutions for their experiments.

In terms of difficulty, Figure 4.1 does show that, on average, the students from School 2 viewed this as the most difficult phase of the module. As previously mentioned, three students from School 1 expressed in interviews that the Experimental Design phase was the most difficult for them. When the Teacher 2 was asked to comment on what she perceived as the most difficult for her students and value of the phases of the module, her response was:

Teacher 2: I think the most difficult was probably the experimental design – designing their own experiment based on what they did. I think it was valuable for them to gain lab experience with some of the tools and be able to use instruments they probably wouldn’t otherwise be able to use. I think it was useful to them to design their own experiment . . . so I think those two pieces were really valuable.
Teacher 2 did offer that felt that the Experimental Design phase was the most difficult for the students, which is consistent with the students’ perceptions. Although Teacher 1 did not talk about what was most difficult for her students, she did comment on what she thought was the most valuable for her students:

Teacher 1: I think the most important part or the most valuable part was the experimental design, because that was the part where I feel like they finally realized, ‘Hey, this is mine. I get to design this and it’s not my teacher telling me what to do. It’s me coming up with this.’

Both teachers consider this a very valuable experience for their students, which is not surprising since both teachers had, up until this time, only utilized traditional types of experiments where students did not have the opportunity to formulate their own experiments and research projects.

The general perception from students about their view of the Independent Research experiments is that they were easy, because they had done similar labs in the Skill Building phase. They report that because they had previously done these types of labs, they did not need help from students in other lab groups or the teacher and could complete the labs by working with their lab partners. They also described dividing up tasks – either individually or in sub-groups – in order to complete their experiments. In Skill Building, students’ descriptions of their lab work tended to be about groups working together and seeking validation for the correctness of their work, whereas in Independent Research, students describe using the tactics of division of labor (defined in Table 3.5) to complete their experiments. In terms of students ranking the difficulty of this phase (Figure 4.1), the average shows it is ranked as being slightly more difficult than the Skill
Building, but not as difficult as the Experimental Design phase. For independence (Figure 4.2), it is ranked as students being more independent than the previous two module phases. This seems to be consistent with their description of their lab work with their lab partners and not having needed to seek help from outside of their lab group.

In the Independent Research phase, the teachers also view their students as being independent as lab groups. They report that they observed that students had assumed roles in their group to get tasks done, which is consistent with employing division of labor. Teachers describe themselves as observers and facilitators in the classroom and state that they did not receive as many questions from students as they had in the Skill Building phase. The teachers did express that students still had a certain amount of dependence on them when it came time to performing data calculations and creating the appropriate graphs. Although teachers admit that some of the issues were related to students’ lack of experience using Excel®, teachers often found it necessary to refer students back to their data calculations from the Skill Building phase of the module. Students tended not to mention needed help in their lab calculations, but both teachers cited that they did have to help students by directing them to their prior instructions and calculations.

For the Results and Poster Presentation phase of the module, both students and teachers describe an environment where each lab group worked independently to report their results in the form of a poster. As seen in Figures 4.1 and 4.2, students generally perceive this as an easier phase of the module and that they worked very independently. As they did in the Independent Research, the students describe how they used division of labor by dividing up tasks to complete their posters. The teachers did not comment on
the division of labor as described by the students, but their perception that lab groups worked together – independent of external help – to complete their posters is consistent with the students descriptions of their work to complete their posters.

To summarize the students’ and teachers’ perceptions of experiences throughout the completion of the CASPiE module, both describe shifts in students’ and teachers’ roles in the classroom. Students and teachers both describe the classroom dynamics during the Skill Building phase as one in which students are very dependent on the teacher for help and for verification that they are following procedures correctly. Although, the teachers seemed to be unaware of an element of classroom dynamics that involved a sort of subculture where students worked along with other lab groups as part of a community effort to complete the tasks during that phase of the module. During the Experimental design phase, both teachers and students describe a classroom dynamic where lab groups work more cohesively with each other and more independent of those outside of their group than in the Skill Building phase. Lab groups engaged in discussions and negotiations to formulate their research project and utilized the teacher for opinions that they could use to evaluate their proposed projects. In this environment, teachers shifted from the role that was more of a director in Skill Building to more of a facilitator in Experimental Design. Both students and teachers describe the Independent Research phase as an environment where lab groups worked almost exclusively independent of any outside help. Most lab groups worked to complete this phase utilizing division of labor for smaller tasks to complete the larger tasks of the module. Teachers also report that they observed lab groups employing division of labor. With division of labor, students assumed roles within their lab groups to complete tasks to
Contribute to the overall completion of this phase of the module, which is a shift from the manner in which the lab groups worked together to complete the Experimental Design. In the Results and Poster Presentation, the classroom dynamics seem to be much the same as what was established during the Independent Research phase. Teachers tended to be more of observers and facilitators and lab groups worked independently with students assuming roles within their lab groups and employing the techniques of division of labor to complete their tasks.

Ethnography

It is evident in examining the data and results presented in this chapter that there was a dynamic culture that developed throughout the completion of the CASPiE module in each of the classroom settings. I describe the culture as dynamic since it seems to be constantly changing and evolving as the students progressed through the four phases of the module. As students completed the Skill Building phase of the module, both classes exhibited a culture of students working together in their lab groups, but also interacting with students in other lab groups for help and assurance as they completed tasks. The students developed a culture amongst themselves in which they described helping each other – regardless of their lab group – so that all the lab groups could succeed in completing their labs. There was also a community, which included the teacher and myself in roles as resources for facilitating the completion of the Skill Building tasks. One difference between the cultures that developed in the two schools is that the students in School 1, who were in a second year chemistry course, had more verbal interactions
during the Skill Building phase as compared to the participants in School 2, who were in a first year chemistry course.

As students progressed through the Experimental Design phase, they seemed to have fewer interactions with students from other lab groups, the teacher and me. In this phase, there seemed to be a subculture that developed within each lab group in each setting. This subculture involved students working together to discuss and negotiate their ideas as they designed their research projects. During this phase the role of the teacher shifted from that of directing students and answering questions to a role where the teacher was more of a facilitator for the students as they worked in their lab groups to propose an original research project and write their experimental procedures to be executed in the next phase. The students became more independent in this phase and relied on the teacher for help mainly with math-based problems.

During the Independent Research phase of the module, a community and culture seemed to develop within each lab group. During this phase students began to assume more defined roles and adopt a work ethic that included division of labor to complete the tasks within this phase. The teacher’s role was, like in the Experimental Design phase, more of a facilitator who was an outsider to each lab group, but was available as a resource when necessary. Similar to their roles in the Experimental Design phase, teachers seemed to be used by students as a resource for math-based problems. This is a significant shift from their role during the Skill Building phase where students were highly dependent on teachers for executing lab procedures.

As students worked to complete the final phase of the module, they tended to work in their groups and continue to use a method of division of labor to present the
results of their projects in the form of a poster. Much like in the previous phase, students worked very independently and rarely sought outside assistance to complete their tasks. Thus it can be seen that each class had a dynamic culture that evolved as they progressed through the module to complete the various task requirements. The roles of the students within the classroom and within their lab groups shifted, their dependence on their lab partners and those outside their lab groups shifted, as did the role of the teacher. The data suggests that these cultural shifts occurred as the tasks change throughout the module and as students’ confidence in their work and ownership of their work increased as they progressed through the module.

Activity Systems

As students completed the CASPiE module, many of the relationships of activity systems theory (see Figure 3.1) were observed and reported by students and teachers alike. It could be considered that the ultimate outcome of the module was the completion and presentation of posters by each lab group. However, I believe it is more appropriate to consider the relationships of activity systems theory as it applies to each phase of the module, each with its own outcome. Much like the dynamic classroom culture, there are also shifts in which relationships are more relevant for students in the completion of each individual phase of the module.

The ultimate outcome for Skill Building was for the students to complete lab tasks that afforded them the opportunity to learn and practice the protocols for measuring antioxidant capacity and vitamin C content in a given sample of tomato juice. This included performing the appropriate data calculations, which, like the experimental
protocols, would later be applied by the students for their projects. Students received the procedures, which is represented as the object in activity systems theory, and were instructed to complete the lab procedures and appropriate calculations. To complete their procedures, students worked within the community that developed within their classroom. This community included relationships that were established between students in each lab group, between students in the class as a whole, between the students and their teacher, and also, at times, the relationships between the students and the researcher. This community was established through students’ use of their cultural tools of language and communication with each other. Communication was the key to establishing the relationships within the classroom community and allowed for students to work with members of the community to utilize the material tools provided to complete the tasks of the Skill Building module. This seemed to be very prevalent during this phase of the module as students often described situations of asking students in other lab groups for help or verifications as they worked through the lab procedures provided. Although it should be mentioned that there are classroom rules, the relationship of the rules of the classroom seemed to be implicit in the students’ work as students completed the module. There were no explicit instructions on what the rules were, but it can be assumed that classroom rules had been established prior to the students completing the module. (This implicit nature of the rules remained consistent throughout all the phases of the module.) In addition, the practices of division of labor were not reported by the students nor observed during this phase. Thus, for the Skill Building phase of the module, the strongest relationships in reaching the goal of completing the tasks required were the relationships between the individual students as part of the classroom
community and the mediating artifacts, which include both the cultural tools of communication and language and the material tools provided in the classroom.

During the Experimental Design phase of the module, the culture of the classroom shifted, as did the relationships that were important to completing this phase of the module. The ultimate outcome for this phase was for each lab group to have developed a research project that explored the antioxidant capacity and vitamin C content in a food of their choice and write the laboratory procedures needed to complete the experimental part of their project. The object in this phase was the set of guidelines that presented the requirements for Experimental Design. During this phase, the classroom community evolved from what had developed in the Skill Building phase. Although an overall community still existed, each lab group now developed as its own community within the larger community of the classroom. Within each lab group community, individual students used communication skills to express their ideas for their group’s project. As students wrote down ideas and discussed these ideas, the students tended to use the cultural tool of negotiation to reach agreement in their lab group and to write their experimental procedures. In writing these procedures, students also had to consider the material tools that would be available to them in the laboratory so that they could decide whether or not their procedures would be feasible. It also should be mentioned that the teacher was still part of the larger classroom community and that individual students and lab groups did have occasion to interact with the teacher for assistance during the Experimental Design phase. It should be noted that the issue of rules in relation to the tasks completed did arise. With the development of the individual research projects, it was reported by Teacher 1 that students did ask questions such as ‘Can we do this?’ as if
to ask permission. This suggests that students may have been conscious of their established rules of the classroom and wanted verification from the teacher that they would still be working within the rules of the classroom. As was the case in Skill Building, division of labor practices seemed to be absent in the process of completing the Experimental Design phase of the module.

The object in the Independent Research phase is the experimental procedure that each lab group prepared in the previous phase. The outcome for this phase is the collection of data by executing the experiments and performing the appropriate calculations as had been done in the Skill Building phase. Similar to the Experimental Design phase, there is the whole class community, but also the community of each lab group. Once again, students used communication – mainly within their lab group – to complete the tasks at hand. These tasks, of course, were completed utilizing the tools available in the classroom. One shift from previous phases was that within each lab group community, students began to assume specific roles in working within their lab groups. This is related to the lab groups using a system of division of labor to complete their tasks. Most lab groups worked as a community and through communication devised a system of division of labor to complete the Independent Research phase of the module. As described previously, working within the rules of the classroom is an implicit part of the classroom itself.

As students worked with their data to display their results and explain their conclusions in poster form during the Results and Poster Presentation phase of the module, the relationships that were established during the Independent Research phase remained unchanged. Once again, the rules of the classroom were an implicit part of how
students completed their tasks. The students communicated – mainly within their own lab group community – to complete their tasks by employing methods of division of labor.

Cognitive Apprenticeship

A cognitive apprenticeship environment must include skills to be learned that are presented in an authentic context, an expert who provides the tools necessary to complete the task with the embedded skills to be learned as well as model the skills to be learned, and an appropriate social context that reflects the environment modeled by the authentic context (Collins, Brown, & Newman, 1989). The CASPiE module implemented in this study provided the scaffold from which a cognitive apprenticeship environment developed in each classroom. The module used in this study was designed such that an authentic context of research in the field of nutritional studies – in this case, specifically antioxidants and vitamin C – was established in an introduction to the module. The teacher and I were the experts who provided the appropriate tools and were able to work with the students and model skills by providing demonstrations, examples, or instructions as needed. The appropriate social context was developed by having students work in lab groups in their classroom in which their peers, the teacher, and I were available as resources. The skills to be learned were embedded into the Skill Building phases of the module. Upon completion of the Skill Building experiments and appropriate data calculations, lab groups applied what they had practiced to design their own research project in the Experimental Design phase of the module. The lab groups had to work to choose a food to study, propose a treatment to be performed on their chosen food, and
write detailed procedures for laboratory experiments in which they would collect data to analyze. In the Independent Research phase of the module, they worked in the lab to perform their experiments as well as the necessary data calculations. The module then culminated with students drawing conclusions based on their analyzed data and creating a poster, which was modeled after posters that would be presented at a scientific conference. Thus implementing the CASPiE module facilitated the establishment of an environment that mimicked the process of real scientific research. This included learning about a specific area of research, practicing the skills needed to engage in experimental research, applying those skills to develop and execute a novel research project, and presenting the findings to peers in that field of research in an appropriate forum.
CHAPTER FIVE: CONCLUSIONS, LIMITATIONS, AND IMPLICATIONS

In this final chapter I will first present assertions based on the coded interview data comparing the classroom dynamics between the Skill Building and Independent Research phases of the module. I will then discuss the limitations of this study. This will be followed by presenting the conclusions of my work and the implications for how this is applicable in science education and future research in this area.

Comparison of Dynamics Between Skill Building and Independent Research Labs

**Assertion 1:** Lab groups become less reliant on the teacher and their peers in other lab groups when completing an Independent Research lab project as compared to the traditional Skill Building indicating that a cognitive apprenticeship environment has resulted in increased independence, as predicted by that framework.

In comparing the coded interview data of student-student interactions between lab groups in the Skill Building and Independent Research (Table 3.6), it can be seen that there is a sharp contrast in the frequency of lab groups interacting with each other. Questions about how to perform lab procedures and checking each other’s work for correctness are very commonplace during the Skill Building phase. However, these are nearly nonexistent in the Independent Research phase. An example of interactions among students in different lab groups during a Skill Building lab can be seen by the following interview response:

Interviewer: So both of you feel like there was a lot of communication between lab groups?
Interviewer: And what about you, did you talk to other lab groups?
Student 6: Yes, we asked often, and I think we talked to the other groups often. We tried to see if they know what is going on.

(Skill Building interview)

This example shows that students were not confident enough with the lab work of their own group, so they often would ask other lab groups to see “if they know what is going on.” There were several responses from students with instances of having interactions with other lab groups. The more specific example below involves three students in different lab groups discussing how they worked together to complete the first set of Skill Building experiments.

Interviewer: How about things you discussed with your lab partners? Were there things you had to ask your lab partners or your lab partners asked you during that time?
Student 11: Yeah, we usually just help each other out. The different groups... one group will ask the teacher then everyone else knows.
Student 10: Kind of like...
Student 11: We all work together pretty much.
Student 10: When we were doing the dilutions, like the .05, when we were doing equations to figure out the dilutions and how much stuff to put in there. Just double checking all that kind of stuff.
Interviewer: That was double checking with...
Student 10: With other groups.
Interviewer: So was there a lot of interaction between other lab groups?
Student 10: Yeah. (nodding)
Student 11: (nodding yes)
Student 3: (nodding yes)
Interviewer: Really?
Student 10: Yeah.
Interviewer: Can you think of specific instances where you went to another lab group or another lab group came to your lab group?
Student 11: For everything new.
Student 10: Yeah. You know, like “are you guys doing this step right now?” “Yeah, this is how much we’re adding.”
The dialogue among these students exemplifies a lack of confidence in their own group members to complete the tasks. The description of double-checking their work with other lab groups shows how unsure they are of their own group’s work, and that validation or consensus by peers in other groups is needed to complete the tasks. Also, a student from one group obtained information from the teacher and shared that with the other lab groups. Thus, the small lab groups are so dependent on each other that they seem to function as one large lab group to complete the lab tasks.

In contrast to the Skill Building interview responses, when students were asked about their interactions with other lab groups in the Independent Research interviews, most students just responded that they only worked with their lab partners and did not go to other groups. For example:

Interviewer: Compare this [Independent Research] to those first Skill Building labs at the beginning.
Student 11: At the beginning we used other groups to compare how to do it and stuff, but now we all know how to do it so we don’t need other groups.”
(Student 5 and Student 6 nod “yes” in agreement)
Interviewer: How do you guys feel about that?
Student 4: Yeah, about the same.
Interviewer: Why do you feel you were able to work more independently?
Student 11: We knew how to do it.
Interviewer: Because you had already done it?
Student 4: Yeah.
Student 6: Because we were more confident. The first time it was more or less (pause) I remember the first time messing with it so we were stumbling along. Now we knew what to do so we could walk right through it.

(Independent Research interview)

It is important to note that the procedures for the Independent Research phase were determined by each group of students themselves, and would likely vary for other groups’ procedures. Although many of the general lab techniques, which were part of the Skill Building labs, employed in each groups’ procedures were the same, each group had a unique set of procedures. Thus it is not surprising that groups would be less likely to ask a question or do the double-checking that they described in the Skill Building interviews. However, students cited that they did not need to consult with other lab groups because they knew what they were doing. One student even mentioned that they (lab group) were able to work more independently of other lab groups because of an increase in confidence from having done the prior Skill Building labs.

Although not as sharp of a contrast, there is a noticeable difference in how much the teacher was needed for questions regarding lab procedures and problems in the lab that students could not solve on their own (Table 3). This is evident when comparing the responses of Teacher 1 in the Skill Building and Independent Research interviews when asked about the labs where students prepared samples for the HPLC for each phase.

Teacher 1: I noticed a lot of procedural questions during that time.
(Skill Building interview)
As compared to:

Teacher 1: I didn’t notice any procedural questions. There were a couple of “Hey, where is this? Where is this located?” but no procedural questions.

(Independent Research interview)

Teacher 1 provides a clear contrast of how students were dependent on her in the Skill Building phase to answer procedural questions, but then notices that in the Independent Research labs there were no procedural questions – only questions about where materials were located in the classroom. This is consistent with a previous student quote that cites an increase in confidence from having performed the Skill Building labs.

Likewise, Teacher 2 provides a contrast of how her students worked during the Skill Building and Independent Research labs. In the Skill Building interview and in her journal she describes how she was bombarded with questions during the Skill Building labs and she noted that she observed lab groups also going to other lab groups for help.

Teacher 2: Yeah, so in terms of frequency, I remember very clearly. Um, that it was like everyone was asking questions all the time. I wasn’t, I wasn’t bothered by it, but it was, I had to move quickly from group to group because students were asking a lot of questions and were unfamiliar with what they were doing. I think a lot of those questions could have been answered by reading the lab, but um, I wasn’t bothered that they asked them because I think a lot of them were just trying to make sure that they knew what they were doing. So even though the procedures were written clearly, they just wanted to double check.

(Skill Building interview)
Teacher 2: I spent a lot of time answering questions about procedural issues. While most of these questions could be answered by the lab procedures provided, I think that many of the students just wanted to carefully double-check before they performed the steps. I thought it was interesting that many students asked other groups during this process what to do if they were stuck on a specific step. They used each other and the teachers in the room to help guide them through the process.”
(Skill Building teacher journal entry)

Both the interview response and journal entry paint a clear picture of how much her students were dependent on her during the Skill Building labs. She comments that not only was she getting a large number of questions, but that most of these questions could have been answered simply if the students reread the procedures. Teacher 2 also noted that she observed lab groups working with other lab groups to complete the labs, which exemplifies how much students relied on their peers in other lab groups in the Skill Building labs.

In contrast to the Skill Building experience and observations of Teacher 2, during the Independent Research Interview she gave this response to a question regarding students’ questions in the lab:

Teacher 2: In terms of the actual lab procedures there were very few questions. Honestly, I felt like I was wandering around and didn’t have much to do. At some points, I mean, there are always students to redirect. You know, in terms of questions about the procedures there weren’t very many.
(Independent Research interview)

It is an interesting comparison of how Teacher 2 describes her students performing the Skill Building and Independent Research labs. In the Skill Building she states that not only was she getting a plethora of questions, but they were questions that could mostly have been answered by reading the procedures. She also mentions her
students seemed to have a need for their work to be checked, which could be seen as a lack of confidence in their work, as well as the observation that students were asking questions of students in other lab groups. This is a sharp contrast to the scene she describes during the Independent Research labs where she felt like she “didn’t have much to do.” Both Teacher 1 and Teacher 2 experienced a shift in their roles in the classroom as student lab groups shifted from being highly dependent on the teacher for the Skill Building labs to where students complete the Independent Research labs more independent of the teacher and with more confidence in their own skills. This shift may be contributed to the cognitive apprenticeship environment that is created by the general approach to teaching the module and which begins during the Skill Building phase of the module. The authentic context of the labs in the field of antioxidant research and the social context of students learning skills with expert guidance of the teacher and a researcher follows the theory of cognitive apprenticeship. The independence of lab groups from seeking help from other lab groups and the teacher suggests that the laboratory skills practiced in the Skill Building phase were successfully learned, thus they were able to apply them independently to the lab tasks for their independent research projects. In addition, this ties into activity systems theory in that lab groups worked more independently for each of their research projects, which contributed to the formation of the community of the classroom that was reflective of a scientific community in which various groups worked on novel research projects in a particular area of research.
Assertion 2: Students rely more on their lab partners to complete an Independent Research lab than the traditional Skill Building lab experiment, because students use their cultural tools of communication with their lab partners and form a community within their lab group, which is reflective of activity systems theory.

One of the most notable differences in comparing how a lab group functioned together as a group and relied on each other can be seen by how much they problem solved with their lab partners. More than double the number of students commented on problem solving with their lab partners during Independent Research labs as compared to the Skill Building labs (Table 3). This may be an indication that more problems arose during the Independent Research. However, the frequency of student-teacher interactions regarding solving problems the group could not solve on their own went down for the Independent Research, which points to a shift in how students are seeking solutions (Table 3). This supports the assertion that students work more cohesively as a lab group during the Independent Research phase. It not only shows that they are more independent of the teacher (and other lab groups), but that they are more reliant on each other to complete the tasks at hand. The following description by a student of how his lab group worked to complete an Independent Research lab exemplifies how lab partners relied on each other:

Student 8: We would kind of separate and do the things to get everything prepared and we’d come back as a team to put everything together.” (Independent Research interview)

Not only does this student describe the group’s interactions, but he also refers to his lab group as a “team.” We can therefore deduce that students are not just assigning each other things to do to get the lab done, but are actually relying on each other like teammates who work together and rely on each other to achieve a goal.
Another example that shows students’ independence from their teacher and peers in other lab groups and reliance on their lab partners can be seen here in a response to an interview questions about how they worked through issues that came up during their Independent Research labs.

**Interviewer:** When questions came up, did you just talk to each other about how to go forward in the lab or did you have to ask other lab groups or the teacher?

**Student 20:** My group, we just basically talked it over then everybody started getting it.

**Student 12:** Well, my group, if we had a question we would just ask our group member and usually one of them would know the answer to it.

**Interviewer:** How about your group?

**Student 16:** We didn’t ask the teacher or facilitators any questions. We just talked it over with each other.

(Independent Research interview)

These three students, who were in different lab groups from each other, all expressed how they utilized their lab partners to work through problems in the lab, and not seeking any help from outside of their group. This shows how lab groups became more cohesive as a team and more independent of outside help during the Independent Research lab as compared to their work during the Skill Building labs. In addition, the description given by students is corroborated in both classrooms by the following interview response from Teacher 2 and researcher field notes from School 1.

**Interviewer:** How did your students work in those [Independent Research] labs?

**Teacher 2:** I think they worked collaboratively and, you know, independently.

(Independent Research interview)
Observations: After getting settled, the groups began working very quietly. It’s the quietest class I have observed. Groups appear to be having discussions. Both all-male groups have divided up responsibilities – labeling tape, making data table, setting up cuvettes. Two students in a group discuss how to set up the data table; one explains that there are 2 trials for each dilution so two columns are needed.

(Researcher field notes, Independent Research lab day 3, School 1)

These statements from a teacher and researcher regarding the students’ lab work in both schools describe observable differences in classroom dynamics in comparing the Skill Building and Independent Research labs. This is consistent with the students’ interview excerpts where students said that they did not seek help from outside of their lab groups to complete their Independent Research labs. This shift in the intra-group dynamics can be described by activity systems theory since it is seen that students used their cultural tools of communication to work with their lab partners to complete their lab tasks. This includes employing a strategy of division of labor within the lab group to complete lab tasks with the goal of gathering their data to complete the Independent Research phase of the module and move on to the final phase module.

Assertion 3: Whether students are completing the Skill Building or Independent Research experiments, students are still reliant on their teacher for analysis of results, which is evidence that the data analysis skills from Skill Building did not transfer as successfully as the laboratory skills.

During their data analysis within both the Skill Building and Independent Research phases, students had interactions with the teacher with questions about their data analysis. In fact, there was actually an increase in these interactions in the Independent Research phase as compared to the Skill Building phase (Table 3). In the
Skill Building phase, students were guided through the calculations and graphing with worksheets, and occasionally, a teacher demonstration projected for the class to see.

Here are two descriptions of how data analysis was guided for the Skill Building labs:

Teacher 2: We got our HPLC lab results back today, and spent about 45 minutes going over the results in our lab groups. I had a step-by-step data analysis guide for them to follow so that they would be able to interpret the peak area and figure out the concentration of Vitamin C in their samples. I feel that the students have a firm grasp on the steps to go through, but they do not understand what the standard curve means or where it came from.
(Skill Building HPLC teacher journal entry)

Teacher 2: Analyzing the TEAC data is more difficult than analyzing the HPLC data. Therefore, I took the whole class through the process one step at a time, using exemplar data (that I made up). I performed the step on the overhead, and then waited until all the groups had performed that calculation with their own data. I noticed that most groups assigned one person to be the “data analyzer” (usually someone that was good with math). Students were very dependent on me during this process, and I was glad we took the step-by-step approach.
(Skill Building TEAC teacher journal entry)

In the Skill Building phase, all students first performed their labs to prepare the same set of samples for the HPLC in order to determine the vitamin C content of that given food substance (in this lab, all students prepared the same tomato juice samples). The students received their data reports after the HPLC run was complete and as described above, had a step-by-step guide to take them through the data analysis process. The next set of Skill Building labs had students prepare samples and collect data utilizing a spectrophotometer. This data was used to determine antioxidant capacity of the tomato juice samples. For this data analysis, Teacher 2 had students follow along to perform
calculations using their TEAC data. Although step-by-step approaches were taken for both analyses, there were still questions from students about graphing, such as this:

Student 24: One of the two teammates doing it was confused as to what kind of graph we should’ve put ‘em on.  
(Skill Building interview)

Although the teacher gave explicit instructions using a projected example of how to create a graph, students still had difficulty comprehending what type of graph to create and how to create it. Another concern was performing calculations based on the trend line from their standard curve graph. Here is an example of a student who had difficulty performing calculations:

Interviewer: Were you able to just start it on your own or did you need help?  
Student 29: Um, we needed help. Cause we’re not all, not all of us are good at algebra, but we can get it if somebody shows us how. We asked the teacher to show us how and we got it.  
(Skill Building interview)

The majority of students’ questions were about getting started with the data analysis. Students needed guidance to do the first step – whether it be a calculation or setting up a graph – after which they were able to complete the analysis tasks. This trend was seen again during the analysis of data in the Independent Research phase.

Even though students appeared to gain confidence when performing their Independent Research labs compared to their Skill Building labs, as described in the previous section, this same trend was not observed with the data analysis. In the data analysis – where manipulation of collected data is involved – students did not gain confidence in their work nor independence from their teachers. Students actually became
more reliant on their teachers for help in the Independent Research phase as compared to the Skill Building phase to complete their data analysis calculations and graphs.

The data analysis in both the Skill Building and Independent Research involved calculations with collected data, graphing of a standard curve, calculations that involved using the equation from a trend line from the standard curve graph, and graphing of results after specific calculations were done with the data. Although students were given explicit instructions on all facets of the data analysis during the Skill Building phase and completed the analysis, they were still very dependent on their teacher to analyze their Independent Research data. The same calculations and graphs were to be made for the Independent Research phase that students made during the Skill Building phase, with the only difference being that they were using their own data collected from their independently designed projects. However, the Independent Research data calculations were still difficult for the students. One example of a teacher observation of the analysis during the Independent Research phase underscores students’ dependence on the teacher:

Teacher 1: I repeated myself with each group in explaining how to reconfigure the spreadsheet I had originally set up for them to accommodate their results from yesterday’s run. I spent the majority of today’s class period in helping each group with Excel® spreadsheet issues that involved how and where to input their data to get the correct values. (Independent Research teacher journal entry)

Teacher 1 expresses that even though she had spreadsheets set up for each student, she still had to go to each group to explain how to manipulate the spreadsheet and input their data. To an extent, student questions tended to be focused on how to use functions in the spreadsheet program.
It is clear that in the Independent Research phase, students still had the same types of issues in the data analysis. In fact, Table 3 shows that students made even more mention of issues with the data analysis during the Independent Research phase as compared to the Skill Building phase. Similar to Skill Building, students had difficulty getting started with their graphing to begin their data analysis of their Independent Research labs.

Interviewer: Were you able to do that data analysis in your group and problem solve or did you have to ask questions outside of your group for that?

Student 9: For the data analysis we did have to ask outside of our group.

Interviewer: So who did you ask?

Student 9: I believe it was [Teacher 2] that helped us out with that.

Interviewer: Was that very often or just one time?

Student 9: She helped us and just stayed with us most of the time that we were making our graphs.

(Independent Research interview)

Not only did this student state that her group had trouble in just starting to make the graph, but the teacher had to stay with their group to help them create the graph for an extended period of time. Although the same graphing, calculations, and spreadsheet functions were done in the Skill Building phase, it was still difficult for students to complete in the Independent Research without getting help from a teacher. This next example also shows that students seemed to have the most trouble in just getting started with their data analysis.

Interviewer: How were you guys all starting out? Were you able to just go in and do everything or did you need some help getting started?

Student 5: We had to have help.

Student 6: We had to ask [Teacher 1].

(Student 5 laughs)

Interviewer: OK, so let’s start with 5’s group. Tell us how you got started or what you had to ask to get started.
Student 5: Let’s see. We knew we had to plug in the information we had, but then we came to (pause) an equation that we had to do that we were really confused with.

Interviewer: So were you able to plot the standard curve first before asking the questions or did you need help before you plotted the standard curve?

Student 5: We asked before.

Interviewer: And that was with [Teacher 1]? Can you describe how she walked you through it?

Student 11: She just told us.

Student 5: Well, she told us to open up the one we had before because it had the example of what we made before.

(Independent Research Interview)

Although calculations and graphing in the Independent Research were the same protocol as in the Skill Building, students still relied on their teacher for help in completing those tasks. In contrast to students’ descriptions of their actual lab work, students did not talk about gaining any confidence in completing their data analysis since they had previously done that work in the Skill Building phase. It seems that they can quickly gain confidence in tasks that involve physical actions, but in manipulation and analysis of data, which involves mathematical operations and reasoning skills, they remain unsure of themselves even in their second time following a set of instructions. The reason for the differences discussed in this section in students completion of experiments and data calculations in the Independent Research as compared to the Skill Building may be related to the way in which the experimental procedures and data analysis procedures were presented to the students. To complete the Skill Building experiments students were given procedures to follow. The teachers may have given some tips on certain parts of the procedures to start class, but the students were on their own to follow the procedures to complete the experiments. Although students did ask a lot of questions of their respective teacher during the Skill Building, they also took the opportunity to work
with their lab partners and students in other lab groups to work through problems and complete lab tasks. During the data analysis calculations in the Skill Building phase, both teachers walked students through how to use a spreadsheet and do the necessary calculations and create graphs. Students were given templates on their computer desk tops to create standard curves and written instructions for calculations. During this instruction of data calculations the students mainly focused on following along with the teacher and occasionally checking their lab partners’ computer screens to check that they had the same graphs and answers, which is a very algorithmic approach to teaching a task. This is not consistent with cognitive apprenticeship in that students were given rigid direction and not given the correct social and authentic context that is reflective of the scientific enterprise. This may have been part of the reason that the data analysis skills were not successfully learned to the point that students could successfully apply them in a different setting.

Summary of Assertions

The assertions described above compare and contrast the two laboratory phases (Skill Building and Independent Research) of the module. In examining the students’ lab work, there are cultural shifts that can be viewed through the lens of activity systems theory. During the Skill Building experiments, students were more reliant on using their mediating artifact of communication (cultural tool) to seek help and verification from other lab groups and their teacher as compared to the Independent Research experiments. In the Independent Research phase students functioned more as a team – independent from the other lab groups and the teacher – and also employed methods of division of
labor to complete tasks. This suggests that students gained confidence in their own work and in the work of their lab partners after completing the Skill Building phase of the module. The evidence for this is that when completing similar experimental procedures from the Skill Building phase in the Independent Research phase the students did not feel the need to have their own work double checked or double check their lab partners’ work with other students or the teacher. Thus, the need for validation as they proceeded through experimental tasks diminished from the Skill Building phase to the Independent Research phase of the module.

The shift described above, however, did not occur in students’ completion of data analysis. Students actually became slightly more reliant on their teacher for help when they had to perform appropriate calculations in analyzing their Independent Research data as compared to their Skill Building data. In terms of cognitive apprenticeship, the students did learn to work in the lab with the teacher (expert) in Skill Building and then were able to apply those skills and work more independently to design their experiments and then perform them in the Independent Research phase of the module. However, the data analysis skills did not transfer from their Skill Building experience and they could not complete their calculations without seeking help from their teacher. The data analysis portion of the Skill Building phase was taught in a very step-by-step and algorithmic manner which was different from the way in which students were instructed in the laboratory and different from the way in which real research scientists would work through their data calculations. This method of instructing students to perform data calculations is inconsistent with authentic science since students were not given the opportunity to work like real research scientists to figure out and perform appropriate
calculations with their data. They had no opportunity to problem solve on their own or to even figure out the correct analytical calculations. Thus, we offer the conclusion that the lab skills performed and practiced in Skill Building were learned by the students and then applied in their Independent Research. The data analysis skills, however, were not successfully learned by the students in the Skill Building phase of the module, and thus not able to be applied to their Independent Research because students were not instructed in an authentic context and cognitive apprenticeship environment. Thus, this aligns with the research presented by Chinn and Malhotra (2002) that for students to gain a truly authentic science experience, they must have the opportunity to engage in every facet of scientific research.

Limitations

One limitation of this study is that it reports trends from the collected data that are drawn mainly from responses from the student focus group interviews and individual teacher interviews. Having previously done a pilot study in which individual student interviews had been conducted, I believe that focus group interviews with this particular population generally promotes more engagement in the interviews and richer data. This scenario, however, is not always true. Focus groups for interviews were created with the help of each teacher such that the groups would be comprised of students from different lab groups and that the students would be comfortable enough talking about their experiences with the others in their focus group. Even with these considerations, there were still occasions when one student would answer a question and the other students would simply reply that they agreed or offered something like, ‘that’s how we did it in
our group, too.’ Students were asked follow up questions to elaborate on their experiences, but they often did not provide full descriptions because it seems they felt that other students’ answers were sufficient in conveying their own experiences. Thus rich data were not obtained from every student in each focus group interview. In addition, this study was done in only two settings with two different teachers. Although the teachers did work with different student populations and had different backgrounds and experience, it still only provides two perspectives from the teacher point of view on which to draw conclusions.

Another limitation of this study is that it simply reports on the perceptions of the participants and the classroom dynamics as the CASPiE module is completed. There is no measure as to the quality of work done by the students. There is no gauge as to whether each project was actually novel, nor does it measure any learning gains. Although I report that cognitive apprenticeship was integral to the completion of the module, there is no measure of students understanding of the embedded skills they practiced and applied for their projects or of their understanding of NOS. At the culmination of this study, students were graded by their teacher on their final poster and presentation. Each teacher graded these using her own rubric. Thus, each teacher had her own set of criteria for grading, neither of which examined the authentic nature of each project nor students’ understanding of NOS.

One other limitation was my field notes as a rich data source. I chose not to use any particular observation protocol for this study. At the onset of the study I attempted to develop a system of recording observations to capture the frequency and types of interactions that were occurring within the classroom. This placed me in the etic role as
the outside observer. The issue was that my role changed early on in this study to an *emic* role as I became part of the classroom culture. Thus, my field notes became reflections that I wrote after class as opposed to observations during class. Although there were benefits to my emic role as part of the class, there was a downside. As I interacted with students and lab groups and gained valuable insight into individuals and specific lab groups, I often did not observe the class as a whole and did not have the opportunity to interact with each lab group on any given day due to my time spent with small groups of students in the class.

**Implications**

The purpose of this study was to examine the classroom dynamics and report the students’ and teachers’ perceptions during the completion of a research-based lab module. This was done to gain an understanding of how students use available resources to complete such a module as well as gain insight into teachers’ perspectives throughout the process. In regard to the importance of gaining students’ and teachers’ perspectives, it may have best been stated by Crawford (2000): “Needed are the voices of the teacher and students, which are vital to developing understanding of the nature of an inquiry-based classroom.” The literature shows that many studies have focused on the outcomes of inquiry and research-based science experiences, but never gave a voice to those who actually experienced it. Thus, it is important first to find out how students and teachers experience authentic science practices – and verify that these truly are authentic practices – before studying what is learned from it.
This study aimed to provide students with the opportunity to experience authentic science practices in a cognitive apprenticeship environment. Although all the components of authentic science (Chinn & Malhotra, 2002) were part of the CASPiE module, the experience still fell short for the students in one particular area. By giving students such step-by-step, algorithmic instructions for their data analysis in the Skill Building phase of the module, students were not given the opportunity to work on their own to perform calculations and create graphs. Thus, when the students had to analyze the data they had collected in the Independent Research phase, they were not confident in their work and often had to ask the teacher for help with the calculations. In contrast to this, students were given procedures for the Skill Building labs, however, they still were able to work with their lab partners and other peers in class to interpret those procedures to perform tasks and learn lab techniques. Thus, when they completed their Independent Research labs the students worked independently within their lab groups to problem solve and complete their labs and rarely sought help from outside of their lab group. Similarly, students were only given guidelines for Experimental Design. The results presented show that – although they may have found it difficult – they still were able to complete this phase by working with their lab partners. As with Experimental Design, students were given guidelines for their final poster and presentation, and they worked much the way they did in designing their experiments. It seems that the boundaries of cognitive apprenticeship may have been crossed in providing too much instruction for data calculations in Skill Building. With that one exception, the students completed the CASPiE module working with their lab partners and utilizing other resources at their
disposal (including peers in other lab groups and the teacher) and formed a community that was reflective of the scientific community.

From the results presented in this dissertation, other inquiry-based and authentic science practice modules can be developed. It is important that these modules be developed such that all the components of authentic science are included and that students have the opportunity to complete the authentic science experience just as a real scientist would. This includes learning about the background of the research area, learning and practicing appropriate lab techniques and protocols, performing data collection and analysis techniques, formulating researchable questions, designing experiments, collecting and analyzing data, drawing conclusions, and presenting results. Throughout the process, however, students must have the opportunity to make mistakes, problem solve, make adjustments, and work through any difficulties that they encounter, just as scientists would do. In creating such a module with these described components, students can experience authentic science and a classroom community will developed that is reflective of the scientific community. Once this has been done and implemented successfully, then future studies can focus on examining things such as gains in NOS and learning outcomes.
LIST OF REFERENCES
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Green, K. F. (2010). The research-based laboratory versus the traditional laboratory: An investigation of students’ understanding of nature of experiments and experimental design. Purdue University, West Lafayette, IN. (UMI 3449754)


Kingery, K. S. (2012). *Gender differences in high school students’ confidence in lab and content knowledge: Investigating the impacts of an authentic science curriculum*. Purdue University, West Lafayette, IN. (UMI 1529705)


Nikstad, L. J. (2009). *The impact of an authentic science module on high school chemistry students' perceptions and mental models of laboratory work in school science and professional science.* Purdue University, West Lafayette, IN. (UMI 1476018)


APPENDICES
Appendix A: CASPiE Antioxidant Module

Phytochemical Antioxidants with Potential Health Benefits in Foods

Created by Jay Burgess
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A CASPiE Project Module
About the Author

Dr. Jay Burgess is an Associate Professor in the Department of Foods and Nutrition at Purdue University. His research focuses primarily on oxidative stress in mammals and the physiological results of such an accumulation of reactive oxygen species.

The Burgess lab has studied the role of reactive species in the pathophysiology of attention-deficit/hyperactivity disorder (ADHD) and has shown that some children with ADHD exhibit certain biochemical abnormalities indicative of cellular oxidative stress. They have also shown, in an animal model for ADHD, that supplementing with antioxidants can reverse the abnormalities and improve behavior.

Another project the Burgess lab has been working on involves flavonoid antioxidants. They have determined that such antioxidants can be helpful in reducing oxidative stress, but are not sufficient to compensate for a deficiency of essential antioxidant nutrients like vitamin E.

From this module, Dr. Burgess would like to gain a greater amount of knowledge of the antioxidant activity from different food products currently on the market. He is particularly interested at this time in the antioxidant characteristics of green tea and green tea mixtures with fruit juices, and what happens to these during digestion.

Recent Publications:


Acknowledgements

The author would like to extend appreciation to S. Messenger and J. Andrade for conducting experiments and procedures to test the methods included in this module, and for extensive help in preparing the initial drafts. Sincere appreciation is extended to C. Russell and G. Weaver for extensive intellectual input into the design and construction of this module and for thorough editing of the document. The author is also grateful to A. Bentley and W. Fornes for editing the document and providing additional information.
I. Introduction

We are constantly being told to eat fruits and vegetables, and that these types of plant foods contain chemicals that are good for us. Now the message is that we need to eat up to nine servings a day of fruits and vegetables because this will help prevent people from getting cancer, heart disease, and other disorders that might afflict us as we age. You may have many questions about these recommendations: Why do people have to eat so many fruits and vegetables to obtain these health benefits? What are the chemical components in fruits and vegetables that provide the proposed health benefits? It is common practice to use vitamin and mineral supplements to replace the need to consume so many fruits and vegetables. Are vitamins and minerals alone necessary to achieve the health benefits fruits and vegetables give us? Is this a misconception or has our understanding changed in recent years? You will explore the science that addresses some of these questions over the next seven weeks.

The picture to the right illustrates an example of a vegetable source that is available in many of your local supermarkets. Note that under the name of the product contents is the description “with long lasting antioxidant activity.” Many would associate antioxidant activity with something good, but what does this really mean? As a consumer looking at this package you might wonder what would this so-called long lasting antioxidant activity do for you? Is it this characteristic of the sprouts that make them good for your health? Are these sprouts really better for you than the less expensive sprouts you can buy in the adjacent bin? The more detailed information on the back of the package identifies a specific compound, sulfurophane GS, as something in these sprouts that provides “long lasting antioxidant and cellular function.” The sulfurophane GS is not an essential nutrient such as vitamin C which is also found in these sprouts. This chemical substance is one of a multitude of substances in plants (called phytochemicals) that are thought to explain an observed association between high fruit and vegetable consumption and lower incidence of killer diseases such as cancer and heart disease. Antioxidant activity is hypothesized to be one mechanism by which these chemical substances might exert this protective effect. This characteristic is cited because many of these chemical substances show potent antioxidant activity in the test tube. Despite these observations many questions remain concerning which chemical antioxidants in fruits and vegetables really contribute to a lower risk for chronic diseases and how the substances from multiple food sources interact with one another in a mixed food diet to provide such benefit.

Figure 1. Label of Brocco Sprouts
This introductory section describes the background information on food, nutrients, and antioxidants and their relationship to health. A second section will describe phytochemicals and indicate the chemical characteristics that make them good antioxidants. The final section will provide an overview of techniques you will use in this module and how these assessments are used to evaluate the potential health benefits of phytochemicals as antioxidants.

1. Essential Nutrients in Food

Food provides chemical substances that are required by heterotrophs¹ to allow for growth, reproduction, and the overall maintenance of health. By the first part of the twentieth century the chemical constituents in food that supported these outcomes for humans were identified and classified into groups: water, carbohydrates, lipids, proteins, vitamins and minerals. It was observed during this time that if one did not consume sufficient amounts of these essential nutrients that specific deficiency disorders would develop. A good example is the disease scurvy, which occurs as a result of insufficient consumption of vitamin C. This scientific discovery process was carried out by chemists, biochemists, physiologists, and nutritionists and eventually led to government-supported recommendations for the minimum amount of these nutrients that healthy people should consume to prevent the development of deficiency diseases. Today most disorders resulting from nutritional deficiencies are uncommon in developed countries, but still often occur in the developing world.

You probably recognize the acronym RDA, which stands for Recommended Dietary Allowance. The RDAs define specific amounts for each nutrient which must be consumed to prevent deficiency in healthy people. The U.S. Dietary Guidelines² and the Food Guide Pyramid³ are more practical tools that consumers can use to select combinations of foods to create diets that provide the RDA for all of the nutrients. In recent years it has become apparent to many scientists who study human health that in addition to providing nutrients that prevent deficiency diseases, consumption of certain foods appears to be associated with a lower occurrence of diseases that occur more frequently as we age. These chronic diseases, which generally require decades to develop, include heart disease, cancer, diabetes, hypertension, and Alzheimer’s disease, and are among the leading causes of death in the U.S. population. Fruits and vegetables are the types of foods that are most often cited as helping to protect people from developing chronic disease. However, the essential nutrient content of fruits and vegetables, although abundant, does not appear to account for all of these health benefits. Thus, over the past decade a great deal of research has been conducted to identify how other chemical constituents in fruits and vegetables might help prevent the development of chronic disease in people.

2. Chronic Disease and Oxidative Damage

¹ Heterotrophs feed partially or exclusively off of other forms of life.
² http://www.health.gov/dietaryguidelines/
³ http://www.nal.usda.gov/fnic/Fpyr/pyramid.html
Understanding the processes by which chronic diseases develop has led to the identification of key mechanisms underlying the development of a disease state. Some of these processes include carcinogenesis, the inflammatory process, over stimulation of programmed cell death, and oxidative stress. Oxidative stress is defined as the accumulation of reactive oxygen species (ROS) in living systems to a sufficient degree to cause measurable damage to cells and tissues. ROS are defined as partially reduced forms of oxygen that are either radical species themselves or can easily form radical species. As aerobic organisms that undergo respiration, humans use oxygen during the process of respiration to obtain energy from fuel sources. Molecular oxygen is reduced by four electrons to produce water in the mitochondria of cells (the proton gradient resulting from this electron transfer process drives the formation of ATP). Neither the starting material, oxygen, nor the final product, water, that results from this reduction process is very reactive with large macromolecules that make up the structure of the cells. But univalent (one-electron) reduction of oxygen can form species that are much more prone to react with cellular macromolecules.

As illustrated in the first equation below, one-electron reduction of oxygen leads to the formation of superoxide.

\[
O_2 + e^- \rightarrow [O_2 \cdot]^-(1)
\]

Addition of a second electron along with a source of protons can lead to the formation of hydrogen peroxide (H₂O₂). Two-electron reduction of H₂O₂ will lead to the formation of the final product, water. Alternatively, addition of only one electron to H₂O₂ leads to the formation of hydroxyl radical and hydroxyl anion.

\[
2[H_2O_2 + 2H_2O = 2H_2O + O_2 \quad (2)]
\]

\[
H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O \quad (3)
\]

\[
O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad \text{(overall)}
\]

Eq. 1

Addition of a second electron along with a source of protons can lead to the formation of hydrogen peroxide (H₂O₂). Two-electron reduction of H₂O₂ will lead to the formation of the final product, water. Alternatively, addition of only one electron to H₂O₂ leads to the formation of hydroxyl radical and hydroxyl anion.

\[
H_2O_2 + e^- \rightarrow \cdot OH + OH^- \quad \text{Eq. 2}
\]

Superoxide, hydrogen peroxide and hydroxyl radical are considered ROS because they will either directly or indirectly react with biological macromolecules in living cells such as proteins, lipids and DNA. This interaction will often alter the structure of the macromolecule and destroy its function.

3. How Antioxidants Prevent Oxidative Damage

In living systems the potential sources of ROS include mitochondrial respiration, enzymes such as NADPH oxidase⁴ and other types of oxidases, and exposure to environmental chemicals/toxins such as alcohol, cigarette smoke and surface ozone.

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⁴ NADPH oxidase is nicotinamide adenine dinucleotide phosphate-oxidase.
substances that directly inactivate ROS. Primary antioxidants donate a neutral hydrogen atom (with one proton and one electron) to a free radical ROS, thereby terminating the radical (allowing for the formation of an electron spin-pair in the outer orbital of the ROS) and preventing the molecule from causing damage. Hydrogen-donating antioxidants form more stable, less reactive radical species than the ROS. Secondary antioxidants prevent damage due to ROS by a variety of other mechanisms including metabolism of non-radical ROS, chelation\(^5\) of transition metals and repairing the damage that ROS cause to lipids, protein and DNA.

A good example of an interaction between an ROS, cellular macromolecules and an antioxidant is observed in foods and biological systems that contain polyunsaturated fatty acids (PUFA). In living systems, PUFA are important constituents of cell membranes and serve as precursors to hormones. The PUFA chemical structure is characterized by a long hydrocarbon chain that contains multiple double bonds separated by methylene groups. This arrangement makes the molecule susceptible to hydrogen atom removal and oxygen incorporation. Interaction with an ROS can initiate oxidation of PUFA, which can then lead to continual formation of more ROS as illustrated generally in Equation 3 below.

\[
\begin{align*}
LH + X^- & \rightarrow L^- + XH & \text{L=PUFA; } X=\text{ROS} \\
L^- + O_2 \rightarrow \text{LOO}^- & \\
\text{LOO}^- + LH \rightarrow L^- + \text{LOOH}
\end{align*}
\]

Eq. 3

A more specific example is given in Equation 4, where oleic acid forms a peroxyl radical by reaction with hydroxyl radical (step one) and then oxygen (step two). The peroxyl radical can continue to damage other molecules of oleic acid.

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_7\text{COOH} + \cdot \text{OH} & \rightarrow \text{CH}_3(\text{CH}_2)_7\cdot \text{COOH} + \text{H}_2\text{O} & (1) \\
\text{CH}_3(\text{CH}_2)_7\cdot \text{COOH} + \text{O}_2 & \rightarrow \text{CH}_3(\text{CH}_2)_7\text{OOH} & (2) \\
\text{CH}_3(\text{CH}_2)_7\text{OOH} + \text{CH}_3(\text{CH}_2)_7\cdot \text{COOH} & \rightarrow \text{CH}_3(\text{CH}_2)_7\cdot \text{COOH} + \text{CH}_3(\text{CH}_2)_7\text{OOH} & (3)
\end{align*}
\]

Eq. 4

\(^5\) Chelation is the process of reversible binding of a ligand to a metal ion, forming a metal complex.
Unless an antioxidant is present to stop this continual chain reaction, all the PUFA present will be modified via incorporation of oxygen which will significantly change their characteristic from being very hydrophobic to more hydrophilic. If this happens in a cell membrane, it will lead to cell death.

A good antioxidant that typically protects PUFA in foods and living systems is vitamin E, which is a group of structurally similar lipophilic molecules collectively known as tocopherols. Each form of tocopherol is indicated by a different Greek letter. The vitamin possesses a phenolic ring structure attached to a hydrocarbon chain. It can readily donate a hydrogen atom to a lipid peroxy radical (such as the product of step 2 in Eq. 4) terminating the radical and preventing the further propagation of what is referred to as the lipid peroxidation process.

\[
\text{LOO}^- + \text{EH} \rightarrow \text{LOOH} + \text{E}^+ \quad (1)
\]

\[
\text{E}^+ + \text{appropriate reducing agent} \rightarrow \text{EH} \quad (2)
\]

Eq. 5

In biological systems chemical antioxidants such as vitamin E and vitamin C (L-ascorbic acid or just “ascorbate”) are constantly reused because other supporting systems help to keep them in a reduced state. These systems are mostly enzymatic and don’t function in foods. Thus, antioxidants are often added to foods that contain PUFA to prevent lipid peroxidation and to preserve the concentration of essential antioxidant nutrients. In fact ascorbate can reduce vitamin E and is sometimes added to foods as a preservative.

**4. Phytochemicals as Antioxidants**

There is a multitude of chemical substances in foods that possess antioxidant properties. These fall into numerous antioxidant classes, as shown in Figure 3.
The various chemicals that behave as antioxidants have different properties, for example some are soluble in fats/non-polar solvents and some are soluble in water/polar solvents. In this module we will focus on a class of substances referred to as flavonoids. Flavonoids all possess a basic 3-ring structure as illustrated in Figure 4.

![Figure 4. Basic Flavonoid Structure.](image)

Different subclasses of flavonoids vary in the structure of the C-ring: it may include a double bond between carbons 2 and 3, a carbonyl group at carbon 4, or a hydroxyl group at carbon 3. Within each subclass the specific species vary based on the substitution of hydroxyl or O-methyl groups at positions 5, 7, 3', 4', and 5'. Because these are polyphenolic compounds they all possess some degree of antioxidant activity when evaluated in a test tube. Generally, those chemical species with a greater number of hydroxyl substituents on the ring possess greater activity in the test tube. The flavonoid called quercetin possesses adjacent hydroxyl substituents on the B-ring at positions 3' and 4'. A proposed scheme for how quercetin acts as an antioxidant is illustrated in Figure 5 in which two lipid radicals are reduced sequentially. The single dot next to some of the O atoms indicates an unpaired electron, which indicates a radical chemical species.

![Figure 5. Flavonoid Antioxidant Mechanism.](image)

---

7 A polyphenolic antioxidant is a compound having multiple phenols, or benzene rings with –OH (hydroxyl) substituents.
Flavonoids are abundant in common fruits and vegetables. Table 1 summarizes which types of flavonoids are found in fruits and vegetables.

Table 1. Flavonoids.

<table>
<thead>
<tr>
<th>Flavonoid subclass</th>
<th>Major Food Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonols (e.g. quercetin)</td>
<td>Onions, kale, broccoli, apples, cherries, berries, tea, red wine</td>
</tr>
<tr>
<td>Flavones</td>
<td>Parsley, thyme</td>
</tr>
<tr>
<td>Flavanones</td>
<td>Citrus</td>
</tr>
<tr>
<td>Catechins (e.g. epicatechin)</td>
<td>Apples, tea</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td>Cherries, grapes</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Soya beans, legumes</td>
</tr>
</tbody>
</table>

5. Measurement of Antioxidant Activity

Several methods have been developed to measure the total amount of antioxidant activity that a pure chemical, complex mixture, food or biological sample possesses. All of the methods involve the generation of ROS and the termination of the ROS by the antioxidant being tested. One common method for assessing total antioxidant activity in a food or biological sample, like serum, is the Trolox equivalent antioxidant capacity (TEAC) assay. This is a method that you will use during this module. This method measures the ability of a test antioxidant to reduce a radical species. The radical species exhibits a characteristic absorption spectrum. Therefore, reduction is associated with loss of color at a specific wavelength. Potential antioxidant preparations are tested at several different concentrations and compared to Trolox, which is also tested at several concentrations. Good antioxidants will reduce the absorbance of the radical species at lower concentrations than poor antioxidants. Figure 6 illustrates the overall antioxidant activity of common fruits and vegetables observed when measuring their TEAC activities. Larger values indicate greater antioxidant activity. Notice the value for strawberries is more than two times greater than the value for onions.
Another method for measuring antioxidant activity focuses on a different class of antioxidants: polyphenolics. This measurement uses a reagent, the Folin-Ciocalteau reagent, that reacts with polyphenolic antioxidants to form compounds that absorb light in the visible region. As the concentration of polyphenolics increases, so does the amount of light absorbed by the solution. As with the TEAC assay, there is a standard that is known as epicatechin. Epicatechin is reacted with the Folin-Ciocalteau reagent at different concentrations to form a standard curve. Then potential antioxidants are also reacted with the reagent at multiple concentrations. The absorbance of the potential antioxidants is used in conjunction with the standard curve of epicatechin to find the concentration of polyphenolics in the sample.

The final analysis method that you will use is **L-ascorbic acid (Vitamin C) quantification via high-performance liquid chromatography (HPLC)**. In this analysis method, you will make a standard curve of L-ascorbic acid as well as multiple concentrations of your food samples. The HPLC will separate the components of your solutions, both standard and sample, and quantify the amount of L-ascorbic acid. You can use this to determine the concentration of L-ascorbic acid in your sample solutions and calculate the amount in your dry samples.

### 6. What we don’t know and what you are going to do in this module

Although a number of studies have been conducted with pure phytochemicals and food sources of phytochemicals, much is still unknown concerning the contribution of these non-nutrient compounds to health. Many questions remain unanswered about how these phytochemicals interact in a complex diet both before and after we consume them. Some of the questions are listed below with example hypotheses statements. When these hypotheses are tested, they may help answer each question.

- Is the antioxidant activity of spices affected by cooking?
  - **Hypothesis:** The antioxidant activity of curry powder, a spice known to contain antioxidants, will not be changed by heating the spice to simulate cooking.
- Are dried fruits effective for use in making antioxidant-rich foods as a substitute for fresh fruits?
Hypothesis: Raisins, the dried analogue of grapes, which are known to be high in polyphenolics, are not high in antioxidant activity.

- Does the digestive process significantly alter antioxidant activity of common foods?
  - Hypothesis: Conditions that simulate digestion such as changing pH, mixing, and exposure to digestive enzymes will significantly reduce the antioxidant activity of a dried fruit or spice.

In this module you will apply several of the fundamental properties of chemistry to address a research question about antioxidant properties of food substances that may be beneficial to health. Specifically, you will be examining the effect of the digestion process on green teas and juice/green tea mixtures. Key chemistry topics that will be used in this module include the properties of mixtures, organic compounds (the atomic properties of carbon), and chemical equilibrium. During the first three lab periods you will learn how to measure total antioxidant activity (via the TEAC method), ascorbate concentration and total phenolic concentration in standard and food samples. During lab four you will begin to plan your own research project. As a means of learning how to best design your own research project, you will review a very recent paper testing the effect of processing on antioxidant activity of a food substance. During this lab period you will also identify the experimental question that your group would like to address and design the experiment that you will conduct during the subsequent laboratories. The following table illustrates this proposed schedule.

### 7. Module Calendar

The three main measurements for the upcoming labs are total antioxidant activity as TEAC, ascorbate concentration (using HPLC), and total polyphenolics in standard and test samples. The TEAC measurement estimates total antioxidant activity contributed by both known (ascorbate and polyphenolics) and unknown chemical components for a given sample. Measurement of total polyphenolics concentration is used in this module to reflect the amount of flavonoid compounds present in a test sample.

You will use a few standard substrates in the TEAC assay: epicatechin, quercetin, and Trolox. Epicatechin is a common flavonoid that is found in apples and tea and it is also a component of compounds that are found in cherries and grapes. Quercetin is one of the more abundant flavonoids in our food supply; one of the richest sources of quercetin is fried onions. Trolox is a more polar form of vitamin E, lacking the long hydrocarbon side chain in -tocopherol. Ascorbate is a known essential nutrient antioxidant that functions as such in the body.
II. Laboratory 1: Making Solutions and Spectral Scanning of the Trolox Equivalent Antioxidant Capacity (TEAC) Substrate.

Overview of this Laboratory Activity
During this laboratory period you will make a variety of stock solutions that you will be using throughout the remaining weeks of this module. You will also determine the exact concentration of a solution, which has been previously prepared for you, by using the spectrophotometer.

1. Introduction to Making Solutions
Understanding how to make solutions and how to measure their properties is fundamental to many kinds of research, simply because so many substances that are important to life are either solutions or in homogeneous mixtures. The liquids and solids that make up living systems and foods are mixtures of two or more substances physically mixed together but not chemically combined. Mixtures possess two important defining characteristics: variable composition and retention of individual component properties. Solutions and colloids are two common types of mixtures. A solution is a homogeneous mixture with each component dispersed evenly throughout the space or phase. Salt dissolved in water is an example of a solution. Heterogeneous mixtures exist in separate phases. A colloid is an example of a heterogeneous mixture in which one component is dispersed evenly as very small particles in the other. Milk is an example of a colloid; to the unaided eye it appears to be a homogeneous mixture. Applying centrifugal force to a
milk sample will separate the phases and reveal the colloidal nature of the food. Solutions and colloids differ because the particles in solutions are individual atoms, ions or molecules whereas in colloids the particles are large macromolecules or aggregates of smaller molecules that are still small enough to remain dispersed. In this module you will encounter both solutions and colloids. The chemical reagents that you will use to carry out the experiments in each lab are solutions, whereas the foods that you will analyze may be either solutions or colloids.

Solutions are usually defined as one substance (solute) dissolved in another (solvent) that is more abundant. The solubility of a solute is the maximum amount of the chemical that will dissolve in a particular volume of solvent, usually 1 liter. (Some substances will mix together in any proportion and are said to be miscible.) A major factor which influences the solubility of a solute in a solvent is the relative strength of the intermolecular forces within and between solute and solvent. These forces and their effects are discussed in detail in most Chemistry textbooks. Here, however, we will discuss issues relating to making solutions of various desired concentrations.

Common conventions for expressing concentration include molarity, molality, percent mass and percent volume.

- Molarity (M) = moles of solute / volume of solution (L)
- Molality (m) = moles of solute / mass of solvent (kg)
- Percent mass = mass of solute / total mass of solution x 100
- Percent volume (v/v) = volume of solute / total volume of solution x 100
- Percent volume (w/v) = mass of solute / total volume of solution x 100

In this module, we will primarily be using molarity to express concentration units, but you will also see some substances described with the other units so it is important to be familiar with them, and the differences between them. When making a solution of a desired concentration, say 25 mM for example, you need to determine two things:

- Total volume of solution that you need
- Mass of solute that you must add to get the desired number of moles.

An important point to remember about solutions is that the concentration of the solution will be the same throughout the whole solution. For example, if you have a liter of solution with a concentration of 50 mM and you pour half of that into another container, then the amount you have poured out also has a concentration of 50 mM (as well as the amount you left in the original container.) This may seem like such an obvious point that it shouldn’t even deserve mention here. However, this simple point has a very practical purpose when you are making solutions in the laboratory. If you need a very small amount of solution (for example, 5 mL) and it will be a very dilute solution, then it may be difficult or even impossible for you to weigh the proper amount of solute to make the proper concentration of solution at only 5 mL. But you could instead make 100 mL of the solution at the desired concentration and simply use 5 mL of it, if 100 mL is a more practical volume for weighing your solute. Alternatively, you could make a more concentrated solution and use what is known as a serial dilution technique to arrive at the more dilute concentration that you desire. We will talk about each of these approaches. In either case, your own common sense is important in deciding how you will arrive at your final desired volumes and concentrations. (For example, if you are going to make a larger volume than you need, don’t pick such a large
volume that you would be wasting large amounts of solute – especially if it is an expensive solute.)

**Proper Technique for Weighing Solids**

You will be using analytical balances for weighing solids in the lab. You should keep in mind that these balances are not reliable below about 10 mg (0.010g). So, if your calculations show that you need to weigh out less than this amount, you should consider making a more concentrated solution and diluting to the required concentration. Once you’ve determined an amount larger than 10 mg to weigh out, you still need to be careful about using proper technique so that you don’t waste materials or weigh your sample incorrectly. (Remember, many of the substances that you will be working with in this module are very expensive – as is often true with chemicals used in research studies.)

When you use the balances, you should first place your weighing paper or weighing boat onto the balance and “tare” the balance (see page xxi of your CHM 116 lab manual for details.) You will then place both hands into the balance, one from each side of the balance. In one hand you will hold the container for the solid sample you are weighing, and in the other you will hold the tool (such as a spatula) with which you are scooping it onto your weighing paper or boat, as shown in the image below.

When you have the desired amount in your weighing paper or boat, leave your sample on the scale but remove both your hands and close the doors. The mass displayed with the doors closed is the correct mass for the amount of sample you have weighed out. Be sure that you have not spilled any solid sample on the weighing pan outside of your weighing boat, since this will give you an incorrect reading.
Using Dilution to Reach a Desired Concentration

You can make a more concentrated solution than you need, and then dilute it volumetrically to reach the concentration you desire. When you are trying to achieve very specific concentrations, you should always be sure to use volumetric flasks to make your solutions (see the CHM 116 lab manual for specifics on using volumetric glassware. In particular, it is very important to never fill past the line on the neck. You cannot fill past the line and then remove some liquid, because that will change your concentration.)

When diluting a solution, you need to keep track of the number of moles (i.e. amount of solute) that you are moving from one place to another. Conveniently, the number of moles can be calculated by multiplying concentration and volume:

\[(\text{conc}) \text{ mol/L} \times (\text{volume}) \text{ L} = \text{mol}\]

Let’s take an example where we will start out with a “stock” solution that has a concentration of 25.0 mM (that is $2.50 \times 10^{-2}$ M). A “stock” solution is the solution that you start with, and you usually will have a relatively large quantity of it so that you will use it repeatedly to make your other solutions. For this reason, it is very important that you make your stock solutions carefully and make sure they do not become contaminated (such as by putting a dirty pipette into them.) Let’s assume that you want to make 25 mL of a 0.50 mM solution starting with this stock solution. This means you will transfer some small amount of your stock solution into a 25 mL volumetric flask, and then carefully dilute it with the proper solvent up to the line on the neck of the flask. The question is – how much should you transfer?

Remember to always think about the moles! A 50.0 mL solution of 0.500 mM concentration has $(5.00 \times 10^{-2} \text{ L}) \times (5.00 \times 10^{-4} \text{ mol/L}) = 2.50 \times 10^{-5}$ mol of solute in it. So, you need an amount of your stock solution that will put this many moles into your new flask. You can find this by dividing this number of moles by the concentration of your stock solution:

\[
\frac{2.50 \times 10^{-5} \text{ mol}}{2.50 \times 10^{-2} \text{ mol/L}} = 1.00 \times 10^{-3} \text{ L} = 1.00 \text{ mL}
\]

Therefore, you need to put 1.00 mL (measured in a volumetric pipette) from your stock solution into your 25 mL volumetric flask, and dilute to the line. Another way to arrive at this calculation is to remember the equation $V_1C_1 = V_2C_2$. In this equation, $V$ stands for volume and $C$ stands for concentration. If you take the volume and concentration of the solution you want as $V_2$ and $C_2$, and the concentration of your stock as $C_1$, then you can rearrange the equation to find out the amount of solution that you need to transfer from the stock to the new flask:

\[
V_1 = \frac{V_2C_2}{C_1} = \frac{(5.00 \times 10^{-2} \text{ L})(5.00 \times 10^{-4} \text{ mol/L})}{2.50 \times 10^{-2} \text{ mol/L}} = 1.00 \times 10^{-3} \text{ L} = 1.00 \text{ mL}
\]

Notice that this yields the same answer and is, in fact, the same calculation, all in one step. You must not forget, however, that you are actually achieving this by making the number of moles equal on the two sides of the equation.
2. Introduction to TEAC Assay: Spectrophotometric Measurement of ABTS$^{+}\cdot$ (the ABTS Radical Cation)

One of the solutions that you will make during this lab period provides the radical substrate for the Trolox equivalent antioxidant capacity (TEAC) assay. (An assay is an analysis to determine the presence, absence or quantity of some component in a mixture or substance.) This assay compares the total antioxidant activity in a sample to a standard, Trolox. Because this assay aims to quantify the total amount of antioxidant activity in a complicated test mixture of interest, the substrate of choice is a radical chemical species that exhibits characteristics that can be exploited for quantification purposes. The radical chemical species of the monocation 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), abbreviated ABTS$^{+}\cdot$, is ideal for this purpose. It is relatively stable, is readily reduced by important antioxidant compounds and exhibits a characteristic absorption spectrum in the visible range of light. This last property is one that can be used for quantification purposes. The ABTS$^{+}\cdot$ is synthesized prior to its use by first making separate solutions of ABTS and potassium persulfate in water. Equal volumes of these two solutions are combined and the reaction (outlined in Eq. 6 below) is allowed to continue until it reaches equilibrium after about 12 hours. One electron is withdrawn from each of 2 molecules of ABTS by potassium persulfate leading to the formation of potassium bisulfate and ABTS$^{+}\cdot$. The product formed has characteristic absorbance at several wavelengths. This type of oxidation reaction with ABTS is utilized in many common medical analyses, such as enzyme-linked immunosorbent assays (ELISA) to measure the concentration of important biomolecules in blood or urine.

\[\text{ABTS} \quad \text{(pale green)} \quad \text{+} \quad \text{K}_2\text{S}_2\text{O}_8 \quad \rightarrow \quad \text{ABTS}^{+}\cdot \quad \text{(deep blue-green)} \]

Eq. 6
Colored solutions absorb light in the visible range (400-700 nm) of the electromagnetic spectrum. A compound that would absorb all wavelengths of light in this range would appear black. Others which absorb only at distinct regions within this range appear as different colors. The ABTS$^+$ actually absorbs at several distinct regions in the visible light spectrum. We will use this property to quantify the amount of the ABTS$^+$ in solution using a spectrophotometer and exploiting the Beer-Lambert Law, or “Beer’s Law”. This law states that the concentration (C) of a chemical compound is proportional to its absorbance (A) divided by a constant (molar extinction coefficient or absorptivity [ε]) and path length (l), as follows:

\[ C = \frac{A}{\varepsilon l} \]

These websites explain this in more detail:
http://www.shu.ac.uk/schools/sci/chem/tutorials/molspec/beers1.htm
http://dl.clackamas.cc.or.us/ch105-04/beer's.htm
According to Re, et. Al, (Free Radical Biology and Medicine, Vol. 26, Nos. 9/10, pp. 1231-1237, 1999), for ABTS$^+$ at a wavelength of 735 nm.

\[ \varepsilon = 1.5 \times 10^4 \text{ mol}^{-1}\text{Lcm}^{-1} \]

You will use this constant to calculate the concentration of ABTS$^+$ in substrate solutions.

3. Pre-Lab Requirements
Write an introduction and experimental section for this laboratory period. Your experimental section should include a description of what you plan to do in lab, in your own words, such that you could follow the instructions direction out of your own lab notebook. In addition to writing introduction and experimental sections for this laboratory, you will also need to calculate the masses and volumes needed to make all solutions and dilutions for this procedure before coming to lab. You should also research proper volumetric techniques especially those techniques involving the use of equipment not used in the previous Chem 116 experiments, namely, measuring pipettes and 3-way safety bulbs.

4. Materials Available
- ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)
- Potassium persulfate
- Trolox, (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)
- Epicatechin, ((2R,3R)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol)
- Sodium carbonate, anhydrous
- Quercetin dihydrate, (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one dihydrate)
- Ethanol, 100%
- ABTS$^+$ that has been incubated for 12 hours
- 25 mL amber vials and 250 mL bottles
- volumetric flasks in 25mL, 50mL, 100mL and 250mL sizes
5 mL measuring pipettes
1000-200 L and 200-10 L auto pipettes ("micro pipettes")
3-way safety bulb
Cuvettes
Waste Jar

5. Waste Disposal
You should have a beaker designated for waste material on your lab bench. After you have performed the determination of maximum wavelength, the ABTS radical should be disposed of in this beaker. All the glassware used in preparing the solutions for the TEAC assay should be rinsed with a small amount of water from your wash bottle and poured into the waste beaker. You can then empty the beaker into the large waste jar found in the main hood.
6. Laboratory Procedures

Part I: Preparation of Solutions for TEAC Assay

You will make two stock solutions that are stable in the dark for several months, ABTS and potassium persulfate. These two solutions are combined together in equal volumes to create the working ABTS\(^{+}\) that has strong spectrophotometric properties in the visible range and is used in diluted form for the TEAC assay. It requires over 12 hours for the formation of ABTS\(^{+}\) to be complete.

**ABTS\(^{+}\) (radical cation) reagent**

Make 25 mL of a 0.014 M ABTS solution in deionized water. Make this solution in a volumetric flask. (A word of caution when using a volumetric flask: do not fill above the line and then try to remove solvent volume! You can fill almost to the line relatively rapidly, but then add drop wise until the meniscus of your solution is at the line for an accurate solution concentration.) Transfer the solution to an amber vial or a clear vial covered with foil to block light for storage, cap tightly, and store in the refrigerator. Put parafilm around the cap of the vial for weekly storage to avoid solvent evaporation.

Make 100 mL of 0.0049 M potassium persulfate, \(\text{K}_2\text{S}_2\text{O}_8\), in deionized water also in a volumetric flask. Store in a bottle. This solution is not light or temperature sensitive.

To make the ABTS\(^{+}\) reagent, mix 5 mL each of the ABTS and \(\text{K}_2\text{S}_2\text{O}_8\) solutions you just made into a foil covered or amber vial. Cap tightly, parafilm, and store in the refrigerator. Label all solutions with contents, your name, room number, date and section number. This solution will be ready to use after 12 hours and is stable if kept in the dark up to an additional 10 days.

**Standard Antioxidants: Making Stock Solutions of Trolox, Epicatechin and Quercetin**

Make 25 mL of 2.5mM Trolox stock solution in ethanol in a volumetric flask. Transfer the solution to an amber vial. Cap tightly, parafilm, and store in the refrigerator. Label all solutions with contents, your name, room number, date and section number. Notice that it would be very difficult to do this in one step because you would need to weigh an extremely small amount of solute (Trolox). However, in a situation such as this, you can make a larger volume and then extract the amount you need. You’ll need to look at the sizes of the volumetric glassware that you have available in order to determine what volume you will scale up to.

Make 25 mL of 2.5 mM epicatechin and 25 mL of 1.0 mM quercetin in ethanol in the same way. Here again, you need to consider the amount of material that you need to weigh in order to make these solutions. One option is to make a more concentrated solution (for which you know the exact concentration) and dilute it. For example, you could make a 25 mM solution of epicatechin in 25 mL, and a 5.0 mM solution of quercetin in 100 mL. Then you can dilute those to end up with the solution concentrations and quantities that you need.

In general, it is important to think about not only the concentration of a solute that you need, but how you will work with the substance when you are making the solution – such as weighing it. You will want to keep this in mind, for lab periods 4-6 when you design your own experimental procedure.
Preparation of Diluted Trolox, Epicatechin and Quercetin Solutions

Make dilutions of the stock solutions of Trolox and epicatechin to 25, 50, 100 and 200 µM and quercetin to 20, 40, 80 and 160 µM (see example in figure below). Be sure to use the appropriate solvent for dilutions. Storage for the dilute solutions is the same as for the stocks. When finished, you should have twelve dilute, or “sample”, solutions. Each of these will be run in a separate TEAC measurement in laboratory activity 2.

Part II: Preparation of reagents for the total phenolics measurement

Dissolve anhydrous sodium carbonate in water to make 100 mL of a 7.0% solution (w/v) and store in a bottle. This solution is not light or temperature sensitive. Label all solutions with contents, your name, room number, date and section number.

Part III: Preparation of samples for the TEAC Assay

(i) Make a 1/2 dilution tomato juice solution with ethanol and mix thoroughly. (This means 1 part tomato juice and 1 part ethanol, so that the tomato juice ends up as ½ of the total volume of the solution.) Note – in the next step you will be diluting this solution, so you need to make sufficient quantity to work with. Centrifuge this dilution for five minutes and decant the supernatant.

Dilute the supernatant to 1/5 with ethanol. Now make additional dilutions of the filtered supernatant with ethanol to 1/2 and 1/5, making 25 mL of each solution. You should now have three tomato juice samples. Store all samples in amber bottles and make sure they are labeled with the group name, date, sample name, and dilution factor.
(ii) Make a 1/2 dilution of orange juice with ethanol and mix thoroughly (calculate the amount of orange juice needed to make all of your dilutions). Centrifuge the mixture for 5 minutes and decant, then dilute the supernatant to 1/5 with ethanol and filter using a vacuum filtration apparatus, shown below.

![Diagram of vacuum filtration apparatus]

Figure 10: Vacuum filtration apparatus

Make additional dilutions of the filtered solution to 1/2 and 1/5 with ethanol to a final volume of 25 mL of each solution. Store samples in amber bottles and label with group name, date, sample name, and dilution factor.

All the glassware used in preparing the solutions should be rinsed with a small amount of water from your wash bottle and poured into the waste beaker. The waste beaker can then be emptied into the large waste jar found in the main hood.

7. Post-Lab Calculations and Analysis of the Results
   a. Calculate the concentration of ABTS⁺ in the solution that you scanned using the absorbance that you obtained and the ε at 735 nm given earlier.

   b. Explain, based on intermolecular forces, the rationale for using water to dissolve potassium persulfate, ABTS, and sodium carbonate rather than ethanol that was used as the solvent for Trolox, epicatechin, and quercetin.
8. Preparation for Next Week
   a. Read next week’s lab.
   b. Prepare your notebook with necessary pre-lab information to carry out the lab.
   c. Carry out any pre-lab or sample calculations that are necessary.
   d. Be prepared to hand in your post-lab from this week.
III. Laboratory 2: TEAC Activity of Epicatechin, Quercetin and Trolox

Overview of This Activity

In this lab you will follow the reaction between ABTS•+ and primary antioxidants over time and estimate strength of antioxidant activity for the flavonoids quercetin and epicatechin in comparison to Trolox. You will derive a value for quercetin and epicatechin known as the “Trolox-equivalent antioxidant capacity” or TEAC value. There are two components to this analysis: (1) the calculation to determine the extent of reaction for each antioxidant, and (2) the comparison of the extent of reaction for each antioxidant with that of Trolox to determine antioxidant strength.

1. Introduction

The study of the extent of reactions, or chemical equilibrium, involves measuring the concentration of reactants and products at a point in time when no further observable change occurs. For the reaction between strong primary antioxidants and ABTS•+ the rate is quite fast, reaching equilibrium in seconds. For weaker antioxidants a much longer time period is required. This reaction can be written in shorthand as Eq. 7, where AH is an antioxidant capable of donating a hydrogen atom.

\[
\text{ABTS}^{•+} + \text{AH} \rightarrow \text{ABTS}^{+} + \text{A}^{•}
\]

Eq. 7

The extent of the reaction in Eq. 7 is measured in the TEAC assay. The extent of the reaction will be correlated with the antioxidant capacity, or strength, of the antioxidant being tested. In the antioxidant research literature for TEAC, the extent of reaction is determined based on the percentage of reactant, specifically ABTS•+ substrate, converted to product at defined concentrations of antioxidant. Measurements are taken for each test compound and compared to the extent of the reaction for Trolox, the water-soluble form of the nutrient antioxidant vitamin E.

The first thing you will need to do is measure the extent of reaction of each antioxidant (Trolox, epicatechin or quercetin) with the radical cation. For this objective, you will mix ABTS•+ substrate with a defined concentration of each antioxidant and their solvent(s) and read the absorbance at 735 nm after 6 minutes. The results of each measurement are first converted to a value of \(\Delta A_{\text{ABTS}^{•+}}\) (using absorbance units), then to the percent of ABTS•+ substrate converted to product, which is expressed as “percent inhibition”. You may wonder why the extent of reaction is represented as percent inhibition. This relates to the functional role of antioxidants in foods and living systems. Consider ABTS•+ as a type of ROS. In complex systems this ROS would interact with and potentially destroy large macromolecules. Reduction of ABTS•+ to ABTSH+ inactivates this ROS and prevents this damage. Therefore, antioxidants inhibit damage caused by ROS, and representing the results as percent inhibition not only reflects the extent of the reaction but also the potential functionality of the antioxidant in biological systems. Therefore, a high percent inhibition implies that the antioxidant has inactivated a large amount of the ABTS•+ substrate ROS.

The second objective that you will accomplish is to compare the strength of the test antioxidants to Trolox. This will be done by plotting the relationship between
percent inhibition and concentration for each antioxidant used in the analysis. From the slope of the straight line that fits this relationship you will determine the value of percent inhibition per unit concentration of the antioxidant. The next step will be to divide the slope of each test antioxidant by the slope obtained for Trolox, as a means to standardize the comparison of different antioxidant preparations to one another. This ratio of slopes is the “TEAC value” for each sample. A larger TEAC value indicates greater antioxidant capacity.

**What is the Purpose of a Control?**

In all of the techniques you will be using in this module, you will need to carefully use “controls”. The purpose of a control is to ensure that your measurement is yielding the proper values for a known substance. In some cases, this means that you need to know what effect your solvent has on the measurement, so that you can subtract it out. In the first three laboratory periods you will be guided about when and how to use control measurements. For your own research project, you will need to carefully plan how you will incorporate controls into your measurements.

**2. Pre-Lab Requirements:**

In addition to writing introduction and experimental sections for this laboratory, as before, you will also need to calculate the volumes needed to make all dilutions for the TEAC assay before coming to lab. Determine if an absorbance measurement at time $t=0$ is necessary for every aliquot of the ABTS$^{\cdot+}$ substrate.

**3. Materials Available**

- Your previously prepared solutions of quercetin, epicatechin, Trolox, and ABTS$^{\cdot+}$
- ethanol, 100%
- volumetric flasks in 25mL, 50mL, 100mL and 250mL sizes
- 25mL amber vials
- spectrophotometer
- cuvettes
- 5 mL measuring pipettes
- 1000-200 µL and 200-10 µL auto pipettes ("micro pipettes")
- 3-way safety bulb
- Waste jar

**4. Waste Disposal**

You should have a beaker designated for waste material on your lab bench. After you have performed the TEAC procedure, all solutions should be disposed of in this beaker. All the glassware used in preparing the solutions should be rinsed with a small amount of water from your wash bottle and poured into the waste beaker. The waste beaker can then be emptied into the large waste jar found in the main hood.
5. Laboratory Procedures

**TEAC Procedure**

Turn on the spectrophotometer and allow it to warm up for at least 30 minutes.

**Preparation and dilution of ABTS$^+$ (radical cation) reagent**

Measure 2.5 mL of the ABTS$^+$ solution into a 250mL volumetric flask and bring to volume with deionized water. This solution will be referred to as the ABTS$^+$ substrate.

**Measurement of substrate quality**

Blank the spectrophotometer using water at 735 nm. Add 2.9 mL of ABTS$^+$ substrate to a clean cuvette. Record the absorbance of the substrate at 735 nm. If the absorbance of ABTS$^+$ is not above 0.65 then your ABTS$^+$ has gone bad. You will need to borrow some for this measurement and you will need to remake your stock solution for next week.

**Measurement of samples**

Next you will measure the absorbance of the Trolox, epicatechin, quercetin and juice dilutions that you made in Lab 1 reacting with ABTS$^+$. Add 2.9 mL of ABTS$^+$ and 100 µL of one sample to a clean cuvette, and mix thoroughly. (You can mix by putting a small piece of parafilm over the top of the cuvette, putting your finger tightly over that, and inverting a few times.) Set aside for approximately 6 minutes and take a reading at 735 nm. You will measure two trials of each of your fifteen sample dilutions.

You also need to run a control sample. In the TEAC procedure, this means running the TEAC assay with 2.9 mL of ABTS$^+$ substrate and 100 µL of the solvent of the sample being tested. The purpose of this measurement is to mimic the conditions of the sample measurements exactly except for the presence of the antioxidant. Be sure to use the correct solvent. If you are running more than one sample with the same solvent, you do not need to do multiple controls.

Run at least two trials of each sample and of the control.

Dispose of all solutions in the waste jar provided. The cuvettes can be thrown in the trash.

6. Post-Lab Calculations and Analysis of Results

**Calculations and Graphing**

To accomplish the first objective, calculate the change ($\Delta$) in [ABTS$^+$] as represented by the change in absorbance on $A_{735}$ described in Eq. 10. Use the data obtained for the 6-minute time point. Repeat for each antioxidant at each concentration. For each sample concentration, average the values of the two trials that you took. Calculate % Inhibition as illustrated in Eq. 11.

\[ \Delta A_{ABTS^+ \text{ 6 min}} = A_{735 \text{ Control}} - A_{735 \text{ Test at T=6 min}} \]  

Eq. 10
\[ A_{735} \text{Control} = \text{Absorbance at 735 nm of the reaction between ABTS}^{+} \text{ and } [\text{AH}]=0.0 \]
\[ A_{735} \text{Test} = \text{Absorbance at 735 nm of the reaction between ABTS}^{+} \text{ and } [\text{AH}]= x \text{ M} \]

% inhibition = \((\Delta A_{ABTS^{+} 6 \text{ min}} / A_{735} \text{Control}) \times 100\) \hspace{1cm} \text{Eq. 11}

Table 3 is an illustration of a spreadsheet calculation in Microsoft Excel© for these results at Time = 6 minutes.

<table>
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<th>[AH] (µM)</th>
<th>A(735nm)</th>
<th>ΔA_{ABTS^{+}} (µM)</th>
<th>Avg</th>
<th>%I</th>
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<tr>
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For Trolox, epicatechin, and quercetin, make a graph of % Inhibition versus antioxidant concentration using linear scales for each antioxidant. Using the “add trend line” feature under the chart menu in Microsoft Excel©, fit a straight line to the data. Under the options tab, check “set intercept = 0,” “display equation on chart,” and “display R-squared value on chart.” The \(R^2\) value obtained should be between 0.95 and 1.00 to indicate a good fit of the line to the data. Figure 8 illustrates a graph of quercetin data.

\[ R^2 \] is a fraction between 0.0 and 1.0, has no units, and indicates how well you can predict the value of Y (%inhibition, in this case) based on a known value of X (concentration). An \(R^2\) value of 0.0 means that knowing X does not help you predict Y and there is no linear relationship between X and Y. When \(R^2\) equals 1.0, all points lie exactly on a straight line with no scatter, so knowing X lets you predict Y perfectly.

For the juice samples, construct a similar graph of percent inhibition versus juice concentration. Express this concentration of amount of juice in microliters per liter of reaction (L/L). Calculate the TEAC values for the juice samples by dividing the slope of the regression line for each by that for Trolox. The units for the TEAC value for the juice samples will be µM Trolox per L of juice sample. During your independent research, if you choose to study a solid sample, it may be more telling to present the TEAC values in µM Trolox per g of food sample.

To accomplish the second objective, you will use the slope of the best-fit line printed on each graph. You can see that the slope is \(\Delta \% \text{ Inhibition per } \Delta \mu\text{M antioxidant}\). Divide the slope for the test antioxidants (quercetin, epicatechin) by that for Trolox to determine the TEAC value.
TEAC value = (Slope sample) / (Slope Trolox)  \[ \text{Eq. 12} \]

Because the units are identical in the numerator and the denominator, this value is a unitless ratio when comparing pure compounds of known concentration.

**Effect of Quercetin concentration on ABTS$^{\cdot+}$ Absorbance**

![Graph showing the effect of quercetin concentration on ABTS$^{\cdot+}$ absorbance. The equation $y = 4.1124x$ with an $R^2$ value of 0.9917 is noted.]

Figure 8. TEAC results plot of % Inhibition versus quercetin concentration.

**Analysis of the Results**

a. Should there be variation in your control absorption values for the substrate? Was there variation in your experiment? Please explain.

b. What TEAC values were obtained for quercetin and epicatechin? Which is a better primary antioxidant?

c. Why can $A_{\text{ABTS}^{\cdot+}}$ be used to represent the change in ABTS$^{\cdot+}$ concentration?

**7. Preparation for Next Week**

a. Find out which ascorbate quantification method you will be using (HPLC or titration) and plan your procedure accordingly. Find out if your instructor will provide juice samples or if you are to bring your own.

b. Write an introduction and experimental section in your notebook.

c. Carry out the pre-lab calculations before going to lab.

d. Be prepared to turn in your post-lab from this week.
**IV. Laboratory Period 3: Determination of Ascorbate (Vitamin C) Concentration and Total Phenolics in Common Foods**

**Overview of This Activity**
In this laboratory activity you will prepare tomato and orange juice samples for ascorbate concentration determination and polyphenolic measurements. For either ascorbate concentration determination method you will prepare a stock solution and dilutions of ascorbate for use in generating a standard curve. You will then treat and dilute samples of tomato and orange juices so that they are suitable for injection into the HPLC or use in the titration method. For the ascorbate analysis by HPLC the standard and test samples will be sent to the instrumentation lab for injection and the results returned to you for later analysis. For the total polyphenolic measurement (and titration analysis) you will conduct the analysis on the same juice samples in the lab today.

**1. Introduction**
Ascorbate (or vitamin C) is an essential nutrient that is found in a variety of plant foods. A lack of sufficient amounts of this nutrient in the diet is responsible for the disease known as “scurvy.” Sir James Lind established the link between scurvy and an essential component of plant foods (primarily citrus) in the 1700s. Lind’s experiments determined that limes contained a substance that would prevent the scurvy, so British sailors began consuming limes during long voyages and acquired the nickname “limeys.” This important substance was ascorbate.

Most animal species can synthesize ascorbate, but humans, guinea pigs, fish and fruit bats require dietary sources of the nutrient. In the human body ascorbate serves two main functions. It serves as a cofactor for reactions that lead to the maturation of collagen, and vitamin C is the primary water-soluble antioxidant in the body. Optimal ascorbate concentrations in the blood approach 75 $\mu$M, and the requirement of up to 90 mg/day is the largest of all the vitamins. Vitamin C is the most common of the single-nutrient supplements and it is often added to many prepared foods to prevent oxidation. Mega doses of the vitamin have been proposed to cure many diseases including the common cold and cancer. Although most of these types of claims are disputable, the importance of the vitamin for maintaining nutritional health is quite clear.

As an antioxidant, ascorbate can donate two electrons sequentially to terminate radicals; the ascorbate is converted to the oxidized species dehydroascorbate.

![Equation 13](image)

In living systems that utilize ascorbate as an antioxidant, enzymatic reactions catalyze the reduction of dehydroascorbate using reducing equivalents supplied by glucose oxidation. This allows the vitamin to be reused multiple times - a common
characteristic of essential nutrients. Since ascorbate is the most abundant nutrient antioxidant in fruits and vegetables, you will determine its concentration in the samples that you test as a basis for comparison. Ascorbate present in foods and supplements will contribute to the overall TEAC activity. Two methods are offered to quantify vitamin C content in foods. The first method involves extraction of ascorbate from the food matrix followed by separation and quantification by high-pressure liquid chromatography (HPLC). The second method is a titration method using a dye compound that is reduced by vitamin C to a colorless liquid. Your instructor will determine which method you will use.

2. A Primer on High Pressure Liquid Chromatography (HPLC)

HPLC is a common analytical technique that is used for many applications including separation, identification, purification, and quantification of various chemical species. It is used in research in the fields of chemistry, biochemistry, biology, toxicology, cosmetics, and pharmaceuticals. The method involves separating molecules dissolved in a solvent that is in motion (mobile phase) at high pressure over a solid support material (stationary phase). This leads to the separation of individual chemical species based on their binding affinities to the solid matrix versus their solubility in the mobile phase. A diagram of the key components of an HPLC system is shown below.

![Diagram of a typical HPLC setup.](image)

The mobile phase is pumped through a column that can sustain high pressures. The column is packed with support material with chemical characteristics that can be varied. The material commonly used has a strong hydrophobic nature and separates molecules based on dispersion forces. Other column materials have ionic characteristics and separate molecules via dipole forces. This lab is written to use a hydrophobic solid support material which is silica-based but has long aliphatic 18-carbon hydrocarbon chains attached at the functional groups (C₁₈ material). Another column material,
pentafluorophenylpropyl, may also be used. The effluent, or outflow, from the column passes through a detector, which can be a spectrophotometer, electrochemical detector, or other device adapted to function with a stream of flowing solvent. The choice of detection methods depends on the characteristics of the chemical species to be analyzed. Ascorbate is active electrochemically and also exhibits an absorption spectrum with a $\lambda_{\text{max}}$ in the UV range at 267 nm. For this lab you will prepare the samples, standards, and mobile phases. These will be delivered to the HPLC facility where the samples will be run. The data will be provided to you for analysis.

3. Determination of Ascorbate Concentration by Titration

This method is based on the ability of ascorbic acid to reduce the oxidation-reduction dye indicator 2,6-dichloroindophenol to a colorless solution. The reaction is shown in Eq. 14.

At the endpoint of this analysis addition of unreduced dye to the reaction mixture yields a rose pink color in acid solution. Since this is a colorimetric reaction involving a non-specific dye indicator, or an indicator that will react with many titrants, there are certain food substances that give erroneous results. Foods that contain other compounds capable of reducing the dye can result in erroneously high values. Foods that contain transition metals (iron, copper) that compete with the dye for oxidation of ascorbic acid can result in erroneously low ascorbate values. Highly colored foods interfere with the determination of the endpoint.

4. Measurement of the Total Polyphenolic Content of Foods

As indicated in the introduction to this module, polyphenolic flavonoids are among the most abundant non-nutrient antioxidants in foods. To estimate the contribution that these compounds make to the TEAC value, you will quantify the total amount of polyphenolics in chosen samples. The measurement is based on a method
which was originally developed over 100 years ago, was upgraded and improved during the 1960’s and 1970’s, and today is commonly used in the wine industry. This analysis involves the use of a spectrophotometer rather than an HPLC or titration.

5. Pre-Lab Requirements

In addition to writing introduction and experimental sections for this laboratory, you will also need to calculate the volumes needed to make all dilutions mentioned below. Pay special attention to the volumes and dilutions described in Total Polyphenolics Measurement – you will need to choose the volumes for many of the solutions. **NOTE:** the balances are not reliable below about 10 mg (0.010g). So, if your calculations show that you need to weigh out less than this amount, you should consider making a more concentrated solution and diluting to the required concentration.

It may be useful to map out the time you will be spending on this lab to determine which parts of the procedure should be attempted first to maximize time efficiency. Some parts require long incubations and should be attempted earlier in the period.

6. Materials Available

- Professionally prepared HPLC standards
- ascorbic acid
- 5% acetic acid with 0.35 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) – also referred to as acetic acid/TCEP solution
- Folin-Ciocalteau reagent
- 7% (w/v) sodium carbonate solution – previously prepared
- Orange juice
- Tomato juice
- 2,6-dichloroindophenol
- Sodium bicarbonate
- Spectrophotometer
- HPLC autosampler vials (for HPLC analysis only)
- Centrifuge
- Cuvettes
- 1 µm syringe filters
- Syringes
- 25 mL, 50 mL, 100 mL and 250 mL volumetric flasks
- 25 mL amber vials
- 5 mL measuring pipettes
- 1000-200 µL and 200-10 µL auto pipettes (“micro pipettes”)Filter paper
- 3-way safety bulb
- 50 mL buret (for titration method only)
- Magnetic stirrers and stir bars (for titration method only)
7. Waste Disposal
You should have a beaker designated for waste material on your lab bench. After you have performed the measurement of Total Polyphenolics, all solutions should be disposed of in this beaker. All the glassware used in preparing the solutions for polyphenolic measurement should be rinsed with a small amount of water from your wash bottle and poured into the waste beaker. The waste beaker can then be emptied into the large waste jar found in the main hood.

8. Laboratory Procedures
Preparation of Ascorbate Standards and Juice Samples (for HPLC or Titration Method)
Record the serving size of the juices as you will need this information for analysis.

You will first make 25 mL of a stock solution of 1.0 mM ascorbate in dilute (5% v/v) acetic acid in 0.35mM TCEP (this is known as the acetic acid buffer). **NOTE: the balances are not reliable below about 10 mg.** So, if your calculations show that you need to weigh out less than this amount, you should consider making a more concentrated solution and diluting to the required 1.0 mM. This ascorbate solution will serve as the stock concentration for either the HPLC method or the titration method. Store in the cold and protect from light. In our experience the stock is stable under these conditions for about 10 days. Note that the standards for either assay method should be prepared at the same time as the test samples (below). All ascorbate solutions prepared with 5% acetic acid/0.35 mM TCEP are stable up to 10 days if refrigerated and protected from light. Without TCEP, ascorbate solutions are extremely unstable.

Ascorbate Measurement by HPLC
**Note:** If you are only performing the titration method, skip this part.

Each group in the laboratory will be responsible for preparing one HPLC standard. Your teaching assistant will assign your group a concentration. There are five 25 mL standards to be made by diluting the ascorbate stock with the acetic acid buffer to obtain working standards at 25, 50, 100, 150 and 200μM. Fill an HPLC vial for each solution; you will need to lightly “flick” the top (wide diameter section) of the HPLC vial with your finger (fingernail side) to ensure that there is no air bubble trapped in the narrow tip at the bottom of the HPLC vial. Make sure to flick the vial gently so as not to break it. Make sure to label your sample with sample name, concentration, group name, section number and date (ask your instructor about sample codes for your class). Calibration standards must be prepared on the day of HPLC analysis. A set of professionally prepared ascorbate standards will also be loaded for comparison with the student made standards.

To make the HPLC test samples
(i) Make a 1/2 dilution tomato juice solution with acetic acid-TCEP and mix thoroughly. (This means 1 part tomato juice and 1 part acetic acid-TCEP solution, so that the tomato juice ends up as ½ of the total volume of the solution.) Note – in the next step
you will be diluting this solution, so you need to make sufficient quantity to work with. Centrifuge this dilution for five minutes and decant the supernatant.

Dilute the supernatant to 1/5 with acetic acid-TCEP. Filter this diluted supernatant with a 1 µm syringe filter to remove fine particles. To do this, pull the solution into a syringe, then put the filter on the tip, and push the solutions through the filter into a clean container. Throw away the filter and return the syringe after use. Fill an HPLC vial with this solution. Now make additional dilutions of the filtered supernatant with acetic acid buffer to 1/2 and 1/5, making 25 mL of each solution. Fill an HPLC vial with each of these two solutions as before. You should now have three HPLC vials with diluted samples. Make sure they are labeled.

(ii) Make a 1/2 dilution of orange juice with acetic acid-TCEP and mix thoroughly (calculate the amount of orange juice needed to make all of your dilutions). Centrifuge the mixture for 5 minutes and decant, then dilute the supernatant to 1/5 with acetic acid-TCEP and filter using a vacuum filtration apparatus, shown below.

![Vacuum filtration apparatus](image)

Pass the filtrate through a 1 µm syringe filter to remove fine particles. Fill an HPLC vial with this filtered solution, and label your HPLC vial. Make additional dilutions of the filtered solution to 1/2 and 1/5 with acetic acid-TCEP to a final volume of 25 mL of each solution. Fill HPLC vials with these diluted solutions and label the vials.

Your HPLC vials will be delivered to the instrument for HPLC analysis. The samples will be loaded into an autosampler and sequentially injected onto the HPLC column. The output from the column is monitored with a UV or electrochemical detector. The signal from this detector is processed with a computer program and
provides several pieces of information. First, a chromatogram, which is a graph of the
output from the detector over time, is printed. A chromatogram for each sample will be
printed and provided to you. Second the computer is programmed to integrate the area of
each peak on the chromatogram. Using the HPLC protocol described in this module,
ascorbate separates from other components in the sample and elutes\(^8\) as a single peak
with a retention time of 4.1 min. Retention time is the elapsed time from injection until
the maximum height for a single peak passing through the detector.

**Ascorbate Measurement by Titration with 2,6-dichloroindophenol**

Note: If you are only performing the HPLC measurements, skip this part.

Dilute the ascorbate stock in acetic acid buffer to obtain 25 mL of working
standards at 500 µM and 1.0mM. The 2 mM stock solution will also be used with this
method. These three concentrations (500 µM, 1.0mM and 2.0mM) will make up the
calibration curve. Prepare three trials of all points to be used in the calibration curve.
Measure enough juice to make all of your dilutions and centrifuge for at least five
minutes. Decant the supernatant and dilute each juice sample 1/2 with acetic acid buffer
and mix thoroughly. If a precipitate forms, remove it by either centrifugation or
filtration. Make dilutions of the resulting clear supernatant with acetic acid buffer 1/2,
1/5, and 1/10. Make a final volume of 25 mL of each solution.

[Note: the indophenol standard will be provided for you by the prep lab]. If you
were to make the indophenol standard solution yourself, you would dissolve 50 mg 2,6-
dichloroindophenol sodium salt in 50 mL water to which has been added 42 mg
\(\text{NaHCO}_3\). Mix thoroughly and, when the salt is dissolved, dilute to 1000 mL with
deonized water. Filter and store in an amber bottle. The solution breaks down upon
exposure to light. Avoid prolonged periods of exposure in clear glassware.

To carry out the determination, place 5 mL of standard ascorbate solution into a
125 mL Erlenmeyer flask containing 5 mL of acetic acid buffer. Fill a 50 mL buret with
indophenol solution. Titrate with indophenol solution to an endpoint of distinct light rose
color. Record the amount of solution used. Repeat for all solutions.

**Total Polyphenolics Measurement**

Dilute the previously prepared epicatechin stock to achieve three or more standards in the
range of 25 to 200 µM in ethanol. Dilute the concentrated commercially prepared Folin-
Ciocalteau reagent 1 part in 10 with deionized water. Always prepare this fresh daily.

Procedure: Measure all test samples (tomato and orange juices from Part 1) and
standards in duplicate. To each tube add 225 µL water (which will serve as the control)
or sample or epicatechin standard. Then add 1.5 mL of diluted Folin-Ciocalteau reagent,
mix thoroughly and let set at room temperature for 7 minutes. Add 1.5 mL of 7% (w/v)
sodium carbonate solution, mix thoroughly and allow to sit for 30 min. Read and record
absorbance in a spectrophotometer with wavelength set at 750 nm.

Dispose of all solutions in the waste jar provided. Cuvettes can go in the trash.

---

\(^8\) To elute is to separate or purify by washing out.
9. Post-Lab Calculations and Analysis of the Results

Ascorbate Measurement by HPLC

The instrument lab will provide two pieces of information from your ascorbate analysis by HPLC. The first will be a profile (similar to Fig. 11) showing the elution of ascorbate from the HPLC column and the resolution of the peak from surrounding peaks. You can use this to verify that the peak quantified as ascorbate was correct and that the quantification of the peak was not compromised by other peaks eluting in close proximity.

The upper figure in Fig. 11 is an overlay profile showing an example of ascorbate standards injected sequentially from low (~25µM) to high concentration (~200µM). The x-axis is time and the y-axis is absorbance at 268nm. Notice that there are two peaks which elute sequentially from the column. The first set of peaks is the flow-through...
containing material in the sample that is not retained by the column. For these injections the acid (used to dilute the standards) is providing this response. The second set of peaks eluting at 4.17 min corresponds to the ascorbate standard. Notice the symmetrical shape, perfect overlap, and increasing size of the peak corresponding to the greater concentrations injected. These observations provide a qualitative affirmation of the success of the ascorbate standards analysis. This will be verified by the quantitative analysis that you will complete in the next section. (Note: you will get separate chromatograms for each sample, they will not be overlayed like this figure.)

The lower chromatogram in Fig. 11 is an example of an overlay of the orange juice analysis. Notice two major peaks are observed along with several smaller peaks not present in the chromatograms of the standards. Notice the first peak, the flow-through, exhibits a relatively constant size which suggests that the solvent containing acid contributes to this peak. Other components of the sample with absorbance at 268 nm may also be contributing to this peak. The peak eluting at 4.17 min is a symmetrical peak that exhibits a larger peak area for 1/10 diluted sample and a much smaller peak area for the 1/100 diluted sample. The peak corresponding to 1/1000 is too small to be visible. Since this peak elutes at the same time, has the same shape, and does not overlap with other adjacent peaks, we assume it is ascorbate and will use the quantification of the area of this peak in comparison to the standard peak areas to determine the concentration of ascorbate in the juice samples.

The second piece of information that will be provided is a table with areas and retention times for each peak present in the chromatogram. The absorbance values measured as the eluting sample passes through the UV detector are converted to an electronic signal in units of mV. The computer program used to analyze the results will integrate the area under each peak and report it. The example that follows is focused on the peak identified as ascorbate. Table 4 and Table 5 below summarize this information for the standards and the two juice samples.

Table 4: Summary of HPLC peak integration and retention times for ascorbate standard.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>Concentration (µM)</th>
<th>Dilution</th>
<th>Peak Area</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STND1</td>
<td>Ascorbate Standard</td>
<td>26.125</td>
<td>5711390</td>
<td></td>
<td>4.167</td>
</tr>
<tr>
<td>STND2</td>
<td>Ascorbate Standard</td>
<td>52.250</td>
<td>13732536</td>
<td></td>
<td>4.167</td>
</tr>
<tr>
<td>STND3</td>
<td>Ascorbate Standard</td>
<td>104.500</td>
<td>27619134</td>
<td></td>
<td>4.167</td>
</tr>
<tr>
<td>STND4</td>
<td>Ascorbate Standard</td>
<td>209.000</td>
<td>55852793</td>
<td></td>
<td>4.167</td>
</tr>
</tbody>
</table>
Table 5: Summary of HPLC peak integration and retention times for juice samples.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Description</th>
<th>Dilution</th>
<th>Peak Area</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP10A</td>
<td>Apple Juice</td>
<td>10</td>
<td>51632744</td>
<td>4.167</td>
</tr>
<tr>
<td>AP10B</td>
<td>Apple Juice</td>
<td>10</td>
<td>53506976</td>
<td>4.167</td>
</tr>
<tr>
<td>AP100A</td>
<td>Apple Juice</td>
<td>100</td>
<td>6339594</td>
<td>4.167</td>
</tr>
<tr>
<td>AP100B</td>
<td>Apple Juice</td>
<td>100</td>
<td>5954836</td>
<td>4.167</td>
</tr>
<tr>
<td>AP1000A</td>
<td>Apple Juice</td>
<td>1000</td>
<td>598612</td>
<td>4.167</td>
</tr>
<tr>
<td>AP1000B</td>
<td>Apple Juice</td>
<td>1000</td>
<td>540179</td>
<td>4.167</td>
</tr>
<tr>
<td>OR10A</td>
<td>Orange Juice</td>
<td>10</td>
<td>35111848</td>
<td>4.167</td>
</tr>
<tr>
<td>OR10B</td>
<td>Orange Juice</td>
<td>10</td>
<td>34649980</td>
<td>4.167</td>
</tr>
<tr>
<td>OR100A</td>
<td>Orange Juice</td>
<td>100</td>
<td>3890611</td>
<td>4.133</td>
</tr>
<tr>
<td>OR100B</td>
<td>Orange Juice</td>
<td>100</td>
<td>3750404</td>
<td>4.200</td>
</tr>
<tr>
<td>OR1000A</td>
<td>Orange Juice</td>
<td>1000</td>
<td>359440</td>
<td>4.133</td>
</tr>
<tr>
<td>OR1000B</td>
<td>Orange Juice</td>
<td>1000</td>
<td>272837</td>
<td>4.167</td>
</tr>
</tbody>
</table>

Use the graph function in Excel to evaluate the fit for the standard curve. The example that follows illustrates a very good fit of the data to a linear regression.

You can use the trend function in Excel to calculate the concentration of each unknown sample. Choose the dilutions of juice which fall into the range of the standard curve. Once this is complete, calculate the concentration of ascorbate in the juice by multiplying the concentration of each sample injected by the dilution factor.

Table 5 does not include the 1/2 initial dilution of the juice samples and this should be accounted for in the calculation. Thus for sample OR10A, the calculation is:

\[
[\text{Ascorbate in OJ}] = (132 \mu M) \times \frac{20 \text{ volume juice dilutions}}{1 \text{ volume juice}} = 2640 \mu M
\]

The first value, 132 µM, comes from the graph in Fig. 12 and its linear regression. The concentration value corresponding to peak area for sample OR10A in Table 5 is 132 µM.
The number 20 results because the OR10A sample is a 1/10th dilution, and there was a 1/2 dilution in the procedure for the juice samples, such that 1/2 x 1/10 = 1/20.

Converting this concentration to a number that can be compared to the information given on the juice nutrition information label can be done as follows:

\[
\text{Ascorbate } F.W. = 176.13 \text{ g mol}^{-1} \quad \text{0.240 L} = 1 \text{ cup}
\]

\[
\frac{0.00264 \text{ mol ascorbate}}{1 \text{ L juice}} \times \frac{0.24 \text{ L}}{1 \text{ cup}} \times \frac{176.13 \text{ g ascorbate}}{1 \text{ mol ascorbate}} = 0.1116 \text{ g / cup}
\]

or 111.6 mg / cup ascorbate

The standard for food labels for vitamin C is 60 mg per serving. The label on a typical orange juice container indicates that the amount per cup is 120% of the RDA. What do the data indicate about the amount of vitamin C in your juice sample? Discuss explanations for any possible discrepancies you find.

Which HPLC dilutions of juices produced usable results? If you were to take another measurement, what concentration should it be? If you decide to use the HPLC method in your research (Part 4 of this module), it would be useful for you to know what dilutions produce usable results. Furthermore, some foods have more vitamin C than others, so in the context of your research project, you will want to think about the relative amount of vitamin C your food product has before deciding on dilution factors.

**Ascorbate Concentration Measurement by Titration**

Construct a standard curve for ascorbate plotting concentration versus volume of indophenol titration solution. Determine the concentration for samples using the best fit straight line for the standards as described above. Note that this method is much less sensitive than the HPLC method.

**Total Polyphenolics**

Construct a standard curve for epicatechin plotting concentration versus absorbance at 750 nm similar to the graph in Figure 12. Determine the concentration for samples using the best fit straight line for the standards in a similar way as for ascorbate described in the previous section. How do these two juice samples compare in terms of the quantity of total phenolics? Does this result differ from the ascorbate results?

**10. Post-Lab Questions and Analysis of Results**

a. What is the purpose of using 5% acetic acid as a solvent for the ascorbate solution? Why is it also needed as a solvent for the orange and apple juices?

b. Did the dilutions used in this period produce usable results? If not, what changes can you make in the future to your dilutions to produce results that will be more valuable?

**11. Preparation for Next Week**

a. There is substantial reading for the next period, so give yourself ample time.

b. Prepare your own unique experimental design based on the techniques you have learned. Be prepared to discuss your design and procedures.
V. Laboratory 4-7: Original Research Project

Overview of this Research Activity

We have described many observations concerning oxidative stress, its role in health and protection by antioxidants in this module. Also, we have introduced various types of antioxidants in foods, and you have learned how to measure the total amount of antioxidant activity as well as the concentration of ascorbate, a key nutrient antioxidant, commonly found in foods. Based on these observations we can formulate a hypothesis concerning total antioxidant activity in ascorbate-rich foods. A testable hypothesis could be stated as follows “fruit samples stored at room temperature for a week will exhibit a 50% greater loss in antioxidant activity compared to similar samples stored at 4°C.” The techniques that you have learned so far, TEAC, HPLC or ascorbate titration, and polyphenolics measurements, can be used to assess this hypothesis. The TEAC assay assesses total antioxidant activity. HPLC, the ascorbate titration, and the polyphenolics measurements are more specific in their assessments. Each of these methods can have a place in examining this hypothesis: TEAC can evaluate changes in total antioxidant capacity, while HPLC, the ascorbate titration, and the polyphenolics measurement can assess changes in specific flavonoid classes. To isolate a specific variable, it is important that as many other factors as possible be kept constant between the samples. For this reason, this experiment will require that the samples are drawn from the same batches of solution, kept in the same kinds of vials, either with or without parafilm (but the same for both conditions), and with the same amount of contact with sunlight and motion. An example experimental design is as follows:

- Make solutions to be tested
- Measure antioxidant activity of the samples using TEAC and HPLC
- Fill two sets of identical amber vials, one for room temperature, one for 4°C
- Put parafilm on the vials
- Store the room temperature vials in a drawer to minimize contact with sunlight and risk of being knocked over; store 4°C vials in the refrigerator, taking precautions if there is direct sunlight
- Note the time
- 7 days later, return and remove the samples at approximately the same time from their conditions, opening them in the same way
- Measure antioxidant activity of the samples using TEAC and HPLC
- Compare antioxidant activities and changes in antioxidant activity

What is Currently Known and Unknown About Antioxidants in Foods?

A search of the peer-reviewed literature reveals many papers published that measure some indicator of total antioxidant activity in food and biological samples. Because our focus is on health benefits, I will use the example of PubMed, the main government-financed database used by researchers in the health field to survey the literature. If one does a search on PubMed using the search terms that describe total antioxidant activity (TEAC, ORAC, & TRAP), 275 references are obtained. (See Appendix B for tips and information about reading scientific research articles – it’s not as hard as it looks!) When these terms are combined with the term “food,” 106 references
are found. Many of these papers report on total antioxidant activity of commonly consumed fruits and vegetables, teas, spices, various grains, food additives, wine, and chocolate. Also, some papers report analysis of uncommon plant parts which may have a basis in traditional medicine. A few papers (not many) have been published testing the effects of processing, storage or digestion of fruits and vegetables on antioxidant activity. A few studies have explored the effects that mixing high antioxidant and high protein foods has on total antioxidant activity. Very few published reports have tested whether antioxidant-rich foods exhibit synergy, whether more highly palatable foods containing fruits or vegetables exhibit antioxidant activity, whether treatments that simulate the digestive process diminish antioxidant activity, or whether common cooking methods (grilling, boiling, sautéing in butter, baking, deep frying, etc.) significantly decrease antioxidant activity.

Any of these questions is a valid research question to test for this module because there is not a lot of information available yet on them, so you would be doing work that could contribute to the known research in this topic. HOWEVER, Dr. Burgess is currently working on the antioxidants in green teas, and in green tea drinks that are mixed with a juice that contains ascorbate (such as lemon juice) that can be found in many supermarkets and convenience stores. He is particularly interested in what effects digestion (i.e. processing through the human digestive tract) has on the antioxidant capacity of these teas and whether the juice makes a difference in this process. You can focus your efforts on this topic for now. (See Appendix A for an in-lab procedure that simulates digestion.)

1. Introduction

In this laboratory period you will go through the process of hypothesis testing in antioxidant research using an example of citrus fruits which are rich in vitamin C. Science is the process by which we try to understand nature by continually testing our theories and refining explanations based on observation. The scientific method is the means by which we try to make this process reliable and consistent. Generally there are several key components: observation and description of phenomena, formulation of a hypothesis to explain the phenomena, performance of experiments to test the hypothesis, rejection or modification of a hypothesis inconsistent with experimental results, and application of a validated hypothesis to explain new observations. Observations can include descriptions but also quantifiable data, which are useful because comparisons can be made and trends observed. A hypothesis which explains observations must be testable and constructed to address one factor at a time. Testable means that the hypothesis statement is written in a way that one can determine it to be incorrect by experimentation. Experiments are procedures carried out generally involving an independent variable that is purposefully manipulated and a dependent variable that is observed or measured. Good experimental design attempts to minimize the effect of bias on the results. Several factors must be considered to be successful here. These include a representative sample, accuracy, precision, and reproducibility. A representative sample is one that truly reflects and encompasses the independent and dependent variables. Accuracy is how close a measured value is to the “true” or “real” value. Precision is the spread in the data of repeated measurements or trials. Reproducibility indicates that the results of an experiment are the same when repeated again at a later time or by another
individual or research group. This lab will apply the scientific method to a question about which chemical species is responsible for the total antioxidant activity that can be measured in one particular vitamin C-rich food.

2. Pre-Lab Requirements

You will need to prepare your own unique experimental design and procedure to share with your group during lab. In addition, look up any pertinent information about the products you are interested in studying to see if some tests would be more telling than others and design your experiment accordingly.

Plan ahead for the mixing of your ABTS⁺ as it will need to be made at least 12 hours but not more than 10 days before your next lab period.

Finally, think about the products you will be using with respect to the appropriate dilution factors. What did you learn about dilutions from previous experiments that could be useful in determining where to start? Are there dilutions that are too dilute or too concentrated when trying to use the line of concentration versus signal from your standards? It may take more than one try to get the dilutions right.

3. Design and Conduct a Research Project on Antioxidants in Food

Your goal is to design an experimental procedure that actually will test a hypothesis. Review the introduction to this laboratory, to remind yourself about the use of controls and about reproducibility. Also, think about what you plan to test and what each of the techniques you learned in weeks 1-3 actually measures? Do you need to use all of them for the hypothesis you want to test? If not, which one or which ones will you use, and why?

Once a research question has been agreed upon, design an experimental protocol and discuss this as a group. Think about the steps you will be taking, and what you will do with your samples. Consider and plan for the procedures, timing, etc., that will be necessary for you to conduct a valid research study. Consult with your peer leader and/or TA about the research question that your group would like to address. Review the recent peer-reviewed literature to help you refine your research question/hypothesis. Refine your hypothesis or procedures if necessary – it is OK to do this when you do research.

Write up the experimental procedures and a list of supplies. Keep in mind that your supplies will be limited to those that you have already used in laboratory periods 1-3. Some additional items can be provided, as long as you include them in your list of supplies. These additional items include: blender, hot plate, cutting board. The food items will be limited to those provided by your instructor (ask your instructor for a list.) It may be possible for you to carry out research on food items that you provide. You must check with your instructor to see if this will be allowed.

For Laboratory Period 4: You should come to lab with an experimental procedure already in mind. In lab, you will meet and discuss this with your group, and with your TA, to finalize a procedure that you will work on for the next 3 lab periods.
4. Your Contribution to the Research of Dr. Burgess

This portion of the module will culminate with data that you can give to the researcher, thus contributing your work to the body of research. Keep in mind that Dr. Burgess would like to be able to *use your data for publication!* Therefore, you should *conduct and report your experimental procedures and results carefully.* For this module, you will provide data that includes the following things:

- Your hypothesis for your research.
- Detailed protocol of your experimental design for testing your hypothesis, including the specific food products researched and the conditions to which they were subjected.
- List of tests performed and procedures carried out.
- Results of the tests, such as numerical values and copies of your spectra.

You will be provided with detailed instructions on how to enter your data into a web-based form that will be sent to Dr. Burgess in electronic form. Be sure to label your data (and all your samples) sufficiently (your group name or number will not mean much to the researcher, so include information about what you studied and when the study was performed and what the data or sample is).

Most of all: have fun!
VI. References
Chemistry 116. Laboratory Manual (2005). Chapter 2, 4 and Appendix A


Appendix A: Procedure to Simulate Digestion

Example of a Test Question: will green tea with lemon preserve antioxidant activity of tea through simulated digestive process?

Ingredients: Lemon Juice. Freshly brewed green tea – two 250 mL cups

Sample Preparation:
   a. green tea as prepared (brewed)
   b. green tea with 2 Tbsp (or around 5-6 mL) of lemon juice per cup
   c. water with 2 Tbsp (or around 5-6 mL) of lemon juice (as a control)

Reserve 25mL in an amber bottle for TEAC and polyphenolics. Samples must be tested for ascorbate immediately. Each preparation must be filtered through 1µm filter, diluted and put into HPLC vials.

Set aside another 10mL of each treatment for Burgess/Ferruzzi test follow-up. Label these as pre-digested or undigested samples and store in freezer. Labeled boxes will be available for students to put their samples in.

In vitro digestion protocol: (Note: If tea is freshly brewed it must be cooled to room temperature to measure the pH. You can cool by putting in an ice bath and monitoring the temperature as it drops.)

1. 30 mL of each test mixture to be tested should be transferred to an 125mL Erlenmeyer flask. Wrap the flask with a single layer of aluminum foil to protect the solution from light. (Consider whether you will have time for duplicates? Will this help with the reproducibility of your experiment?)
2. check the pH of your mixture and if it is above 2.5 adjust the pH to 2.5 by the dropwise addition of 1.0 M HCL,
3. add 2.0 mL of porcine pepsin mixture (40 mg/mL in 0.1 M HCl, supplied),
4. flush the flask with nitrogen and stopper,
5. transfer mixture to shaking water bath at 37°C and incubate for 15 min at 95 rpm
6. next raise the pH of mixture to 5.0 by the addition of 2.0-2.5 mL of 0.9 M sodium bicarbonate,
7. add 9 mL pancreatin enzyme mix (2.0 mg/mL in 0.1 M sodium bicarbonate, supplied) and mix,
8. increase pH of mixture to 6.5 by the addition of 1M sodium hydroxide, dropwise
9. flush with nitrogen and stopper tightly,
10. incubate at 37°C with shaking for 45 min,
11. filter the mixture using a Buchner funnel and P5 filter paper.
12. Transfer two 2.0 mL aliquots of the filtrate to new tubes labeled (post digestion) Burgess lab for potential follow-up catechin quantification. Transfer the remaining filtrate to a clean tube for analysis the following week. Store all of these samples in the freezer.

After Digestion: TEAC and polyphenolic assay should be performed on each sample. Compare Results of TEAC after simulated digestion versus before to determine potential impact of lemon on green tea antioxidant. Dilution factor will need to be considered in before/after comparison.
Appendix B: Reading and Interpreting a Research Paper

A Paper about Processing Effects on Antioxidants in Food Sources

Consider the paper cited below (published in 2005) testing the effects of fermentation and heat treatment on the antioxidant activity of cowpea flours. The purpose of reviewing this paper is not to get bogged down in the details but to review the key characteristics of a study design. Hopefully this will help you to develop your research question and design your own study using the tools and skills you have learned so far in this module.


Cowpea (Vigna sinensis L. var. Carilla) flours were obtained by fermentation with inoculum Lactobacillus plantarum (PF) or with the natural microorganisms present in the flour (NF) and subsequently heat treated in an autoclave. The flours were prepared to study the effect of fermentation on the antioxidant vitamin content and on the antioxidant capacity. Bacterial counts and pH values, vitamins C and E, carotenoids, glutathione (GSH), superoxide dismutase-like activity (SOD-like activity), peroxyl radical-trapping capacity (PRTC), lipid peroxidation in unilamellar liposomes, and Trolox equivalent antioxidant capacity (TEAC) were evaluated in raw and processed cowpea flours. Gamma-Tocopherol and delta-tocopherol were found in raw cowpea, whereas vitamin C and carotenoids were not detected. An increase in the vitamin E activity was observed in PF, whereas vitamin C and carotenoids were not detected in fermented cowpea flours. Fermentation or heat treatment in an autoclave after fermentation produced processed cowpea flours with lower PRTC, glutathione content, and SOD-like activity than those of the raw seeds. However, those processes increased the capacity to inhibit the lipid peroxidation in unilamellar liposomes and TEAC. According to the results obtained in this study, the fermentation of cowpeas (naturally or with L. plantarum) and fermentation and subsequent heat treatment in an autoclave are good processes to obtain functional cowpea flours having higher antioxidant capacity than the raw legume.

What is the main question addressed in this paper?

*Answer:* What is the effect of fermentation and subsequent heat treatment on the antioxidant activity present in a bean flower (cowpea)

Why is this important?

*Answer:*
- Antioxidant capacity is an aspect of food that may provide health benefits
- Cowpea a staple of many cultures especially those in the developing world
- Fermentation is believed to improve nutritional quality of processed legume food products, and several examples (soy sauce, tempeh, miso, natto, etc.) are now being consumed more commonly in the West
- Heat treatment after fermentation is used to reduce antinutritional factors derived from this legume
Cowpea is a type of legume which possesses many nutritional and health promoting properties but its antioxidant activity has not been characterized.

What was the hypothesis that the authors tested?  
**Answer:** The authors did not clearly state a hypothesis, but based on the extensive introductory material one could write a hypothesis statement for this research. We’ll call this an implied hypothesis: Fermentation will improve the antioxidant capacity of cow pea flour.

How was the research question addressed?  
**Answer:**
- Measurements were taken of the sample of interest before the treatment
- Each step in the process of preparing the final product was analyzed, i.e., raw seeds, flour, fermentation (two alternatives were tested), and heat treatment by autoclaving
- Enough replications (trials) were conducted to do statistical analysis
- Multiple tests were conducted to improve confidence in the results. These included
  - Measurement of the concentration of four-six different antioxidants in each sample
  - Using three different methods to estimate total antioxidant capacity
- Careful attention was paid to the completeness of the methods used. An example of this is table 5 in which the authors carefully extracted the sample with multiple solvents starting by using a very hydrophilic solvent and ending with a very hydrophobic solvent. Each extract was then tested for antioxidant capacity.

What was the interpretation of the results?  
**Answer:** Fermentation of cow pea flours increased antioxidant capacity as indicated by two of the methods used, but decreased this capacity as measured by a third. The effect of heat treatment by autoclaving the fermented products resulted in variable effects (increase or decrease) depending on the method of measurement. Both fermentation and heat treatment decreased the concentration of the known antioxidant nutrients (tocopherols and glutathione) in the cow pea flour. The implication here is that other non-quantified components in the cow peas is responsible for the antioxidant activity and that some of these compounds actually increase in concentration due to fermentation and possibly heat treatment. The authors of the paper discuss this idea and refer to other published work that implicates certain flavonoid species. The authors conclude: “According to the results obtained in this study, the fermentation of cowpeas (naturally or with L. plantarum) and fermentation and subsequent heat treatment in an autoclave are good processes to obtain functional cowpea flours having higher antioxidant capacity than the raw legume.”
Appendix C: Preparation Techniques for Different Foods

Methods for Extraction of Food Using Homogenization, Precipitation and Extraction to Obtain Experimental Samples for Study

Obtaining a representative sample is key to conducting a valid experiment to test a hypothesis. Many food samples are quite complex mixtures of material possessing both characteristics of solutions and colloids. It can be quite difficult to obtain a representative sample from a whole food. Below are listed several sample preparation protocols that can help guide you in obtaining adequate representative samples for your research project. The emphasis is on obtaining samples with good recovery of the more water-soluble antioxidant compounds, including most of the flavonoids. Although the TEAC assay has been tested on fat-soluble antioxidants, its use for testing these compounds is much less common. Some sample protocols follow.

General Supplies
- Knife
- Cutting board
- Cheesecloth
- Syringe and syringe filters
- 5% acetic acid with 0.35 mM TCEP
- Ethanol, 100%
- Filter paper
- Hot plates
- #5 stoppers
- Aluminum foil
- Shaker/oven
- pH meter
- Nitrogen gas source
- Blender
- Shaker
- Oven

Spices and Herbs
Weigh a little more than 10g of sample and grind (even if using pre-ground) in mortar with pestle. Carefully weigh 10g and dissolve in 100mL of appropriate solvent. If you are unsure whether you’re isolating a water soluble or insoluble antioxidant or both, divide the sample and dissolve 5g in 50mL of buffer and the other 5g in 50mL of ethanol. Stir sample mixture with magnetic stirrer for 30-60 minutes. After stirring, centrifuge the sample for fifteen minutes. Decant and perform syringe filtration on the supernatant. Make dilutions for testing; multiple dilutions over a large range will maximize the possibility that one of the dilutions will fall in the range of the standard curve.
Dried, Frozen, and Canned Fruits

Drain the fruit with cheesecloth, if needed. Weigh a little more than 10g of sample and cut it into small pieces, as necessary. Carefully weigh 10g and dissolve in 100mL of appropriate solvent. If you are unsure whether you are isolating a water soluble or insoluble antioxidant or both, divide the sample and dissolve 5g in 50mL of buffer and the other 5g in 50mL of ethanol. Blend in blender for 30-60 seconds. Stir sample mixture with magnetic stirrer for 30-60 minutes. After stirring, centrifuge the sample for fifteen minutes. Decant and vacuum filter supernatant. Perform syringe filtration on the filtrant. Make dilutions for testing; multiple dilutions over a large range will maximize the possibility that one of the dilutions will fall in the range of the standard curve.

Dry Solid Sample Preparation

Dietary Supplements, Dried Herbs, and Spices

Pulverize ~10g of sample using a mortar and pestle

If isolating water soluble antioxidants:
Dissolve 10g in 100 mL of acetic acid buffer *
Stir with magnetic stir rod 30-60 min or until dissolve (whichever comes first)

If isolating water insoluble antioxidants:
Dissolve 10g in 100 mL of 100% ethanol *
Stir with magnetic stir rod 30-60 min or until dissolved (whichever comes first)

Centrifuge for 15 minutes

Decant supernatant into beaker

For HPLC only:
Perform syringe filtration on supernantant

Dilute in acetic acid buffer or ethanol
Perform appropriate tests
Remember to run standards for comparison
High Moisture Food Sample Preparation

Fresh, Frozen, or Canned Fruits and Vegetables

- Drain food in cheesecloth if necessary
- Cut into small pieces as necessary
- Weigh 10g of sample

If isolating water soluble antioxidants:
- Blend sample with 1:10 w/w acetic acid buffer
  - Blend for 30-60 seconds

If isolating water insoluble antioxidants:
- Blend sample with 1:10 w/w 100% ethanol
  - Blend for 30-60 seconds

- Stir sample with magnetic stir rod 30-60 min
- Centrifuge sample for 15 minutes
- Decant and filter supernatant using vacuum filtration

For HPLC only:
- Perform syringe filtration on filtrant

- Dilute filtrate with either acetic acid buffer or ethanol
  - Perform appropriate tests
  - Remember to run standards for comparison
Liquid Food Sample Preparation

Juice and Tea

- If isolating water soluble antioxidants:
  - Dilute 1:1 with acetic acid buffer
  - Mix well

- If isolating water insoluble antioxidants:
  - Dilute 1:1 with 100% ethanol
  - Mix well

- If juice has large particulate matter:
  - Centrifuge 5 minutes, decant
  - Centrifuge supernatant 15 min if not clear

- If juice has small particulate matter:
  - Centrifuge 15 minutes

- For HPLC only:
  - Decant, perform vacuum filtration
  - Perform syringe filtration on filtrate

- Dilute supernatant in acetic acid buffer or ethanol
  - Perform appropriate tests
  - Remember to run standards for comparison
Appendix D: Master List of Equipment, Materials, and Reagents

**Equipment/Materials**
- 25 mL amber vials
- 250 mL bottles
- Volumetric flasks in 25mL, 50mL, 100mL and 250mL sizes
- 5 mL measuring pipettes
- 1000-200 µL and 200-10 µL auto pipettes (“micro pipettes”)
- 3-way safety bulb
- Cuvettes
- Waste Jar
- Professionally prepared HPLC standards
- Spectrophotometer
- HPLC autosampler vials (for HPLC analysis only)
- Centrifuge
- 1 µm syringe filters
- Syringes
- 50 mL buret (for titration method only)
- Magnetic stirrers and stir bars (for titration method only)

**Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>CAS Number</th>
<th>Toxicology/Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)</td>
<td>30931-67-0</td>
<td>Irritant</td>
</tr>
<tr>
<td>Potassium persulfate</td>
<td>7727-21-1</td>
<td>Oxidizing, harmful</td>
</tr>
<tr>
<td>Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)</td>
<td>53188-07-1</td>
<td>Irritant</td>
</tr>
<tr>
<td>Epicatechin ((2R,3R)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol)</td>
<td>490-46-0</td>
<td>Irritant</td>
</tr>
<tr>
<td>Sodium carbonate, anhydrous</td>
<td>497-19-8</td>
<td>Irritant</td>
</tr>
<tr>
<td>Quercetin dihydrate (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one dihydrate)</td>
<td>6151-25-3</td>
<td>Toxic</td>
</tr>
<tr>
<td>Ethanol, 100%</td>
<td>64-17-5</td>
<td>Highly flammable</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>50-81-7</td>
<td></td>
</tr>
<tr>
<td>5% Acetic acid with 0.35 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP)</td>
<td>64-19-7, 51805-45-9</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Folin-Ciocalteau reagent</td>
<td>F9252 (From Sigma)</td>
<td>Corrosive</td>
</tr>
<tr>
<td>Orange juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-dichloroindophenol</td>
<td>620-45-1</td>
<td></td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>144-55-8</td>
<td></td>
</tr>
</tbody>
</table>
Appendix B: Skill Building Procedures

CASPiE – *Skill Building HPLC*

LAB 1: Measuring the Amount of Vitamin C in Tomato Juice Using HPLC

**DAY 1 PROCEDURE – Making HPLC Test Samples**

1. In a small beaker, combine 5 mL of tomato juice with 5 mL Acid/TCEP and mix thoroughly. Calculate the concentration of this solution as a fraction, convert to its decimal form and record:

2. Carefully measure the 10 mL of the tomato juice mixture and pour equal amounts into two centrifuge tubes.

3. Label both tubes with your group letter – use a Sharpie marker.

4. Bring your samples over to the centrifuge. DO NOT operate the centrifuge on your own. Centrifuge for 6-7 minutes.

5. When you receive your sample, it should be separated into a bottom “solid” layer and a top “liquid” layer. You need the liquid stuff! Measure 7 mL of the liquid (supernatant) in a 10 mL graduated cylinder. Use a dropper pipet, if needed, to draw as much of the liquid off the solid layer as possible.

6. Pour the 7 mL of liquid supernatant into a small, clean 50 mL beaker and then add 28 mL of Acid/TCEP into the beaker. Mix well. (See Mrs. Nern if you do not have 7 mL of liquid.)

7. Calculate the concentration of the tomato juice in the 50 mL beaker as a fraction, convert to its decimal form, and record below:

8. Gather another clean and dry 50 mL beaker, a funnel, and a piece of filter paper. Pour the tomato juice solution through the filter and catch the filtrate in the clean and dry 50 mL beaker.

9. Pour the filtered TJ solution into a clean and dry brown storage bottle. Label the bottle with your group letter and the concentration (in decimal form) of the tomato juice. Use a piece of masking tape to do this.

10. This solution will be refrigerated until your class resumes.
DAY 2 PROCEDURE

1. One person in your group should gather 3 HPLC vials and caps, a sharpie marker, a syringe, and a 1um syringe filter.

2. Another person in your group should get the brown bottle of filtered tomato juice from Friday.

3. Another person should gather the following equipment/supplies:
   - disposable dropper pipet, three clean small beakers (either 50mL or 100mL), a 10mL graduated cylinder, and one additional clean small beaker with approximately 20mL of Acetic Acid/TCEP in it

4. You will need to fine filter your solution that has been stored since Friday in the brown bottle.
   - Pull the solution from your brown bottle into the syringe. You need to filter all of this solution.
   - Attach the flat 1um syringe filter to the tip of the syringe and gently push the solution through the filter into one of the small clean beakers.
   - Throw away the flat 1um syringe filter and return the syringe.

5. Using a disposable pipet, fill the first HPLC vial with this solution just as it is. Label the HPLC vial with both your group’s letter and the concentration expressed as a decimal value.

6. Pour 5mL of the LEFTOVER filtered solution into a small beaker and add 5mL of the Acetic Acid/TCEP to it. Calculate the concentration of this solution as a fraction, convert to its decimal form and record below:

7. Using a disposable pipet, fill the second HPLC vial with this solution. Label the HPLC vial with both your group’s letter and the concentration expressed as a decimal value.

8. Pour 2mL of the LEFTOVER filtered solution into a small beaker and add 8mL of the Acetic Acid/TCEP to it. Calculate the concentration of this solution as a fraction, convert to its decimal form and record below:

9. Using a disposable pipet, fill the third (last) HPLC vial with this solution. Label the HPLC vial with both your group’s letter and the concentration expressed as a decimal value.

10. Fill out the HPLC form in duplicate and return your vials with the paperwork to the tray.
PROCEDURE

1. Using a 10-mL graduated cylinder, combine 5-mL of tomato juice with 5-mL ethanol and transfer to a clean 50-mL beaker. Mix thoroughly.

2. Carefully pour all 10-mL of the tomato juice mixture into two (2) centrifuge tubes.

3. Label both tubes with your group letter. Bring both samples to the centrifuge. DO NOT operate the centrifuge on your own. We will run the samples for 7-8 minutes.

4. While your sample is running, someone from your group should measure 28-mL of ethanol into a clean 50-mL beaker to use later in this lab (See step #9 below). Clean and use the 50-mL beaker that you just used to mix the tomato juice with ethanol.

5. While your sample is running, someone else from your group should label three small amber vials (like the ones in which we placed the Tro and Epi dilutions) with your group letter, 0.1 TJ, 0.05 TJ, and 0.02 TJ.

6. While your sample is running, the third person from your group should clean and carefully shake dry the 10-mL graduated cylinder that you just used to measure the ethanol.

7. When you receive your sample back from the centrifuge, it should be separated into a bottom “solid” layer and a top “liquid” supernatant layer. You need the liquid supernatant.

8. Measure 7-mL of the liquid supernatant in the clean and dry 10-mL graduated cylinder. (From step #6 above.)

9. Pour the measured 7-mL of liquid into the 50-mL beaker with the pre-measured 28-mL of ethanol. (From step #4 above.) MIX WELL!!!

10. Filter all 35-mL of this new mixture (from step #9) through a funnel lined with filter paper into a clean 100-mL beaker.

11. Measure 10-mL of the filtrate (the liquid that comes through the filter paper) using a clean graduated cylinder and transfer to the amber vial labeled 0.1 TJ.

12. Measure 5-mL more of this filtrate and transfer to the amber vial labeled 0.05 TJ. To this 5-mL of filtrate, add 5-mL of ethanol. Cap and shake.
13. Measure 2-mL more of this filtrate and transfer to the amber vial labeled 0.02 TJ. To this 2-mL of filtrate, add 8-mL of ethanol. Cap and shake.

14. Bring all 3 small amber vials to the designated area to be stored until we begin our TEAC analysis with the spectrophotometers.
GROUP _____: 25µM Epicatechin

PROCEDURE

1. Safety glasses of course! 😊

2. Obtain 6 amber vials and label them 25µM Epi.

3. Measure out 25-mL of ethanol in a clean 25-mL graduated cylinder. (The ethanol is under the fume hood.)

4. Get a 25-mL volumetric flask, one square of parafilm, and a micropipette with a disposable tip.

5. In the 25-mL volumetric flask, pipet 0.25 mL (250 microliters) of 2.5mM Epicatechin stock solution. (The stock solution can also be found under the fume hood by the EtOH.)

6. Dilute to 25-mL with the ethanol. Remember to use the line of the flask as a guide.

7. Cover the 25-mL volumetric flask with a piece of parafilm by stretching the parafilm over the mouth of the flask to create an airtight seal. With your thumb securely over the parafilmed mouth, invert the flask several times to thoroughly mix the dilution.

8. After the dilution in the flask has been mixed, transfer the entire contents into one of the 6 amber vials that you labeled at the beginning of class.

9. Transfer a total of 4-mL of your dilution to each of the other amber vials. You can use your micropipette to do this. Put a new tip on the micropipette. Set the pipette to 1000 µL (1 mL) and use this setting to pipet four times into each amber vial.

10. When each of the 6 amber vials have 4-mL of 25µM Epicatechin, bring all 6 vials to the designated area in the classroom. (NOTE: The first amber vial that you filled will contain 5-mL of this dilution because we had a total of 25-mL. 5 vials times 4-mL is 20-mL plus the original 5-mL makes 25-mL.)
PROCEDURE

1. Check to see that the spectrophotometer is ON and warming up. There are six (6) set-up around the room and each group will get to use their own. 
   The spectrophotometer will measure the level of antioxidant capacity in each of your solutions.

2. The spectrophotometers should be set to a wavelength of 735 nm. Check this setting on your spectrophotometer. If it is not set to the correct wavelength, use the button marked “nm” with either the UP or DOWN triangle.
   735 nm is the wavelength of light that will go through your samples to obtain the absorbance (A_{735}) readings.

3. (Teammate #1) First, you will need to measure out 75-mL of ABTS⁺ solution (using a 100-mL grad. cylinder) into a clean 100-mL beaker and take it to your lab area. Next, prepare a data table for 2 sets of 12 absorbance (A_{735}) readings. Use the blank sheet of paper attached to this handout. See the board for an example to help you get started. You are free to set up your own data table in whatever manner you would like.

4. (Teammate #2) Get one brown vial each (from the front of the room) of: 25 Tro, 50 Tro, 100 Tro, 200 Tro, 25 Epi, 50 Epi, 100 Epi, and 200 Epi. You also need to get the tomato juice samples your group prepared last Thursday. Take them all back to your lab area.

5. (Teammate #3) Line up 2 rows of 13 clean and wiped cuvets (26 total cuvets) on the counter.
   a. Take some masking tape and put a long strip down on the lab table in front of the cuvets.
   b. Label the masking tape in front of each set of cuvets in the following order:
      i. Blank
      ii. Control
      iii. 25 Tro
      iv. 50 Tro
      v. 100 Tro
      vi. 200 Tro
      vii. 25 Epi
      viii. 50 Epi
      ix. 100 Epi
      x. 200 Epi
xi. 0.1 TJ  
xii. 0.05 TJ  
xiii. 0.02 TJ

c. Put on a pair of purple latex gloves. Fill each of the 2 “Blank” cuvets with 3-mL of DI water. (Put a tip on the micropipette and make sure it is set to 1000µL. Use this setting 3 times with the DI water to fill these 2 cuvets. When finished, eject tip into “Waste Tip” beaker.)

d. Fit your micropipette with a new tip. You will now be filling each of the 2 sets of 12 remaining cuvets with 2.9-mL of ABTS•⁺ solution. **Use this tip until you are DONE filling the cuvets with ABTS•⁺.**

i. For 2.9-mL of ABTS•⁺ solution, put 2000 (1000µL 2 times) in each cuvet, then adjust the micropipette to 900 and put 900µL more in each.

ii. When finished, eject tip into “Waste Tip” beaker.

e. Fit your micropipette with a new tip. (You will start using LOTS of tips at this point! **MAKE SURE TO CHANGE THE TIP BETWEEN EACH SAMPLE!!**) Adjust the micropipette to 100µL. Add 100µL of each dilution into the corresponding cuvets. **REMEMBER TO CHANGE TIPS AFTER EACH SET!**

i. (2) Control = 100µL of ethanol  
ii. (2) 25 Tro = 100µL of the 25 Trolox solution  
iii. (2) 50 Tro = 100µL of the 50 Trolox solution  
iv. Continue in this pattern until all the cuvets have their correct sample added to the 2.9-mL of ABTS•⁺ solution.  
v. Eject all tips into “Waste Tip” beaker.

6. (Teammates #1 and #2) While Teammate #3 is filling the cuvets, the other team members should mix them. Follow this procedure:

a. Take one square of parafilm and carefully cover the cuvet so that no liquid cans escape.

b. Hold the cuvet carefully with your thumb on the bottom and your forefinger on the top. Turn the cuvet gently up and down to mix. **Pick up cuvets by the grooved or frosted sides. The clear side should always face you when you put it in the spectrophotometer.**

c. Dispose of all used parafilm in the “Waste Tip” beaker.

d. After mixing the (2) Control vials, watch the time for 6 minutes to let the mixtures react. Write the time that will be 6 minutes from now in the space below.
e. After 6 minutes have passed from the time you mixed the Control vials, your group can start taking readings with the spectrophotometer.

7. When you are ready to get your spectrophotometer readings:
   a. Wipe the clear sides of the “Blank” cuvet with a Kimwipe.
   b. Place the “Blank” cuvet in the spectrophotometer and close the lid.
   c. Hit the “zero abs/100% T” button.
   d. Do NOT open the lid and remove the cuvet until the screen shows the 0.000 for the absorbance.
   e. When your “Blank” is removed, you can test your samples next.
   f. Be sure to wipe each cuvet with a Kimwipe before putting it into the spectrophotometer. You can reuse the Kimwipes.
   g. Be sure that when you put the cuvet in the spectrophotometer, the clear side of the cuvet is facing you.
   h. Someone in your group should fill in the absorbance (A_{735}) readings in the chart that was made at the beginning of the class period.
Appendix C: Student Semi-Structured Interview Protocols

Student Interview #1

You have just completed the **Skill Building** section of the Antioxidant module. I’m going to ask you questions about your experience in completing those labs. You can refer to your reflection sheets.

1. The first lab you did in this module was preparing tomato juice samples for the **HPLC**. The first questions I’m going to ask you are about that lab.
   - Do you recall any particular questions or types of questions you asked your teacher during those lab days?
   - Do you recall any questions or types of questions you asked me?
   - Did you have questions that you asked your lab partners?
     - Were you able to answer as a group or had to go elsewhere?
   - Did you ask students from other groups questions or for help?
   - Did other groups ask you questions or for help? Were you able to answer?

2. After (snow days) you then had to **analyze your data for the HPLC** using the graphs that were printed out for you.
   - Could you describe how you worked to do the data analysis.
   - Did you ask your teacher for help?
   - Did you ask me for help?
   - Did you ask your lab partner for help? Did lab partners ask you?
   - Did you ask other groups? Did other groups ask you?
   - Did you understand what you were doing in the data analysis? Why or why not?

3. Next you prepared samples of Trolox and tomato juice for the **TEAC** analysis.
   - Do you recall any particular questions or types of questions you asked your teacher during those lab days?
   - Did you recall any questions or types of questions you asked me?
   - Did you have questions that you asked your lab partners?
     - Were you able to answer as a group or had to go elsewhere?
   - Did you ask students from other groups questions or for help?
   - Did other groups ask you questions or for help? Were you able to answer?

4. Then the last thing you did as part of this skill building portion of the module, you did **data analysis of your TEAC** results.
   - Could you describe how you worked to do the data analysis.
   - Did you ask your teacher for help?
   - Did you ask me for help?
   - Did you ask your lab partner for help? Did lab partners ask you?
   - Did you ask other groups? Did other groups ask you? Did you understand what you were doing in the data analysis? Why or why not?
Student Interview #2

You have just completed the Experimental Design section of the Antioxidant module. I’m going to ask you questions about how you designed your experiment for the labs you are going to do for your independent research.

1. How did your group work together to decide what food or beverage you were going to test in your independent research? (Describe the process.)
   Did you ask any questions to your teacher, me, or students in other lab groups or did you work within your group?

2. How did you decide what your treatment would be (or what you would be comparing) for your independent research?
   Did you ask any questions to your teacher, me, or students in other lab groups or did you work within your group?

3. How did your group go about writing the procedures for your actual experiment?
   Did you ask any questions to your teacher, me, or students in other lab groups or did you work within your group?
   Did you all work together as a group or did you divide up tasks?
   Did you refer back to the HPLC and/or TEAC procedures you had done in the skill building section of the module?

4. How did you decide on what dilutions you would make?
   Were you able to calculate how to make your dilutions?

Other Questions to consider if not asked above
- How much do you think you relied on your teacher during this section?
- How much did you rely on your lab partners? (Others?)

- What was the most helpful thing in writing the procedures?

- Do you feel like you were just repeating the procedures already done, or do you feel like you came up with something new for your lab (or parts of your labs you will do)
Student Interview #3

1. In preparing your TEAC samples, could you describe how your group worked (divide up tasks or did everything together?)

2. In preparing your TEAC samples for your independent research, do you recall questions you asked:
   - to your lab partners
   - to other lab groups
   - to your teacher
   - to one of the researchers
   - figured out on your own

3. In your data analysis of your TEAC, could you describe how your group worked?

4. When you analyzed your TEAC data in the computer room, do you recall questions you asked:
   - to your lab partners
   - to other lab groups
   - to your teacher
   - to one of the researchers
   - figured out on your own

5. In preparing your HPLC samples, could you describe how your group worked (divide up tasks or did everything together?)

6. In preparing your HPLC samples for your independent research, do you recall questions you asked:
   - to your lab partners
   - to other lab groups
   - to your teacher
   - to one of the researchers
   - figured out on your own

7. In your data analysis of your HPLC, could you describe how your group worked?

8. When you analyzed your HPLC data in the computer room, do you recall questions you asked:
   - to your lab partners
   - to other lab groups
   - to your teacher
   - to one of the researchers
   - figured out on your own
Student Interview #4

1. You had 3 days to make your posters and prepare your presentation. Could you describe what you first did to design your posters? (How did your group work?)
   *Did you divide up the work on the electronic poster and tri-fold poster?
     - Did you look at data and discuss results?
     - Did you plan out your format?
     - Did you use other resources (other posters, people in other lab groups)
     - Did you discuss any ideas with your teacher?
     - Work together or divide up tasks?
     - Ask any questions to your teacher or other lab groups?
   1A. What did you get done on that first day?

2. How did you work as a group on the second day?
   - Work together or divide up tasks?
   - Interactions with other lab groups?
   - Interactions with teacher or researcher(s)?
   2A. What did you get done on that second day?

3. On the third day, did you finish your poster or practice your presentation?
   - Work together or divide up tasks?
   - Interactions with other lab groups?
   - Interactions with teacher or researcher(s)?
   3A. What did you get done on that third day?

4. Now that you’ve completed the entire module – Skill Building, Experimental Design, Independent Research, and Data Analysis with the creation of the poster, could you summarize how you worked through each of those sections (in terms of how you worked and how you felt as you were working.)

5. Which was the hardest phase for you? Why?

6. Which was the easiest phase? Why?

7. Which was your favorite phase? Why?

8. If you had to do this again, what would you do differently?

9. If you knew someone who was going to do this module, what piece of advice would you give them?

10. Did you feel like you did “real science”?

11. Anything else you want to say about your experience completing the CASPiE antioxidant module?
Appendix D: Teacher Semi-Structured Interview Protocols

Teacher Interview #1

1. Could you describe the procedures you went through before the students began their skill building HPLC labs? This can include any prelab exercises and instruction in addition to what you did at the beginning of class each day they were in the lab.
   - How would you describe your role.
   - How would you describe your students role/work ethic

2. During the HPLC sample preparation labs for the tomato juice, do you recall any the types of questions asked by your students or specific interactions you had with your students?
   Possible follow ups:
   - How did you answer these questions?
   - How frequent were the questions?
   - Was the same question asked by multiple students?
   - Did you ask your students any questions?

3. Could you describe the classroom procedures for getting started on the HPLC data analysis?
   - How would you describe your role.
   - How would you describe your students role/work ethic

4. During the HPLC data analysis for the tomato juice, do you recall any the types of questions asked by your students or specific interactions you had with your students?
   Possible follow ups:
   - How did you answer these questions?
   - How frequent were the questions?
   - Was the same question asked by multiple students?
   - Did you ask your students any questions?

5. Could you describe the procedures you went through before the students began their skill building HPLC labs? This can include any prelab exercises and instruction in addition to what you did at the beginning of class each day they were in the lab.
   - How would you describe your role.
   - How would you describe your students role/work ethic
6. During the **TEAC sample preparation labs** for the tomato juice, do you recall any the types of questions asked by your students or specific interactions you had with your students?
   Possible follow ups:
   - How did you answer these questions?
   - How frequent were the questions?
   - Was the same question asked by multiple students?
   - Did you ask your students any questions?

7. Could you describe the classroom procedures for getting started on the **TEAC data analysis**?
   - How would you describe your role.
   - How would you describe your students role/work ethic

8. During the **TEAC data analysis** for the tomato juice, do you recall any the types of questions asked by your students or specific interactions you had with your students?
   Possible follow ups:
   - How did you answer these questions?
   - How frequent were the questions?
   - Was the same question asked by multiple students?
   - Did you ask your students any questions?

9. How would you describe the extent to which your students were independent during the **skill building phase** of the module?
   - Did you try to make your students work independently or as a group?
   - What, if anything, did you do to get your students to work independently or become less dependent on you for answers?
Teacher Interview #2

1. Could you describe your classroom procedures for the days when your students were completing the **experimental design** phase of the module?
   Possible follow-ups:
   - How would you describe your role.
   - How would you describe your students role/work ethic

2. During the **experimental design** phase of the module, do you recall what types of questions your students asked you?
   Possible follow ups:
   - How did you answer these questions?
   - How frequent were the questions?
   - Was the same question asked by multiple students?
   - Did you ask your students any questions?

3. Could you please compare how independent your students were during the **experimental design** phase as compared to the **skill building** phase of the module?
   Possible follow ups:
   - What, if anything, did you do to get your students to work independently/become less dependent on you during this phase?
   - Were students naturally more dependent or independent in this phase? Describe.
Teacher Interview #3

1. Could you describe your classroom procedures for students to complete the **independent research** TEAC phase of the module?
   - How would you describe your role.
   - How would you describe your students role/work ethic

2. During the **TEAC labs** do you recall any the types of questions asked by your students or specific interactions you had with your students?
   - Possible follow ups:
     - How did you answer these questions?
     - How frequent were the questions?
     - **Was the same question asked by multiple students?**
     - **Did you ask your students any questions?**

4. Just based on your recollection of those labs, how would you describe the way in which your students worked to complete the labs?

3. During the **TEAC analysis** in the computer lab of your students’ **independent research lab**, do you recall any the types of questions asked by your students or specific interactions?
   - Possible follow ups:
     - How did you answer these questions?
     - How frequent were the questions?
     - **Was the same question asked by multiple students?**
     - **Did you ask your students any questions?**

5. Could you describe your classroom procedures for students to complete the **independent research** HPLC phase of the module?
   - How would you describe your role.
   - How would you describe your students role/work ethic

6. During the **HPLC labs** do you recall any the types of questions asked by your students or specific interactions you had with your students?
   - Possible follow ups:
     - How did you answer these questions?
     - How frequent were the questions?
     - **Was the same question asked by multiple students?**
     - **Did you ask your students any questions?**

7. Just based on your recollection of those labs, how would you describe the way in which your students worked to complete the labs?
8. During the **HPLC analysis** in the computer lab of your students’ independent research lab, do you recall any the types of questions asked by your students or specific interactions?

   Possible follow ups:
   - How did you answer these questions?
   - How frequent were the questions?
   - **Was the same question asked by multiple students?**
   - **Did you ask your students any questions?**

9. Now that the skill building, experimental design, and independent research parts of the module are complete, could you compare the way in which your students worked in those three sections (in terms of independence, etc.)

   Possible Questions:
   10. Do you feel like students were trying to work through problems in their groups or coming to you or the researchers for help whenever problems came up in the labs?

   11. Any thoughts on why they worked differently in the independent research part?

   12. Do you have any comments on whether or not students were confident in their lab work?

   13. How do you think your students felt about being able to do their own independent research project?
Teacher Interview #4

1. Could you describe your classroom procedures (or how did you introduce this final phase) for students to complete the data analysis/poster creation phase of the module?
   - How would you describe your role.
   - How would you describe your students role/work ethic

2. Could you describe what went on in your classroom on the first day of the poster completion that took place in your room?
   - Were there many questions?
   - Could you describe your interactions with students?
   - Any specific observations you could make about how your students were working (were groups talking to each other or did all groups work independently?)

3. On the second day, your students worked in the computer lab to get started on their posters. Could you describe how your students worked on that day?
   - What were your interactions with students on that day?
   - What did you feel like your role was on that day?

4. On the third day, your students worked in the computer lab to get started on their posters. Could you describe how your students worked on that day?
   - What were your interactions with students on that day?
   - What did you feel like your role was on that day?

5. Could you take us through the 4 phases – Skill Building, Experimental Design, Independent Research, and Data Analysis/Poster Completion – in terms of what you observed with your students?

6. Could you take us through the 4 phases – Skill Building, Experimental Design, Independent Research, and Data Analysis/Poster Completion – in terms of what your role was as you moved through these phases?

7. What do you feel was most valuable for your students (could be more than one thing) by completing this module?

8. What was most valuable for you throughout this module?

9. If you were going to do this again, what would you do differently?

10. What piece of advice would you give to a teacher who was going to implement this module for the first time?

11. Anything else you would like to say regarding the CASPiE antioxidant module and your experience with it in your classroom?
Appendix E: Reflection Sheets

Name: ______________________________

HPLC Skill Building – Reflection Questions

1) When questions or problems came up in the lab, how did you go about solving them?

2) Describe how the members of your group worked to complete the tasks in the lab.

3) On a scale of 1 – 10 (with 10 being your highest quality of work), how would you rate yourself on the tasks you performed? Why did you rate yourself this way?
Name: ___________________________

TEAC Skill Building – Reflection Questions

1) When questions or problems came up in the lab, how did you go about solving them?

2) Describe how the members of your group worked to complete the tasks.

3) On a scale of 1 – 10 (with 10 being your highest quality of work), how would you rate yourself on the tasks you performed? Why did you rate yourself this way?
Name: ______________________________

Experimental Design – Reflection Questions

1) How did your lab group decide on what to test and your treatment for your independent research?

2) Did you have any discussion with your teacher or the researchers about your experimental design? If so, please describe.

3) Do you feel that your experimental design will yield good results? Why or why not?

4) Now that your experimental design is complete, do you feel that you could perform all the labs by yourself (no lab partners)? Why do you feel this way?
Name: ______________________________

TEAC Independent Research – Reflection Questions

1) When questions or problems came up in the lab, how did you go about solving them?

2) Describe how the members of your group worked to complete the tasks.

3) On a scale of 1 – 10 (with 10 being your highest quality of work), how would you rate yourself on the tasks you performed? Why did you rate yourself this way?
HPLC Independent Research – Reflection Questions

1) When questions or problems came up in the lab, how did you go about solving them?

2) Describe how the members of your group worked to complete the tasks.

3) On a scale of 1 – 10 (with 10 being your highest quality of work), how would you rate yourself on the tasks you performed? Why did you rate yourself this way?
Poster and Data Analysis – Reflection Questions

1) Describe the steps your lab group took to create your poster for your independent research? (What order did you complete tasks – did you look at your data first or design the layout of your poster? Did you divide up responsibilities or work together?)

2) Did you have any discussion with your teacher or the researchers about your data analysis/conclusions or poster? If so, please describe.

3) Do you feel confident in the data and discussion presented on your poster? Why or why not?

4) Now that your poster is complete:
   a. Do you feel that you satisfied with the results from your independent research? Why or why not.

   b. Is there something that you wish you had done differently in your experiments? If yes, please describe.
CASPiE Module Reflection

1. You have completed the 4 phases of the CASPiE Antioxidant Module. Please rank them in order of difficulty from #1 – 4 with #1 being “easiest” and #4 being the “most difficult.”

   _______ **Skill Building** (Completing HPLC and TEAC with tomato juice and analyzing the results)

   _______ **Experimental Design** (Picking your own project and writing the procedures for the labs)

   _______ **Independent Research** (Completing labs you wrote in experimental design)

   _______ **Conclusions and Poster** (Taking the information from your independent research analysis and drawing conclusions and discussing your findings and summing them up on a poster)

2. Please rank (#1 – 4) the sections according to where you felt least independent #1 (this means where you relied the most on your teacher or people outside of your lab group.) to most independent #4 (this could mean independent as an individual or working as a lab group)

   _______ **Skill Building** (Completing HPLC and TEAC with tomato juice and analyzing the results)

   _______ **Experimental Design** (Picking your own project and writing the procedures for the labs)

   _______ **Independent Research** (Completing labs you wrote in experimental design)

   _______ **Conclusions and Poster** (Taking the information from your independent research analysis and drawing conclusions and discussing your findings and summing them up on a poster)
VITA
Matthew Pilarz was born and raised in southern New Jersey. He attended Tufts University in Medford, MA where he earned a Bachelor of Science in Chemical Engineering. After earning his degree, Matt decided that he wanted to teach high school chemistry instead of pursuing an engineering career. To make this transition, he enrolled in the post-baccalaureate teacher certification program at Rowan University in Glassboro, NJ, where he earned his New Jersey certification to teach high school physical science. He then went on to teach chemistry at Eastern Regional High School in Voorhees, NJ. While teaching, Matt earned his Master in Chemistry Education from the University of Pennsylvania in Philadelphia, PA. After eleven years, Matt left high school teaching to enter a doctoral program in the Department of Chemistry at Purdue University.

At Purdue, Matt conducted his dissertation under the tutelage of Dr. Gabriela Weaver in the Division of Chemical Education. His research project focused on high school students’ experiences completing research-based experiments. Also during his time at Purdue, he was a member of Purdue Swim Club, served as a representative on the Graduate Student Advisory Board, and was a member and officer of Phi Lambda Upsilon, the graduate chemistry honorarium.

Matt has accepted a post-doctoral position with Marilyne Stains at the University of Nebraska in Lincoln, which he will begin upon graduation.
Submitted for Publication to Journal of Research in Science Teaching

Examining Student-Student and Student-Teacher Interactions During a High School Chemistry Research-Based Experiment

Abstract

In every classroom, a community develops through interactions that occur among students and teachers and the roles they play during daily class activities. The relationships that develop within the classroom community play an important part in students’ learning experiences. For this study, we implemented a research-based lab module into two high school chemistry classes to study the effects on the classroom dynamics. The module used was developed by the Center for Authentic Science Practice in Education (CASPiE) and was originally intended for use in undergraduate laboratories. The researchers and cooperating teachers worked together to modify the module for appropriate use in a high school setting. The results of this study reveal that there is a shift in the classroom dynamics and lab group dynamics when comparing the introductory skill building labs to the student generated independent research labs. In the introductory labs, student lab groups were very dependent on students in other lab groups and their teacher to complete the lab tasks. In the independent research labs, lab groups functioned more like teams and became more independent of peers in other lab groups as well as the teacher. One contrasting finding that we report is that students do not gain independence and confidence when it comes to the analysis and interpretation of their collected data. However, the overall shift in the community is one that reflects a more authentic science environment in which scientists function as part of a research group with the goal of presenting their results to the larger scientific community.

Keywords: laboratory science, cooperative grouping, sociology, school culture

Introduction and Background

It has long been recognized that the laboratory plays a vital role in the chemistry classroom (Hofstein & Lunetta, 1982). However, research has shown that there are many shortcomings to the actual role the laboratory plays in the chemistry classroom (Hofstein & Lunetta, 2004). One major shortcoming that has been identified and researched is that school science does not provide an accurate depiction of the actual scientific process. Gaskell (1992) points out that there is a clear distinction between authentic science and school science, concluding that school science needs to become more authentic to reflect actual science practices. Traditional “cook book” labs are a misrepresentation of the scientific enterprise, and thus students are not given a true picture of real scientific work (Hodson, 1996; Hodson, 1998).
In an effort to improve the lack of authentic scientific experiences in the classroom, the National Research Council (NRC) published the National Science Education Standards (NSES). These standards include the use of more inquiry activities in an effort to model the scientific enterprise so that students could gain a better understanding of the nature of science (NOS) (NRC, 1996). Although improved understanding of NOS may have been the major goal, research has shown that there have been other benefits with the implementation of inquiry activities. For example, students completing inquiry activities tend to ask more in-depth questions than students completing traditional labs (Hofstein, Shore, & Kipnis, 2004). In addition to asking more in-depth questions, students involved in inquiry activities have also been seen to put more time and effort into completing an evaluation of their lab experience compared to their peers that completed a traditional lab (Hofstein, Navon, Kipnis, & Mamlock-Naaman, 2005). Crawford (2000) reported that inquiry resulted in students’ development of ownership of the activity and also saw a shift in roles of students and teachers in various tasks. Another study reported that a positive effect of inquiry is the increase in discussions and interactions in lab groups which resulted in improved group dynamics (Gormally, Brickman, Hallar, & Armstrong, 2011).

Beyond inquiry labs are research-based, or authentic, labs. The distinction between the two is that inquiry labs have the purpose of simulating the discovery process for the student, though the experiment itself may have a known outcome. On the other hand, research-based labs involve actual scientific research and engage students in designing and executing novel experiments (Weaver, Russell, & Wink, 2008). As with inquiry, research-based labs have been shown to yield positive effects on students and classrooms. Roth and Roychoudhury (1993) reported improvement in process skills that did not need to be explicitly taught separately from the lab. O’Neill and Polman (2004) reported improved practice-based science literacy. Another positive effect observed in the research-based laboratory classroom is more student engagement in the lab and a better understanding of the goals and content of the labs as compared to students in traditional lab settings (Cacciatore & Sevian, 2009). Other noteworthy findings in the implementation of research-based labs are increased interest in the subject, better understanding of the connections between research and real-life applications, and improved perceptions of the understanding of the content (Scantlebury, Li, & Woodruff, 2011).

In considering the positive effects of both inquiry and research-based labs presented here, it cannot be ignored that these effects develop as students work together as part of a lab group and with the teacher as part of a classroom community. Little work has been carried out to study how these effects emerge and how they are linked to classroom dynamics. To do so, it would be necessary to look at the laboratory classroom from a sociological perspective. It has been found that social conversation among students – not just about chemistry – is important in a laboratory class because these interactions help students form the community in that classroom (Del Carlo & Bodner, 2009). The social interactions that take place within a lab group are important for students adapting to working together in that group (Falk, Fishbacher, & Gachter, 2010). It has been shown that working in lab groups give students a more authentic science experience because they work in an environment that is more like the sociological reality.
of scientists, which includes such factors as using effective communication, collaboration with colleagues, and division of labor for task completion (Cunningham & Helms, 1998).

Other studies have looked more closely at the types of interactions that occur in the classroom. One study in a traditional laboratory environment revealed that student-student interactions in a lab group contribute to the learning experience of the students and that student-teacher interactions affect the perceptions of what students deem important in completing a lab (Högström, Ottander, & Benckert, 2010). Krystyniak and Heikkinen (2007) compared student-student and student-teacher interactions between an open-inquiry and non-inquiry class. In examining the types and frequency of interactions, they noticed the open-inquiry students worked more independently of the instructor (Krystyniak & Heikkinen, 2007). In examining student and teacher interactions in a dynamic inquiry activity, Zion and Slezak (2005) found that the teacher’s role shifts during different stages of inquiry. Dependence on the teacher was not a constant throughout the dynamic inquiry. These shifts in teacher dependence and student-teacher interactions created the community of the classroom (Zion & Slezak, 2005). Similarly, Enyedy and Goldberg (2004) concluded in their study that the daily interactions during an inquiry activity shape and reshape the social structure within the classroom. The interactions that occur within the rules of the classroom are what develops the community and defines the roles of both teacher and student in that context (Enyedy & Goldberg, 2004).

The study presented in this paper examines the interactions among students and between students and teachers during the implementation of a research-based lab module. More specifically it compares the student-student and student-teacher interactions in the traditional introductory laboratory to those during a researched-based laboratory that occurs later the same year. A comparison of types and frequencies of interactions in the two lab environments is used to examine if the culture and community of the class is affected by shifting to the research-based approach.

Methods

Description of the Module

The module used in this study was originally developed for the Center for Authentic Science Practice in Education, CASPiE (Weaver, Wink, Varma-Nelson, Lytle, Morris, Fornes, et al., 2006). CASPiE was developed by a National Science Foundation grant to give college students the opportunity to have research experience as part of their first or second year chemistry course. The module implemented in this study was a modified version of “Phytochemical Antioxidants with Potential Health Benefits in Foods” (Burgess, 2011). Modifications were made to the module for two reasons: 1. to make the module appropriate for the level of understanding of high school sophomores and juniors; 2. to make the labs that were designed for three hour time blocks fit into each high school’s schedule constraints. Revisions went through several iterations between one researcher and both teachers who participated in the study. A pilot trial of the module was done in each school in the academic year prior to the implementation and data collection for this study. Upon completion of the pilot study, the module used in this study was finalized by the researcher and both teachers. This module is divided into the
following four phases: 1. Skill Building; 2. Experimental Design; 3. Independent Research; and 4. Results and Poster Presentation.

In the Skill Building phase of the module the students are first given an overview of the entire module. Lab groups (3 or 4 students each) then complete two experimental protocols, which they will later use to design and implement their own research project. One protocol is preparation of samples to be analyzed with high performance liquid chromatography (HPLC) to determine vitamin C concentration for a given food substance. In addition to the lab, students learn how to make the appropriate graphs and calculations needed to determine the vitamin C concentration. The second protocol is preparing samples for spectrophotometric analysis to determine antioxidant capacity via the Trolox equivalence antioxidant capacity (TEAC) method for the same food substance. This data analysis has a graphing component and calculations that apply Beer’s Law.

In the Experimental Design phase, lab groups apply the protocols they learn in Skill Building to design a novel experiment. They are given a list of materials and equipment available to them. They are instructed that they are to pick a food or beverage to study. Students have to determine a treatment to do to that food or beverage, make a hypothesis on how it will affect its vitamin C concentration and antioxidant capacity, then design a procedure for that treatment to perform in the laboratory.

For the Independent Research Phase, each lab group performs the labs that they have written in the Experimental Design phase of the module. This includes the appropriate calculations for data analysis.

In the final phase, Results and Poster Presentation, lab groups interpret their results and make a poster to present their Independent Research results. Students are given a template of what general sections should appear on a poster that is presented at a scientific conference. In addition they have access to seeing examples of actual posters that present research results. The culmination of the module is a poster session in which students must present and defend their findings to their peers.

**Research Questions**

The study presented in this paper examines how the classroom dynamics of the traditional Skill Building lab experiments compare to the dynamics of the Independent Research lab experiments. This research was guided by the following research questions:

1. What, if any, differences are there in how a group of students performs an independent research based lab as compared to a traditional lab experiment?
2. What, if any, differences are there in the interactions among students from different lab groups in a research-based lab as compared to a traditional lab experiment?
3. What, if any, differences are there between student and teacher interactions in a research-based lab as compared to a traditional lab experiment?

**Theoretical Frameworks**

The theoretical frameworks applied in this project are ethnography (Patton, 2002; Bhattacharyya, 2007) and activity systems (Engeström, 1987). Ethnography was chosen because the guiding research questions seek to examine the culture and dynamics of the laboratory classroom. They were designed to explore the way in which lab groups...
function as part of the society within the classroom of peers and teachers. Thus ethnography is a fitting framework as it provides the cultural prospective of the classroom as it develops through the completion of lab experiments. Activity systems is sometimes referred to as a “second generation activity theory” (Smidt, 2009) which was developed from Vygotsky’s original activity theory (Vygotsky, 1976; Vygotsky 1987). Both activity systems and activity theory consider the relationship between subject (learner), object, and artifact. Activity theory classifies actions of the learner to be artifact-mediated, in which the learner uses cultural tools such as language and communication, and object-oriented, in which the learner experiences an activity using material tools. Activity systems goes beyond activity theory by distinguishing that there are both individual and collective activities. Activity systems expands from the basic relationship between learner, object, and mediating artifacts to include the rules, community, division of labor, and the ultimate outcome. (Engeström, 1999). These additional relationships are important in this study as they reflect how the culture of a high school laboratory setting develops. A modified version of the relationships in activity systems (Engeström, 1999) can be seen in Figure 1.

![Modified activity systems diagram](image)

**Figure 1. Modified activity systems.**

*Settings and Participants*

Two settings and groups of participants were used in this study (summarized in Table 1). School 1 is a small, rural junior-senior high school in central Indiana. The research-based laboratory module for this study was implemented into a second year high school chemistry class at school 1. Classes met 45 minutes a day, five days a week. The curriculum for this class would be considered a traditional high school curriculum that consisted of lectures and “cookbook” labs. The class consisted of thirteen high school juniors who had all completed a first year of chemistry the previous school year. Eight
students and the teacher agreed to be participants in this study. The teacher from this school will be referred to as Teacher 1.

School 2 is an academy within a large, urban high school in central Indiana. The curriculum of the academy follows a model of project-based learning. In addition, all classes are two-subject integrated with two different content teachers in each classroom. The research-based lab module for this study was implemented into the biochemistry class at school 2. The chemistry taught is the equivalent of a first year high school chemistry course in Indiana. Classes met for 80 minutes, five days a week. The class consisted of 32 students, most of whom were sophomores. Twenty three students and the chemistry content teacher (who will be referred to as Teacher 2) agreed to be participants in this study.

<table>
<thead>
<tr>
<th>School 1</th>
<th>School 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small rural junior-senior high school</td>
<td>Academy within a large urban high school</td>
</tr>
<tr>
<td>Second year chemistry course</td>
<td>First year chemistry course</td>
</tr>
<tr>
<td>Small class size ( &lt; 15)</td>
<td>Large class size ( &gt; 30)</td>
</tr>
<tr>
<td>45 minute class periods</td>
<td>80 minute class periods</td>
</tr>
<tr>
<td>Traditional curriculum</td>
<td>Project-based learning</td>
</tr>
<tr>
<td>N = 8</td>
<td>N = 23</td>
</tr>
</tbody>
</table>

**Professional Development**

Professional development for the teachers in this study occurred during the summer prior to the pilot implementation. Each teacher completed the two skill building labs, including the appropriate calculations necessary for data analysis and interpretation. In addition to the labs, teachers were also given materials that included instructions and handouts for the module as well as tips on how to facilitate each phase. Since one researcher observed every class throughout the module, he also met with each teacher as necessary to help with the implementation.

**Data Collection**

The primary data sources for this study were interviews with students and teachers, carried out upon completion of each of the four phases of the module. Student interviews were conducted in a focus group format, which generally consisted of three students from three different lab groups. Each focus group completed four interviews at each school respectively. Teacher 1 and Teacher 2 each participated in four individual interviews, which occurred at the end of each phase of the module. All interviews were transcribed verbatim and entered into NVivo®, the software that was used for qualitative analysis. Secondary data sources were collected in the forms of journals kept by each teacher, reflection sheets completed by students throughout the module, and researcher field notes.
Coding and Analysis

For the purpose of this study, we are only making a comparison between the Skill Building and Independent Research phases of the module, since the research questions are focused on the comparison of the classroom dynamics and culture of the traditional Skill Building labs to the student-generated research-based labs performed during the Independent Research phase. Findings reported here are based only on interview data from the Skill Building and Independent Research phases. Other interview data and previously mentioned data sources were used for triangulation and to support the findings reported.

Prior to coding all responses to interview questions, interview data were categorized in the following manner:

1. Responses describing interactions among students within the same lab group;
2. Responses describing interactions among students between two or more lab groups;
3. Responses describing interactions between a student or students and the teacher.

In this study, interactions include verbal interactions such as asking and answering question, explanations, and discussions, as well as non-verbal interactions such as watching a specific lab technique that is being performed. In the event an interview response described multiple categories, it was put into all that were appropriate.

Open coding was first done for each of the three categories described above using the constant comparison method described by Glaser and Strauss (1967). After open coding was complete, the data were grouped based on similarities and patterns seen when comparing all open coded responses. A more structured coding scheme was developed as a result of the relationships seen after open coding. Definitions for these codes were developed between two researchers and all data were recoded by both researchers based on these definitions.

Interrater reliability tests were done with another researcher who had not previously seen the data. This researcher was given the definition of each code and an example of data for each code. This researcher was then given a portion of data from each of the three categories to code individually. Initial interrater reliability was 87.4% agreement. Each piece of data in disagreement was discussed until 100% agreement was reached. This process included referring back to the interview transcript to give the piece of data more context and discussing the definitions of the codes. The definitions for these codes can be found in Table 2.
Table 2
Coding Definitions

<table>
<thead>
<tr>
<th>Code</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Procedures</td>
<td>An instance of asking a question about explaining or executing a lab procedure that is explicitly part of a written procedure</td>
</tr>
<tr>
<td>Problem Solving</td>
<td>An instance of asking a question about or working with other students to solve a problem that cannot be answered by reading the lab procedures</td>
</tr>
<tr>
<td>Checking Work</td>
<td>An instance when students ask for their work to be checked by another student or the teacher, or check the work of another student</td>
</tr>
<tr>
<td>Community Effort</td>
<td>An instance when students state that they work together with students in other lab groups or help students in other lab groups where the common goal of all involved is completing the lab</td>
</tr>
<tr>
<td>Division of Labor</td>
<td>An instance when lab groups split up work to complete tasks individually or in sub-groups. This includes descriptions of instances when groups come back together to complete the lab as a whole combining the results of their individually completed tasks</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>An instance of asking a question pertaining to calculations with and graphing of collected data</td>
</tr>
</tbody>
</table>

In analyzing the data to formulate assertions there were three main factors considered. The first was the distinct number of student participants who made a comment corresponding to each code. The second was the total number of occurrences of coded interactions. By occurrences, we mean that a student may have described more than one situation within a phase of a module that falls into the same code. Table 3 presents the counts for number of student participants and occurrences for codes in the category of interactions among students within a lab group, student-student interactions among students in different lab groups, and interactions between a student or students and the teacher. The third factor considered in data analysis was the strength of the data. Once all interview data of the students were coded, the data were read for the richness of detail to support assertions. After assertions were made, the secondary data sources of teachers’ journals and research field notes were considered for triangulation and support.
Table 3
Coding Counts: the number of participants represented for each code (N) and the number of occurrences (O) of descriptions for each unique instance that was coded in that category. Interactions are listed as SSW (student-student interactions among students within their lab group), SSB (student-student interactions between lab groups), and ST (student-teacher interactions).

<table>
<thead>
<tr>
<th>Code</th>
<th>Interaction</th>
<th>Skill Building</th>
<th>Independent Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Procedures</td>
<td>SSW</td>
<td>12 14</td>
<td>2 2</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>19 32</td>
<td>6 6</td>
</tr>
<tr>
<td>Problem Solving</td>
<td>SSW</td>
<td>8 9</td>
<td>19 24</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>21 28</td>
<td>8 10</td>
</tr>
<tr>
<td>Checking Work</td>
<td>SSW</td>
<td>15 20</td>
<td>8 9</td>
</tr>
<tr>
<td></td>
<td>SSB</td>
<td>19 32</td>
<td>1 1</td>
</tr>
<tr>
<td>Community Effort</td>
<td>SSB</td>
<td>7 10</td>
<td>0 0</td>
</tr>
<tr>
<td>Division of Labor</td>
<td>SSW</td>
<td>22 34</td>
<td>28 62</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>ST</td>
<td>10 13</td>
<td>14 18</td>
</tr>
</tbody>
</table>

Findings

Assertion 1: Lab groups become less reliant on the teacher and their peers in other lab groups when completing an Independent Research lab project as compared to the traditional Skill Building lab.

In comparing the coded data of student-student interactions between lab groups in the Skill Building and Independent Research (Table 3), it can be seen that there is a sharp contrast in the frequency of lab groups interacting with each other. Questions about how to perform lab procedures and checking each other’s work for correctness are very commonplace during the Skill Building phase. However, these are nearly nonexistent in the Independent Research phase. An example of interactions among students in different lab groups during a Skill Building lab can be seen by the following interview response:

Interviewer: So both of you feel like there was a lot of communication between lab groups?
Student 4: Yeah
Student 5: Yeah
Interviewer: And what about you, did you talk to other lab groups?
Student 6: Yes, we asked often, and I think we talked to the other groups often. We tried to see if they know what is going on.
This example shows that students were not confident enough with the lab work of their own group, so they often would ask other lab groups to see “if they know what is going on.” There were several responses from students with instances of having interactions with other lab groups. The more specific example below involves three students in different lab groups discussing how they worked together to complete the first set of Skill Building experiments.

Interviewer: How about things you discussed with your lab partners? Were there things you had to ask your lab partners or your lab partners asked you during that time?
Student 11: Yeah, we usually just help each other out. The different groups... one group will ask the teacher then everyone else knows.
Student 10: Kind of like...
Student 11: We all work together pretty much.
Student 10: When we were doing the dilutions, like the .05, when we were doing equations to figure out the dilutions and how much stuff to put in there. Just double checking all that kind of stuff.
Interviewer: That was double checking with...
Student 10: With other groups.
Interviewer: So was there a lot of interaction between other lab groups?
Student 10: Yeah. (nodding)
Student 11: (nodding yes)
Student 3: (nodding yes)
Interviewer: Really?
Student 10: Yeah.
Interviewer: Can you think of specific instances where you went to another lab group or another lab group came to your lab group?
Student 11: For everything new.
Student 10: Yeah. You know, like “are you guys doing this step right now?” “Yeah, this is how much we’re adding.”
Student 3: And [Student 10], we asked you how to fold the filter paper, didn’t we?
Student 10: Yeah and I asked [Teacher 1]
Interviewer: So let me see if I get this straight. [Student 3] you asked [Student 10] and then you [Student 10] went to Teacher 1 and you brought the information back to the other group?
Student 10: Yeah, pretty much.
Interviewer: OK, did that happen often.
Student 3: Yeah.
Student 10: Yeah
Student 11: (nodding yes)
The dialogue among these students exemplifies a lack of confidence in their own group members to complete the tasks. The description of double-checking their work with other lab groups shows how unsure they are of their own group’s work, and that validation or consensus by peers in other groups is needed to complete the tasks. Also, a student from one group obtained information from the teacher and shared that with the other lab groups. Thus, the small lab groups are so interdependent on each other that they seem to function as one large lab group to complete the lab tasks.

In contrast to the Skill Building interview responses, when students were asked about their interactions with other lab groups in the Independent Research interviews, most students just responded that they only worked with their lab partners and did not go to other groups. For example:

Interviewer: Compare this [Independent Research] to those first Skill Building labs at the beginning.

Student 11: At the beginning we used other groups to compare how to do it and stuff, but now we all know how to do it so we don’t need other groups.”

(Student 5 and Student 6 nod “yes” in agreement to Student 11’s statement)

Interviewer: How do you guys feel about that?

Student 4: Yeah, about the same.

Interviewer: Why do you feel you were able to work more independently?

Student 11: We knew how to do it.

Interviewer: Because you had already done it?

Student 4: Yeah.

Student 6: Because we were more confident. The first time it was more or less (pause) I remember the first time messing with it so we were stumbling along. Now we knew what to do so we could walk right through it.

(Independent Research interview)

It is important to note that the procedures for the Independent Research phase were determined by each group of students themselves, and would likely vary for other groups’ procedures. Although many of the general lab techniques, which were part of the Skill Building labs, employed in each groups’ procedures were the same, each group had a unique set of procedures. Thus it is not surprising that groups would be less likely to ask a question or do the double-checking that they described in the Skill Building interviews. However, students cited that they did not need to consult with other lab groups because they knew what they were doing. One student even mentions that they (lab group) were able to work more independently of other lab groups because of an increase in confidence from having done the prior Skill Building labs.

Although not as sharp of a contrast, there is a noticeable difference in how much the teacher is needed for questions regarding lab procedures and problems in the lab that students could not solve on their own (Table 3). This is evident when comparing the responses of Teacher 1 in the Skill Building and Independent Research interviews when asked about the labs where students prepared samples for the HPLC for each phase.
Teacher 1: I noticed a lot of procedural questions during that time.
(Skill Building interview)

As compared to:

Teacher 1: I didn’t notice any procedural questions. There were a couple of “Hey, where is this? Where is this located?” but no procedural questions.
(Independent Research interview)

Teacher 1 provides a clear contrast of how students were dependent on her in the Skill Building phase to answer procedural questions, but then notices that in the Independent Research labs there were no procedural questions – only questions about where materials were located in the classroom. This is consistent with a previous student quote that cites an increase in confidence from having performed the Skill Building labs.

Likewise, Teacher 2 provides a contrast of how her students worked during the Skill Building and Independent Research labs. In the Skill Building interview and in her journal she describes how she was bombarded with questions during the Skill Building labs and she noted that she observed lab groups also going to other lab groups for help.

Teacher 2: Yeah, so in terms of frequency, I remember very clearly. Um, that it was like everyone was asking questions all the time. I wasn’t, I wasn’t bothered by it, but it was, I had to move quickly from group to group because students were asking a lot of questions and were unfamiliar with what they were doing. I think a lot of those questions could have been answered by reading the lab, but um, I wasn’t bothered that they asked them because I think a lot of them were just trying to make sure that they knew what they were doing. So even though the procedures were written clearly, they just wanted to double check.
(Skill Building interview)

Teacher 2: I spent a lot of time answering questions about procedural issues. While most of these questions could be answered by the lab procedures provided, I think that many of the students just wanted to carefully double-check before they performed the steps. I thought it was interesting that many students asked other groups during this process what to do if they were stuck on a specific step. They used each other and the teachers in the room to help guide them through the process.”
(Skill Building HPLC journal entry)

Both the interview response and journal entry paint a clear picture of how much her students were dependent on her during the Skill Building labs. She comments that
not only was she getting a large number of questions, but that most of these questions
could have been answered simply if the students reread the procedures. Teacher 2 also
noted that she observed lab groups working with other lab groups to complete the labs,
which exemplifies how much students relied on their peers in other lab groups in the Skill
Building labs.

In contrast to the Skill Building experience and observations of Teacher 2, during the
Independent Research Interview she gave this response to a question regarding students’
questions in the lab:

Teacher 2: In terms of the actual lab procedures there were very few
questions. Honestly, I felt like I was wandering around and didn’t
have much to do. At some points, I mean, there are always
students to redirect. You know, in terms of questions about the
procedures there weren’t very many.

(Independent Research interview)

It is an interesting comparison of how Teacher 2 describes her students
performing the Skill Building and Independent Research labs. In the Skill Building she
states that not only was she getting a plethora of questions, but they were questions that
could mostly have been answered by reading the procedures. She also mentions her
students seemed to have a need for their work to be checked, which could be seen as a
lack of confidence in their work, as well as the observation that students were asking
questions of students in other lab groups. This is a sharp contrast to the scene she
describes during the Independent Research labs where she felt like she “didn’t have much
to do.” Both Teacher 1 and Teacher 2 experienced a shift in their roles in the classroom
as student lab groups shifted from being highly dependent on the teacher for the Skill
Building labs to where students complete the Independent Research labs more
independent of the teacher and with more confidence in their own skills.

Assertion 2: Students rely more on their lab partners to complete an Independent
Research lab than the traditional Skill Building lab experiment.

One of the most notable differences in comparing how a lab group functioned
together as a group and relied on each other can be seen by how much they problem
solved with their lab partners. More than double the number of students commented on
problem solving with their lab partners during Independent Research labs as compared to
the Skill Building labs (Table 3). This may be an indication that more problems arose
during the Independent Research. However, the frequency of student-teacher interactions
regarding solving problems the group could not solve on their own went down for the
Independent Research, which points to a shift in how students are seeking solutions
(Table 3). This supports the assertion that students work more cohesively as a lab group
during the Independent Research phase. It not only shows that they are more
independent of the teacher (and other lab groups), but that they are more reliant on each
other to complete the tasks at hand. The following description by a student of how his
lab group worked to complete an Independent Research lab exemplifies how lab partners relied on each other:

Student 8: We would kind of separate and do the things to get everything prepared and we’d come back as a team to put everything together.”
(Independent Research interview)

Not only does this student describe the group’s interactions, but he also refers to his lab group as a “team.” We can therefore deduce that students are not just assigning each other things to do to get the lab done, but are actually relying on each other like teammates who work together and rely on each other to achieve a goal.

Another example that shows students’ independence from their teacher and peers in other lab groups and reliance on their lab partners can be seen here in a response to an interview questions about how they worked through issues that came up during their Independent Research labs.

Interviewer: When questions came up, did you just talk to each other about how to go forward in the lab or did you have to ask other lab groups or the teacher?
Student 20: My group, we just basically talked it over then everybody started getting it.
Student 12: Well, my group, if we had a question we would just ask our group member and usually one of them would know the answer to it.
Interviewer: How about your group?
Student 16: We didn’t ask the teacher or facilitators any questions. We just talked it over with each other.
(Independent Research interview)

These three students, who were in different lab groups from each other, all expressed how they utilized their lab partners to work through problems in the lab, and not seeking any help from outside of their group. This shows how lab groups became more cohesive as a team and more independent of outside help during the Independent Research lab as compared to their work during the Skill Building labs. In addition, the description given by students is corroborated in both classrooms by the following interview response from Teacher 2 and researcher field notes from School 1.

Interviewer: How did your students work in those [Independent Research] labs?
Teacher 2: I think they worked collaboratively and, you know, independently.”
(Independent Research interview)
“After getting settled, the groups began working very quietly. It’s the quietest class I have observed. Groups appear to be having discussions. Both all-male groups have divided up responsibilities – labeling tape, making data table, setting up cuvettes. Two students in a group discuss how to set up the data table; one explains that there are 2 trials for each dilution so two columns are needed.”

(Researcher field notes, Independent Research lab day 3, School 1)

These statements from a teacher and researcher regarding the students’ lab work in both schools describe observable differences in classroom dynamics in comparing the Skill Building and Independent Research labs. This is consistent with the students’ interview excerpts where students said that they did not seek help from outside of their lab groups to complete their Independent Research labs.

Assertion 3: Students gain more trust in their lab partners’ work when completing a lab they have designed themselves.

There are two main indicators that students trusted their lab partners’ work more in the Independent Research Lab compared to the Skill Building lab: 1. the increase in division of labor to get the labs completed; and 2. the decrease in students checking each other’s work. Here is an example of how students described their work during the Skill Building labs:

Interviewer: Did you do any steps individually on your own? Meaning you read a procedure and said “OK, I’m going to do this because I know what I’m going to do or need to do” or did you always have somebody in your lab group working with you?
Student 10: We always had someone double checking it.
Student 11: Yeah (nodding)
Student 3: Yeah.
Student 11: We always double check each other’s work.
Student 10: I don’t want to screw something up.
Interviewer: OK so you never divided up things and said “you do this”
Student 10: Nope.
Student 3: We always had somebody to check.
(Skill Building interview)

All three students, who were all in different lab groups from one another, had similar experiences in the Skill Building labs. They all commented that they did not divide up tasks for students to do individually and that they always got their work double checked or double checked someone else’s work as they were completing steps in the lab. This shows a lack of trust and confidence in their own work and their lab partners’ work in completing tasks individually in the lab. In their Independent Research phase interview, these three students presented a different picture of how their lab groups worked to complete labs.
Interviewer: Did you divide up responsibilities or did you all work together?
Student 10: Yeah, I mean, we were like “hey, can you go get this while I do the stuff with the, you know, pipet’ and stuff like that.”

Student 3: We spent our whole time squooshing up pomegranates, so we kind of. . . one person going and heat, another would check the centrifuge, and another would continue to squoosh pomegranates, so we kind of divided it up.

Student 11: Our group, we split up our tasks to get it done faster and more efficient.

(Independent Research interview)

In contrast to their Skill Building experience of constantly checking each other’s work and working together, in the Independent Research phase the students focused on dividing up tasks to complete the labs and make no mention of checking each other’s work. This shows a shift in how lab partners trust each other to perform tasks correctly in the lab.

This gain in trust amongst lab partners is also supported by the following teacher interview excerpt:

Teacher 1: In the Skill Building they seemed very dependent on the teacher. They weren’t sure of themselves with the procedure and they were asking a lot of questions and they seemed to, maybe not trust each other as much in the groups. . . and then with the Independent Research I felt like they had become even more independent and that was the best they were working together so far. That they each kind of had roles to do and that I could really take a step back and watch them all working at that point.

(Independent Research interview)

Here, Teacher 1 describes the contrast of how students worked in Skill Building and Independent Research phases. The teacher’s perception of the shift in trust amongst lab partners and the lab group dynamics is reflective of the experience described by the students in previous excerpts. Thus, this assertion is supported by both students’ comparative descriptions of how their groups worked in the Skill Building and Independent Research labs and the observations of Teacher 1 in both phases.

**Assertion 4: Whether students are completing the Skill Building or Independent Research experiments, students are still reliant on their teacher for analysis of results.**

During their data analysis within both the Skill Building and Independent Research phases, students had interactions with the teacher with questions about their data analysis. In fact, there was actually an increase in these interactions in the Independent Research phase as compared to the Skill Building phase (Table 3). In the
Skill Building phase, students were guided through the calculations and graphing with worksheets, and occasionally, a teacher demonstration projected for the class to see. Here are two descriptions of how data analysis was guided for the Skill Building labs:

“We got our HPLC lab results back today, and spent about 45 minutes going over the results in our lab groups. I had a step-by-step data analysis guide for them to follow so that they would be able to interpret the peak area and figure out the concentration of Vitamin C in their samples. I feel that the students have a firm grasp on the steps to go through, but they do not understand what the standard curve means or where it came from.”
(Teacher 2, Skill Building HPLC data analysis journal entry)

“Analyzing the TEAC data is more difficult than analyzing the HPLC data. Therefore, I took the whole class through the process one step at a time, using exemplar data (that I made up). I performed the step on the overhead, and then waited until all the groups had performed that calculation with their own data. I noticed that most groups assigned one person to be the “data analyzer” (usually someone that was good with math). Students were very dependent on me during this process, and I was glad we took the step-by-step approach.”
(Teacher 2, Skill Building TEAC data analysis journal entry)

In the Skill Building phase, all students first performed their labs to prepare the same set of samples for the HPLC in order to determine the vitamin C content of that given food substance (in this lab, all students prepared the same tomato juice samples). The students received their data reports after the HPLC run was complete and as described above, had a step-by-step guide to take them through the data analysis process. The next set of Skill Building labs had students prepare samples and collect data utilizing a spectrophotometer. This data was used to determine antioxidant capacity of the tomato juice samples. For this data analysis, Teacher 2 had students follow along to perform calculations using their TEAC data. Although step-by-step approaches were taken for both analyses, there were still questions from students about graphing, such as this:

Student 24: One of the two teammates doing it was confused as to what kind of graph we should’ve put ‘em on.
(Skill Building interview)

Although the teacher gave explicit instructions using a projected example of how to create a graph, students still had difficulty comprehending what type of graph to create and how to create it. Another concern was performing calculations based on the trend line from their standard curve graph. Here is an example of a student who had difficulty performing calculations:
Interviewer: Were you able to just start it on your own or did you need help?
Student 29: Um, we needed help. Cause we’re not all, not all of us are good at algebra, but we can get it if somebody shows us how. We asked the teacher to show us how and we got it.

(Skill Building Interview)

The majority of students’ questions were about getting started with the data analysis. Students needed guidance to do the first step – whether it be a calculation or setting up a graph – after which they were able to complete the analysis tasks. This trend was seen again during the analysis of data in the Independent Research phase.

Even though students appeared to gain confidence when performing their Independent Research labs compared to their Skill Building labs, as described in the previous section, this same trend was not observed with the data analysis. In the data analysis – where manipulation of collected data is involved – students did not gain confidence in their work nor independence from their teachers. Students actually became more reliant on their teachers for help in the Independent Research phase as compared to the Skill Building phase to complete their data analysis calculations and graphs.

The data analysis in both the Skill Building and Independent Research involved calculations with collected data, graphing of a standard curve, calculations that involved using the equation from a trend line from the standard curve graph, and graphing of results after specific calculations were done with the data. Although students were given explicit instructions on all facets of the data analysis during the Skill Building phase and completed the analysis, they were still very dependent on their teacher to analyze their Independent Research data. The same calculations and graphs were to be made for the Independent Research phase that students made during the Skill Building phase, with the only difference being that they were using their own data collected from their independently designed projects. However, the Independent Research data calculations were still difficult for the students. One example of a teacher observation of the analysis during the Independent Research phase underscores students’ dependence on the teacher:

“I repeated myself with each group in explaining how to reconfigure the spreadsheet I had originally set up for them to accommodate their results from yesterday’s run. I spent the majority of today’s class period in helping each group with Excel® spreadsheet issues that involved how and where to input their data to get the correct values.”

(Teacher 1, Independent Research data analysis journal entry)

Teacher 1 expresses that even though she had spreadsheets set up for each student, she still had to go to each group to explain how to manipulate the spreadsheet and input their data. To an extent, student questions tended to be focused on how to use functions in the spreadsheet program.

It is clear that in the Independent Research phase, students still had the same types of issues in the data analysis. In fact, Table 3 shows that students made even more mention of issues with the data analysis during the Independent Research phase as compared to the Skill Building phase. Similar to Skill Building, students had difficulty
getting started with their graphing to begin their data analysis of their Independent Research labs.

Interviewer: Were you able to do that data analysis in your group and problem solve or did you have to ask questions outside of your group for that?
Student 9: For the data analysis we did have to ask outside of our group.
Interviewer: So who did you ask?
Student 9: I believe it was [Teacher 2] that helped us out with that.
Interviewer: Was that very often or just one time?
Student 9: She helped us and just stayed with us most of the time that we were making our graphs.

(Independent Research interview)

Not only did this student state that her group had trouble in just starting to make the graph, but the teacher had to stay with their group to help them create the graph for an extended period of time. Although the same graphing, calculations, and spreadsheet functions were done in the Skill Building phase, it was still difficult for students to complete in the Independent Research without getting help from a teacher. This next example also shows that students seemed to have the most trouble in just getting started with their data analysis.

Interviewer: How were you guys all starting out? Were you able to just go in and do everything or did you need some help getting started?
Student 5: We had to have help.
Student 6: We had to ask [Teacher 1].
(Student 5 laughs)
Interviewer: OK, so let’s start with 5’s group. Tell us how you got started or what you had to ask to get started.
Student 5: Let’s see. We knew we had to plug in the information we had, but then we came to (pause) an equation that we had to do that we were really confused with.
Interviewer: So were you able to plot the standard curve first before asking the questions or did you need help before you plotted the standard curve?
Student 5: We asked before.
Interviewer: And that was with [Teacher 1]? Can you describe how she walked you through it?
Student 11: She just told us.
Student 5: Well, she told us to open up the one we had before because it had the example of what we made before.

(Independent Research Interview)

Although calculations and graphing in the Independent Research were the same protocol as in the Skill Building, students still relied on their teacher for help in completing those
tasks. In contrast to students descriptions of their actual lab work, students did not talk about gaining any confidence in completing their data analysis since they had previously done that work in the Skill Building phase. It seems that they can quickly gain confidence in tasks that involve physical actions, but in manipulation and analysis of data, which involves mathematical operations and reasoning skills, they remain unsure of themselves even in their second time following a set of instructions.

Discussion and Conclusions

In reflecting on our research questions, we do see that there is a shift in student-student interactions within and between lab groups and a shift in student-teacher interactions when comparing the traditional lab to an authentic lab experience. Students became more independent from students in other lab groups and their teacher and worked more confidently and cohesively as a team in the authentic Independent Research labs as compared to the traditional Skill Building labs. The exception to this shift was the student dependence on the teacher for data analysis procedures.

These interactions that we have studied are part of the classroom dynamic that shapes the classroom community. The shifts described in this paper are grounded in the framework of activity systems. Regardless of the type of lab, students had to work within the rules of the classroom. The overall community of the classroom shifted in comparing the dynamics of the Skill Building and Independent Research activities. In the Skill Building phase, the class seemed to be one large community, whereas in the Independent Research Phase, there were several small communities (each lab group) that formed and interacted within the rules of the classroom and thus shifted the dynamics of the community of the entire class. This shift was related to the students’ communication and negotiation with each other on how they would use the available materials and resources to complete their lab tasks. In the Skill Building labs, there was a sense that the labs were a collective activity of the whole class community. Students from different lab groups worked together to complete these labs. In the Independent Research labs, each lab group formed its own community. This is a shift towards a community and culture that is more reflective of an authentic research environment rather than a typical high school chemistry class. In this community, the collective activity was the completion of the lab for each lab group, not the class as a whole. Within these collective activities of the group were individual activities performed by students. These individual activities included simple activities such as gathering materials and larger activities such as performing part of a lab that was necessary to complete the lab as a whole. It was these larger activities that we have presented in this paper as division of labor. In addition, each lab group worked toward the ultimate outcome to successfully complete their Independent Research labs and analyze their data so that they could draw conclusions that they could later present to the entire class community in a poster session.

We have found it interesting that the students and teachers from the two vastly different school settings described similar experiences in the completion of the research-based CASPiE module. These similar experiences reveal similar trends in the shifts in classroom dynamics during the module. Thus we conclude that research-based labs can be successfully implemented into a variety of high school chemistry class environments.
In addition, understanding where students are more or less dependent on their teachers and peers outside of their lab groups could prove to be useful in future implementations of and studies on research-based labs in high school chemistry classes.

Acknowledgments

The authors would like to thank the National Science Foundation for their support for this research through grant CHE-0418902 as well as the support from the Discovery Learning Research Center. We would also like to thank Emily DiNoto for her help in data analysis during this project. In addition, we would like to thank the teachers and students who participated in this study as well as the school districts and administrators who made this research possible.

References


