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The impact of utilizing peer mentoring interactions, new laboratory experiments, and writing-to-learn practices in undergraduate chemistry education

Dulani Samarasekara

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By

Dulani Samarasekara

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By

Dulani Samarasekara

Approved:

Debra Ann Mlsna
(Major Professor)

David O. Wipf
(Committee Member)

Steven R. Gwaltney
(Committee Member)

Xin Cui
(Committee Member)

Tianlan Wei
(Committee Member)

Joseph P. Emerson
(Graduate Coordinator)

Rick Travis
Dean
College of Arts & Sciences

Name: Dulani Samarasekara

Date of Degree: December 13, 2019

Institution: Mississippi State University

Major Field: Chemistry

Major Professor: Debra A. Mlsna

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Candidate for Degree of Doctor of Philosophy

High attrition rates in Science, Technology, Engineering, and Mathematics (STEM) fields are major challenges in undergraduate education. Many students enrolled in STEM fields end up switching their majors to non-STEM fields or leave college without earning any academic qualification. Due to these reasons, the United States is facing a critical shortage of future talented STEM personnel in the domestic workforce. Therefore, graduating a sufficient number of talented students in STEM fields has come to national attention. It is important to examine strategies for improving STEM-major retention and undergraduate education in STEM disciplines.

The main purpose of this study was to investigate methods to improve students' social and peer-mentoring interactions within the undergraduate chemistry program at Mississippi State University to improve student learning and their attachment to chemistry and the STEM major.

In Chapter II, a study performed to examine peer-mentoring interaction patterns that occur between laboratory partners in the General Chemistry I laboratories is discussed. In this study, five different laboratory partnership types were created. In the development of some partnership types, Math ACT score and lecture section were used as metrics for matching lab

partners to create supportive peer-mentoring interactions. Also, students were encouraged to participate in external study groups during the semester. This research study determines whether valued peer-mentoring interactions in the laboratory could support students to be more successful in their chemistry coursework and to have improved social interactions.

In Chapter III, a peer review writing assignment that mimics the publication process is presented. This writing assignment supports students to improve their writing skills by reviewing peer write-ups and practicing critical analysis of their work. This assignment is introduced to upper-level undergraduate students to improve their scientific literacy skills in order to prepare them for future scientific communication.

In Chapters IV and V, two new laboratory experiments that are connected to real-life scenarios are presented. These laboratory experiments are designed to improve student interest in laboratory learning and to enhance their learning in chromatography techniques and hands-on experience with the GC-MS instrument.

DEDICATION

To my husband, Narada Bombuwala Dewage, and my parents, Nandasena Samarasekara and Chandrika Nanayakkara, for their continuous support, encouragement, and love. To my kids, Shameela Bombuwala Dewage and Shaveen Bombuwala Dewage, for their love and inspiration.

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CHAPTER I

INTRODUCTION

1.1 National issues in STEM retention in undergraduate education

Over the last several decades, education and economy experts have claimed that young American students do not excel enough in STEM (science, technology, engineering, and mathematics) disciplines. Also, in terms of STEM education and training, the United States lags behind other highly industrialized nations, and many economic forecasts predict a growing shortage of STEM-trained professionals to fill the future needs in the domestic workforce.[1] To face this STEM crisis, graduating a sufficient number of students in STEM fields has come to the national attention. According to the President's Council of Advisors on Science and Technology's (PCAST) released report, fewer than 40% of students who enter college aiming to major in STEM fields pursue a STEM degree.[2] Moreover, most STEM majors in the first or second year of college leave college without completing a degree or a certificate or make the decision to switch out of their major.[2,3] The most influential reason for switching out of a STEM major was found to be students' negative experiences in their freshman science courses. Therefore, improving introductory courses can be one of the best remedies to increasing STEM-major retention.[3,4]

According to PCAST, increasing STEM retention from 40% to 50% would single-handedly produce three-quarters of the targeted additional STEM degrees the nation requires over the next decade. They have also identified that reducing STEM attrition in colleges is the best and far most effective way to produce the STEM professionals that are required by the current workforce. In order to improve STEM retention, they recommend institutions adopt new teaching strategies that enhance student active engagement, supply necessary tools for all students to carry out their research, and diversify the pathways to a STEM degree.[2] According to the U.S Department of Education, the United States can access a life-long high-quality STEM workforce by building a strong foundation for STEM literacy, increasing diversity and equity in STEM, and preparing the STEM workforce for the future.[5] Moreover, their expectation from young STEM professionals is that they need to be equipped with skills to solve problems, realize information, and identify how to gather and assess information to make decisions in order to be fit for their future careers.[6] As a result of these reforms, numerous significant modifications have been done in the United States to improve the quality of student learning.

1.2 The need for different learning styles

Learning is a life-long activity and fundamental to education. Basically, learning is considered as gathering and constructing new knowledge, improving understanding of a specific subject, sharpening skills, and enhancing performance.[7] To achieve meaningful learning with these aspects, instructor involvement in the learning process is really important. However, all teachers have their own styles of teaching in the same way the students have their own habits of learning. As a result, learning processes have become very complicated and teachers struggle to communicate their concepts and ideas to students.[8] Also, all students come to learning settings with their unique experiences and backgrounds, which determines how well and how easily they comprehend subject materials. To deepen their understanding of the subject matter, some students need different learning strategies such as visual presentations, hands-on activities, and one-on-one attention while other students need only be told once.[9,10] These differences in student learning behaviors cause the teaching process to be more difficult and thus, educators try to understand the learning process better through educational theories focused on making the teaching processes more effective.

1.3 Related learning theories

Constructivism is a learning theory that utilizes many educational environments to achieve meaningful learning. In a classroom, there can be two types of constructivism: cognitive constructivism and social constructivism. In cognitive constructivism, knowledge is constructed in an individual through a personal process. According to this theory, knowledge is constructed within the learner's capability of constructing new knowledge individually and the ability to resolve conflicts. The four different stages of cognitive development are introduced in the Piaget's theory of cognitive constructivism and they are (1) sensorimotor stage, which is from zero to two years old, (2) preoperational stage, which is from two to seven years old, (3) concrete operational stage, which is from seven to eleven years old, and (4) formal operational stage, which is from eleven years old to adulthood. As the theory of different stages of cognitive development shows, learning changes based on the logical development in an individual. Therefore, educators need to understand that each individual, even an adult, has a different characteristic level of understanding and a characteristic learning pace.[11]

In social constructivism, learning occurs due to the interactions of the learner with other social components (instructors and peers) in the learning context.[11] Social constructivists believe that two aspects of social context, (1) "Historical developments inherited by the learner as a member of a particular culture", and (2) "The nature of the learners' social interaction with knowledgeable members of the society" are impactful on the nature and the level of one's learning.[12] Social constructivism, which uses collaboration and social interactions, has been found to be an effective learning method since it can be beneficial to students at all levels within the discipline. Also, it was found that these both cognitive and social components are important for easy understanding of concepts. Therefore, educators need to utilize both cognitive and social constructivism in the learning environment effectively to achieve meaningful learning.[11]

The cognitive load theory of learning is a recently developed theory by Chandler and Sweller, which explains how to approach teaching more appropriately.[11] According to this theory, everyone learns a little differently from one another and reacts differently to newly exposed materials. Therefore, their suggestion is to minimize the students' exposure to cognitive overload, more specifically extraneous cognitive overload, when presenting new information to students. Extraneous cognitive overload denotes the total effort applied and used by an individuals' working memory to process unnecessary new information.[13,14] This theory also suggests that the learning process can be more challenging if the learning task needs more capacity in the working memory as one's working memory capacity is limited.[14]

1.4 Chemistry learning

Most of the concepts in chemistry are abstract. Prior research findings make evident that many students fail to accurately understand fundamental chemistry concepts.[15,16] This poor understanding leads to difficulties in chemistry learning and thus, many students fail to succeed.[16] Typically, science students prefer to learn individually, and they prepare for exams by themselves, reading the assigned textbook and materials. However, this method is not effective for many students.[17] As students have their own preferential learning styles, the learning process cannot be the same for all students. For this reason, chemistry educators have implemented diverse learning styles in their teaching processes to attract and retain students in the program. Use of small group learning activities is found to be a good method to maintain diverse learning styles among students on top of being more effective than traditional teaching methods.[18–20] In chemistry undergraduate education, various teaching and learning methods, such as in-class student-centered collaborative learning,[17,18,21] flipped or inverted classrooms,[20,22–25] personal response systems,[26–28] and out-of-class activities, such as peer-led team learning,[29,30] and web-based practice and assessment systems[31–38] have been introduced and studied. Machine learning techniques, such as intelligent tutoring systems, have also been shown to enhance student learning, engagement, and effort in the classroom.[39,40] Even though various novel active learning strategies have been developed as alternatives to traditional lecture-based instruction, there is a doubt whether students' chemistry learning has improved or new strategies really work better than old methods.[19,41]

1.5 The laboratory in chemistry education

1.5.1 Historical background and reforms

The first chemistry teaching laboratory was established in Britain at the University of Edinburgh in 1807. In the nineteenth century, teaching laboratories were first introduced in universities to train students in research-based experiments. Gradually, teaching laboratories were integrated into schools in England and laboratory work was considered as an essential requirement for science teaching in England. Most universities in England and North America have adopted teaching laboratories to provide students the skills needed for industries and research.[42] After three decades from the laboratory introduction, the opinion towards chemistry teaching laboratories was changed and educators switched back to demonstrations performed by the teacher as they thought the repetitive individual practical work a waste of time.[42] Some educators still believed that laboratory activities help students construct chemistry knowledge. Moreover, these instructors believed that to achieve meaningful learning in science laboratories, students must get the opportunity to manipulate equipment and materials by themselves.[43] Most chemists and educators now agree that laboratory work is an integral part of chemistry education though there are different arguments about what the goal of the laboratory experience should be.[44,45]

Carnduff and Ried outlined the necessity of a laboratory component in the undergraduate chemistry courses. They suggested that goals of the chemistry laboratory include explaining key concepts, visual experience in chemistry, familiarizing equipment, working out specific practical skills and safety, teaching experimental design, emerging observational skills, developing assumption and interpretation skills, developing group working skills, showing how theories are built from experimentation, reporting, presenting, data analysis and discussion, improving time management skills, enhancing motivation and confidence, and improving problem solving skills.[42,46] However, according to Carnduff and Ried, at the undergraduate level, chemistry laboratories rarely achieve all these outlined tasks or objectives in their teaching.[42] Later, at the end of the twentieth century, more sophisticated teaching laboratory formats began to include pre-laboratory experiences, films/video experiments, computer-based pre-laboratories, and post-laboratory exercises.[42] Yet the traditional laboratories were not always able to successfully integrate cognitive (thinking) and psychomotor (doing) domains to ensure meaningful learning.[44,45]

The President's Council of Advisors on Science and Technology disclosed the necessity of making a change in undergraduate gateway courses to improve the quality of student laboratory experience.[44] As a result, several successful laboratory reforms, such as process-oriented guided inquiry learning (POGIL)[47,48], cooperative, problem-based laboratories (PBLs)[49–51], and course-based undergraduate research experiences (CURE)[45,52,53] have been introduced.

In POGIL laboratories, students work in small groups of three or four students and perform activities that are carefully designed to deepen their understanding of chemical concepts and to improve learning and other interpersonal skills. In this context, the role of the instructor is to guide students in developing their understanding and process skills without delivering content to students.[47,48] In cooperative, problem-based laboratory environments, students are tasked to use their chemistry knowledge to comprehend a given real-life scenario in order to design an experiment with all the features of data collection, analysis, and data interpretation. This practice will allow students to get laboratory experience that is very close to a research experience though controlled for a course setting.[44] In CURE laboratory design, students engage in novel research projects and educators and students do not have an exact idea about the outcome of the research project. Students working in CURE laboratories need to be more responsible for what they are doing than the students in other types of research experience embedded laboratories as they need to make decisions throughout their work. However, they have the opportunity to get research experience in a particular area from development of the research question all the way through to a publication.[45]

Overall, these laboratory designs with research experience are growing in popularity as educators believe these designs support learning goals in the laboratory. However, there is a practical issue in implementing these kinds of laboratories in universities, where large annual undergraduate student enrollments occur, due to the need for more sophisticated instruments. Student success also depends significantly on the support they receive by their research group members. Although many institutions have implemented research-based laboratories based on the success they have shown, further studies are needed to ensure student success within this new laboratory curriculum.[45,54]

1.5.2 The effectiveness of laboratory experiment styles

The different laboratory instruction styles (Traditional labs, Inquiry, Discovery and Problem Based) have been introduced into the undergraduate Chemistry curriculum with the goal of improving student learning.[49,55,56] These laboratory instruction styles can be mainly categorized into four styles based on the nature of the outcome, approach, and procedure of experiments performed in the laboratory. The four instruction styles are expository, inquiry, discovery, and problem-based. Descriptors of the four main laboratory instruction style are given in Table 1. These laboratory instruction styles are often categorized as traditional (expository) and non-traditional styles (inquiry, discovery, problem-based).

Table 1.1 Descriptors of the four main laboratory instruction styles

Style	Descriptor		
	Outcome	Approach	Procedure
Expository	Predetermined	Deductive	Given
Inquiry	Undetermined	Inductive	Student-generated
Discovery	Predetermined	Inductive	Given
Problem-based	Predetermined	Deductive	Student-generated

See reference[57]

The effectiveness of these instruction styles has been compared by different educators. Yeghia Babikian found that traditional laboratory teaching is more effective than discovery instruction style concerning overall student achievements.[58] Later, a meta-analysis conducted by Gerald W. Lott showed that traditional and non-traditional approaches are not significantly different in terms of overall student learning, although some studies have shown that non-traditional instruction is superior to traditional instruction.[59] However, a later study conducted by

Rubin showed that non-traditional instruction is significantly better in various aspects, such as content knowledge, critical thinking ability, and attitudes when compared to traditional instruction. Rubin also found a conceptual knowledge difference between these two groups.[56] Nevertheless, a recent study conducted by Cox and Junkin showed that the addition of conceptual questions into expository laboratory procedures and allowing students to discuss these questions with their group members significantly improved students' conceptual understanding.[60] Therefore, educators need to realize that each laboratory instruction style is different and possesses different limitations on what specific learning outcomes can be achieved within each discipline. It is educators' responsibility to implement the desired instruction style with varied instructional techniques to meet the requirements outlined by the National Science Education Standards and support the development of student understanding.[56,61]

Although expository instruction style is highly criticized, it is the most widely used instruction style in many institutions as it is designed in a way that a large number of students can work simultaneously in the lab under minimal instructor involvement and low operational cost.[57] Also, the studies performed to investigate students' affective experiences in laboratories to improve their interest, showed that experiments with real-life connections can positively affect student attitudes towards chemistry and laboratory learning.[62] Therefore, introducing new laboratory experiments with new techniques, real-life connections, and questions that improve student conceptual understanding within any kind of instruction will be beneficial.

1.6 Peer mentoring interactions

Retention in STEM fields is difficult for many undergraduate students. According to early research findings, students' ability to make an attachment to their major and to create social interactions with peers in the discipline are two main factors that determine their STEM retention.[63] Therefore, to address STEM retention, different kinds of group/collaborative learning approaches consisting of different mentoring interactions have been integrated into STEM undergraduate teaching.[64–68] "Collaborative pedagogy" possesses many definitions in the literature. However, it can be defined broadly as the "attempt of two or more students to learn something together". Group exercises in classrooms and laboratories are considered a part of collaborative pedagogy assist students to learn through experiences, through leveraging the perceptions of their peers, and through creating their own ideas via social constructivism.[69] Past research studies have also shown that collaboration or small-group learning has a positive impact on student accomplishments, self-esteem, and attitudes towards learning.[70]

In general, peer instruction (PI),[71–73] problem-based learning (PBL),[74–77] team-based learning (TBL),[78,79] and process-oriented guided inquiry (POGIL)[80–83] are some of the different group work formats used in undergraduate science classrooms.[27] Research studies vary on the type of the group work implemented, how groups are used in the class, how groups are formed, what students do, and how the groups are assessed. Particularly, in such environments, student performance can be affected by the way groups are formed, including whether students self-select into groups or the instructor forms groups. That is because when students get the opportunity to make their own groups, they commonly get into groups according to similar ethnicity, gender, or class achievements and thus, suffer from lack of diversity.[84] However, well-designed student-centered group work processes provide improved cognitive learning outcomes and enhanced student motivation and engagement compared to traditional lecture-based methods.[20,84] Additionally, student-centered teaching approaches that integrate problem-based learning and collaborative problem-solving activities enhance student knowledge construction and develop student success more than traditional learning methods do. Peer discussions in student-centered learning environments help students explain gaps in background knowledge that are necessary to understand and apply class material. Similarly, these discussions encourage students to regularly assess their own levels of understanding and skills at handling concepts or problems in a particular discipline.[17,19] These facts reveal that improved student interactions can enhance student chemistry learning and thus improve student attachments to their major, which remediates the STEM attrition.

Laboratory learning has become a compulsory component in many undergraduate General Chemistry programs as it is a requirement by the American Chemical Society accreditation.[85] Also, since students have more time in laboratories to engage in cognitive and social knowledge construction process, General Chemistry laboratories can be a better place for students to enhance their learning. It is also believed that instructor-student interactions are improved in laboratories than in lectures.[85] Most of the time, in General Chemistry laboratories, students work in small groups of two or three students. Therefore, General Chemistry laboratories can be a better place for students to make connections with their peers in order to get help for their personal critical thinking process. In addition to this collaborative learning, the social network that students create due to laboratory partnership interactions might help students enhance their attachment to major and institution and hence, improves STEM retention in the undergraduate Chemistry program.

1.7 Writing skills as a life-long goal

Science is not just doing experiments and discovering new things; communicating outcomes into the general public or the scientific community is essential.[86,87] The recommendation of National Science Foundation (NSF) for science educators is to implement new teaching practices to enhance learning, create supportive learning environments, build inquiry, use communication and teamwork, encourage critical thinking, and set skills into learning experiences.[88] Communication and life-long learning skills outlined in this framework include scientific literacy that students need to have in order to communicate with the general public and the scientific community. Therefore, helping science undergraduate students to develop their written communication is important as they need writing skills in their future careers regardless of the path they would choose after graduation.[89] In terms of communication with the general public, science journalism is the key conduit for the dissemination of scientific information. In these kinds of media, scientific information needs to be written at level that a general audience can understand. Writing to the non-scientific community is not an easy task for scientists without formal writing training when they possess increased specialization in a particular field over a long period.[87] Therefore, in the undergraduate curriculum, science writing skills needed to communicate with the general public are usually practiced by integrating different writing assignments into general lecture courses. In some assignments, students are tasked to read a recent scientific paper and to summarize the key points into an article while in some assignments students are tasked to publish their writings in class blogs or newsletters created by the course instructors.[87]

In terms of communication with the scientific community, students need to have the ability to effectively write peer-reviewed journal articles, grant proposals, and literature review articles.[86] Generally, graduate students are encouraged to publish their work early and often during the graduate program. Also, scientists are generally evaluated by the number of papers published and the number of citations those papers receive.[90] Scientists who can communicate effectively are well recognized and well treated by the members of their own community, research funding agencies, and the wider society.[86] As scientific writing is crucial for both graduate studies and future careers, it is important to provide a sound experience in scientific writing to undergraduate students. Therefore, assisting undergraduate students to become better scientific writers has been a concern for many years.[91] However, scientific writing is often neglected in many science curricula as much focus is given on enhancing student concept learning and problem-solving skills. Additionally, the reluctance of instructors to adopt writing assignments into their programs due to the time taken for reviewing and commenting on student writings and ambiguity associated with the grading of student reports have also been shown to impact the poor implementation of scientific writing.[86,92]

During the past few decades, various "writing-to-learning" practices, that can enhance student learning and engagement with STEM discipline have been introduced to the undergraduate curriculum. These various writing approaches include scientific writing training in laboratory courses for writing short scientific reports,[86] formal journal-style full lab reports including abstract, material, and method sections[89,93] using students' own data they gathered from laboratory experiments, and scientific literature review reports[94–96]integrated into lecture courses.

Studies have shown that writing scientific reports on a topic related to subject matter develops a student's ability to gather scientific ideas as well as permitting the coverage of the subject matter.[95] Some educators integrate writing assignments into the undergraduate curriculum to develop students' scientific writing skills needed for manuscript publication.[97] These assignments have been designed so that they mimic the publication process and train students in different layers of the publication processes; some were limited to mimic the peer-reviewing process[98] while some assignments asked students to write manuscripts strictly following guidelines for a major journal[99] and some included 'letter of inquiry'[100] to notify the significance of the subject matter to their instructor. Other undergraduate students have the opportunity to write publications or theses after contributing to faculty-mentored research projects.[101] These kinds of writing assignments have been found to be more effective than literature report writing assignments as they can increase students' self-efficacy towards performing various writing tasks and critical-thinking, in addition to the significantly improved quality in their scientific writing.[86,101] Almost all writing approaches mentioned above are often followed by peer-reviewing to make students' scientific writing more perfect in content and grammar. Moreover, the peer-reviewing process can improve students' critical evaluation skills, which is a life-long scientific skill they may require for their future career.

1.8 Peer reviewing process in student writing

Integration of well-designed scientific writing practices into the undergraduate science curricula have been found to be very effective, as it improves students' discipline-specific writing skills, scientific literacy skills, self-efficacy, conceptual understanding, knowledge acquisition, and cognitive skills in the science disciplines.[86,92,96,102,103] However, these student achievements obtained due to scientific writing practices are often deepened by peer-reviewing processes. In peer-reviewing, students critique one another's work with the intention of supporting their peers by providing feedback. According to the social constructivism theory, learning is constructed in students due to exchanging, sharing, and negotiating of ideas in both personal inner process and social aspect.[104] That indicates that the peer-review process can help construct new knowledge in students. The peer-review process in scientific writing assignments provides support to both student authors and reviewers.[95] The student who receives peer feedback may benefit from getting external ideas on his or her writing from a different viewpoint while the student who reviews other's work might benefit from obtaining experience in reading and analyzing the work of a peer as it provides ideas for improving their own work.[105] The peer review process is considered an important tool for undergraduate studies by many educators and has been adapted into the curricula to improve student writing, reduce the instructor workload, and improve students' positive attitudes towards the peer-review process.[97,104] Most importantly, some educators embedded literature review writing assignments followed by peer review processes into their syllabi in order to switch the learning process from a teacher-centered approach to a student-centered approach as it offers students the ownership of their learning.[95]

In the early peer-review implementation stage, the quality of peer feedback has been highly criticized due to several reasons.[88] One reason was that students were not able to provide honest feedback about the writing of their peers in their face-to-face reviewing processes as they could not hurt others' feelings and thus, the feedback was biased. Also, the quality of feedback was uneven, as students with better writing abilities showed better reviewing ability on peers' writing. Therefore, students with higher writing capabilities showed less preference to accept peer feedback from peers known to have lower capabilities compared to them.[104] In response to these concerns, several new peer-reviewing approaches consisting of electronic communication, anonymous peer review, and multiple reviewers in the process have been introduced.[104] The use of electronic communication has provided many advantages over the traditional face-to-face approach, such as the expansion of the classroom boundaries, enabling students to work from anywhere, anytime, promoting equal participation of group members, and ensuring the anonymity of the participants.[104] Chemistry is in the news (CIITN)[106] is an example of an electronic communication tool that can be used for both studying and peer-reviewing processes.[88]

Calibrated peer review (CPR) is a popular instruction tool that is used by many educators to obtain better peer-reviewing quality.[89,107] CPR assignments consist of four main stages: text entry, calibration, peer review, and self-assessment. In the CPR process, students first upload their writing into the CPR web site. Then, they are tasked to read several calibration writings, which are written in variable quality levels, and to grade those works. According to the reviewing quality that a particular student shows on the calibration documents, a reviewer competency index is produced. In the next stage, students are tasked to review a selected number of anonymous scientific writings. Finally, students assess their own writing and grade their work.[89] In this way, uneven grading of student reports can be avoided as peer reviewing and self-assessment grades are corrected by the reviewer competency index. Grading rubrics can also be used to assess peer reports in an even and fair way.[88] Adopting these improved peer-reviewing process into undergraduate curricula is important to enhance student scientific writing ability as it trains them how to communicate with peers by arguing and criticizing their work.

1.9 Outline of the dissertation

Chapter II of this dissertation will discuss a study that focuses on determining the effects of five different laboratory partnerships established in General Chemistry I laboratories at Mississippi State University. In this study, Chemistry lab sections were randomly designated into one of the five different laboratory partnerships; (1) free choice (FC)- students found their partners, (2) random assignment (RA)- lab partners were assigned randomly, (3) side-to-side assignment (SS)- kept Math ACT score constantly among student pairs (4) high-low assignment (HL)- kept Math ACT score variable among student pairs (5) lecture section-based assignment (LB)-students paired with partners in the same lecture section. In General Chemistry I lectures, students were encouraged to join study groups outside the classroom in order to improve their academic performance. Academic performances will be compared among the created partnership groups to examine whether any partnership type benefits over the other partnership types. How students' attitudes change according to their laboratory partnership type and how it affects their study group interactions outside the classroom will be discussed. Finally, how peer mentoring interactions were impacted with each partnership type and how low-performing students are benefited in those partnerships will be discussed.

Chapter III will discuss a literature review writing assignment incorporated into upper-division Environmental Chemistry course at Mississippi State University. This writing assignment used peer review and response to reviewer comments to improve students' writing skills. The process employed an anonymous and timed in-class peer review format. In addition to editing peer papers, students were tasked to create a response to reviewer comments document, which the authors used to mimic the peer-review process required for scientific publication. The response to reviewer comments document was designed to have students think critically about their writing and defend their choices concerning peer edits. Results of essay quality, reviewing quality, and student surveys will be presented.

Chapter IV will present a simple and inexpensive paper chromatography experiment that separates and identifies major organic acids in wine and fruit juices. This new laboratory experiment was developed for introductory organic undergraduate students to teach the basics of chromatography. This experiment reinforces several concepts for students such as compound separation via extraction and chromatography, intermolecular forces and acidity, and a comparison of organic acid polarities related to structure. Also, the separation of acids within wine and/or fruit juices enhances student understanding of real-world organic acids present in foods. Citric, malic, tartaric and lactic acids, all potential components of fruit juices and wine samples, are the focus of this experiment that includes a description of the malolactic fermentation occurring in wine samples. Retention factor calculation and identification of acid types present in selected samples encourage student understanding of overall acidity and the relationship of pKa values to the acid structure. How students achieved these learning objectives and how they rated their feelings about this new laboratory experience in the survey will be discussed.

In chapter V, a new laboratory experiment designed for upper-level analytical chemistry undergraduate students to help improve their proficiency with instrumental analysis via GC-MS will be presented. This laboratory experiment helped students to understand real-world application of analytical techniques and fundamental theoretical principles while improving their analytical thinking skills. In this laboratory experiment, students extract xylitol from both fresh and chewed gum sticks followed by direct aqueous injection GC-MS analysis. Students learn the proper steps and techniques required for sample extraction and preparation, GC-MS analysis, and determine concentrations of xylitol present in gum samples. Identification and quantification of the chemical components in gum extracted via GC-MS analysis will be discussed. Also, a comparison of external and internal standard calibration methods to quantify xylitol in chewed and unchewed gum samples will be presented. Levels of student understanding on sample injection techniques, quantification of xylitol, correct use of calibration method, and student opinions about the new laboratory experience will be discussed.

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CHAPTER II

IMPLEMENTATION AND EVALUATION OF DESIGNATED LABORATORY PARTNERSHIPS IN AN UNDERGRADUATE CHEMISTRY LABORATORY

2.1 Introduction

Laboratory components have long been included in undergraduate curricula as the hands-on nature and collaborative interaction are supportive of chemistry learning. Many research studies have been done in the past decades to investigate and improve learning in the undergraduate chemistry laboratory, especially to improve student active engagement[3–8] and attitudes[4,9–15] toward science. Within these research studies, the priority has focused on changing the format of lab using different instructional styles, such as expository, inquiry, discovery, and problem-based curricula.[3,6,8,16–19] Moreover, analysis of student perceptions on their learning,[20–23] faculty goals for laboratory learning,[24–26] the role of graduate teaching assistants,[27–30] implementation and examination of virtual laboratories,[31–33] use of scientific instrumentation,[34,35] and research-based laboratory curricula[36–39] have also been performed. However, although the "cookbook" nature of the traditional expository laboratory has been greatly criticized, it is still the most widely used style of laboratory instruction as activities can be performed simultaneously by a large number of students with minimal instructor intervention, cost, and time (typically within a two-to three-hour time frame).[19]

According to constructivist theory of knowledge, knowledge cannot be transferred from one individual to another, it must be constructed in the learner through interactions with the environment.[19] The interactions of students with their instructors and other students can facilitate the construction of knowledge.[40] But as research studies reveal, in a traditional laboratory the instructor-student engagement can be limited.[1] To support mentoring interactions, peer-led team learning, which allows students to work in small groups and actively engage with a trained undergraduate leader, has gained much attention.[1,2,41] Successful learning for students is built by the interactions of individuals with different skills, ideas, and backgrounds.[2] However, few research studies have investigated the impact of peer mentoring in laboratory partnerships on student academic performance and attitudes in chemistry.

Studies on pedagogical agent design show that student interactions with social models having similar attributes, such as gender, ethnicity, and competency have predictive significance on their efficacy beliefs and achievements.[42] Also, research studies on analyzing the faculty-student interactions in undergraduate settings show that differences in racial/ethnicity matching impacts interactions between students and faculty members.[43,44] This research study was based upon frequent observation of how undergraduate students chose their own laboratory partners at our institution. The first day in our laboratory classes, students pair up in predictable partnerships, female with female, male with male, African American with African American, White with White. Not all of these observed partnerships seem to support students toward success and we often notice a pattern where student partners fail together.

Our goal with this research study was to see if fostering valued peer-mentor interactions in the laboratory could support students to be more successful in their chemistry coursework. We used Math ACT score and lecture section as metrics for matching lab partners to create supportive peer-mentoring interactions. In total, five different laboratory partnerships were created. Free Choice sections (FC) could pick their own lab partners. Random Assignment sections (RA) had laboratory partners randomly assigned from the roster in the class. Lecture Based (LB) partners were randomly paired within a lab section with other students enrolled in the same lecture section. If no other students from the same lecture section were available, we paired students that had the same lecture instructor. As each instructor had differing exams and examination schedules, the Lecture-Based partners could potentially study for exams together. The Math ACT sections were split and arranged in two different ways: Side-to-Side (SS) ranked all students in a section by Math ACT score (low (#1) to high (#24) and paired the lowest student (#1) with mid-point (#13); student #2 was paired with student #14 and so on (Figure 2.1). This established lab partnerships that would theoretically be of consistent difference and created a lower-higher Math ACT score pairing. The High-Low Partnership (HL) ranked all students in a section by Math ACT score (low #1) to high (#24) and paired students lowest (#1) with highest (#24). The next partnership paired second-lowest (#2) with second-highest (#23) and so on (Figure 2.1). This established lab partnerships of varying Math ACT score difference with some partnerships showing large differences in Math ACT score while others were close in score.

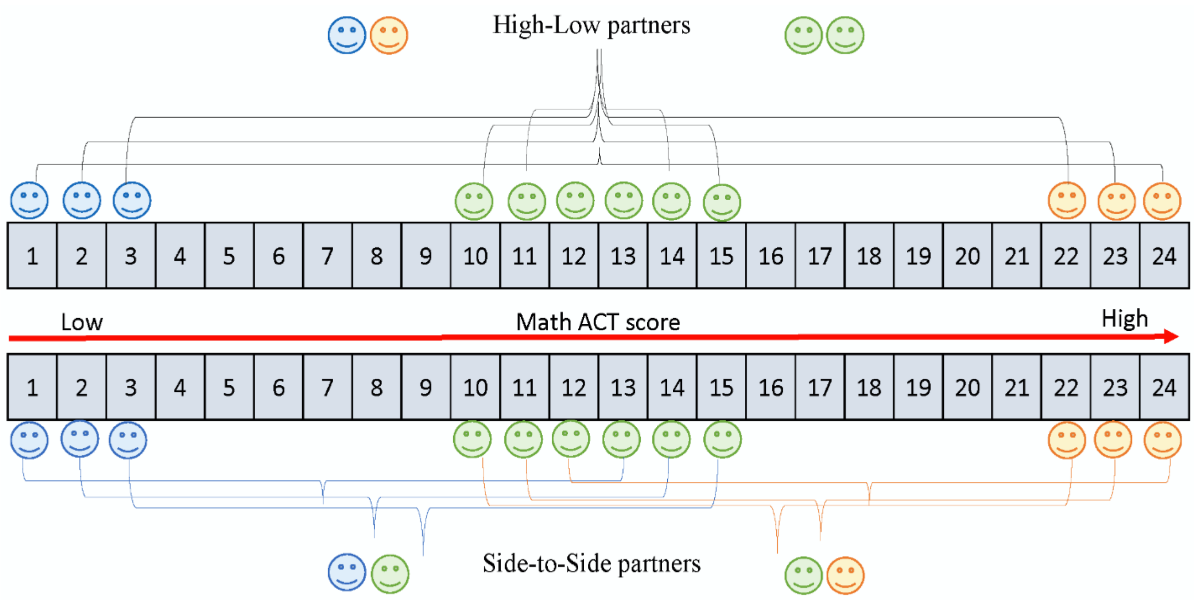


Figure 2.1 Representation of Math ACT score pairing in Side-to-Side and High-Low partnerships.

Students were assigned or chose laboratory partners in the first laboratory meeting and were monitored by the Graduate Teaching Assistants (GTAs) throughout the semester. Graduate Teaching Assistants were not informed on how partners were matched; partner names were listed on a roster given to the GTA, or GTA was assigned to section where students selected own partners. In addition, researchers performed observations in the laboratory once per semester to evaluate lab partner dynamics. For the external study group portion, students were encouraged to participate in study groups during the semester. The information on study group participation is included in Figure A.1. To facilitate study group interactions, students could choose to be included on a master list for each lecture section that included name, email address, major, and housing designation. Students could use this list to contact others if they wished.

2.2 Research questions

The focus of this research study was to determine if laboratory partnerships could support peer-mentor interaction and support students academically in their chemistry course.

The study was guided by three main research questions:

1. Do assigned lab partnerships impact student academic performance through peer-mentoring interactions?
2. Do assigned lab partnerships encourage students to find external study partners?
3. Are student attitudes towards general chemistry impacted based on lab partner assignment?

2.3 Methods

2.3.1 Participants

Students enrolled in General Chemistry I laboratory course in Fall 2012 (*N* = 1234), Spring 2013 (*N* = 881), Fall 2013 (*N* = 1338), and Spring 2014 (*N* = 945) participated in this study with partner designations. Only consented students (*N* = 631, 385, 700, and 406 respectively) were included for analysis. Ninety percent (90%) of consented students completed the end-of-course survey (*N* = 1913). Student sections were randomly selected for the five different laboratory partnerships with variability in day, time, and graduate teaching assistant. Number of consented participants for each assignment were as follows: Free Choice (*N* = 455), Random Assignment (*N* = 513), Side-to-Side assignment (*N* = 302), High-Low assignment (*N* = 295), and Lecture Based assignment (*N* = 557). The sections designated based on Math ACT score were split, with half the sections partnered via the High-Low assignment, and the other half of the sections partnered via Side-to-Side assignment. For some of our analyses, the SS and HL groups were combined into one partner type (Combined Math ACT; CM).

2.3.2 Demographics

Student demographic matrices of gender, ethnicity, classification (year in school), and Math ACT score were summarized according to semester (Table A.1) and lab partnership type (Table 2.1). All students who do not belong to the ethnicity groups African American or White, were bundled collectively due to low individual sample sizes. Here, the ‘Other’ ethnicity group includes Asian, Hispanic or Latino, Hawaiian, American Indian, and multiracial students. Math ACT competency levels were defined as low-performing: Math ACT < 24, mid-performing: Math ACT = 24 -26, and high-performing: Math ACT > 26. These designations were categorized based on historic data of predicted student performance in our General Chemistry I classes.

Table 2.1 Comparative demographic information of students in different laboratory partnerships.

Demographic categories and variables		Student population in different laboratory partnerships, % (Number of students)					
		Four major partnership types				Combined Math ACT Breakdown (CM)	
		Free Choice (FC)	Random Assignment (RA)	Combined Math ACT (CM)	Lecture Based (LB)	Side-to-Side (SS)	High-Low (HL)
Gender	Male	50.8 (231)	53.7 (275)	54.0 (322)	58.8 (326)	50.8 (153)	57.3 (169)
	Female	49.2 (224)	46.3 (237)	46.0 (274)	41.2 (228)	49.2 (148)	42.7 (126)
Ethnicity	White	72.1 (326)	76.2 (390)	78.0 (461)	76.3 (422)	82.2 (245)	73.7 (216)
	African American	20.6 (93)	15.2 (78)	13.9 (137)	14.0 (77)	11.1 (33)	16.7 (49)
	Other ethnicities	7.3 (33)	8.6 (44)	8.0 (48)	9.7 (54)	6.7 (20)	9.6 (28)
Classification	Freshman	77.4 (350)	75.2 (385)	77.5 (458)	74.9 (414)	75.5 (225)	79.5 (233)
	Junior	2.9 (13)	8.2 (42)	6.1 (36)	6.1 (34)	6.0 (18)	6.1 (18)
	Sophomore	17.5 (79)	13.5 (69)	14.4 (85)	15.6 (86)	17.4 (52)	11.3 (33)
	Senior	2.2 (10)	3.1 (16)	2.0 (12)	3.4 (19)	1.0 (3)	3.1 (9)
Competency level (according to the college entrance exam grades)	Low, Math ACT < 24	35.3 (151)	34.4 (166)	35.0 (203)	31.7 (167)	35.7 (101)	33.6 (94)
	Mid, Math ACT = 24 - 26	25.9 (111)	26.3 (127)	29.8 (173)	30.5 (160)	31.4 (89)	27.1 (76)
	High, Math ACT > 26	38.8 (166)	39.3 (190)	35.2 (204)	37.8 (199)	32.9 (93)	39.3 (110)

2.3.3 Laboratory course descriptions and student assignments into different lab partnerships

The General Chemistry I Laboratory course used for this study uses structured inquiry experiments designed to support lecture material. A typical lab activity will include quiz, experiment, data sheet, and post-lab questions. We initially attempted to analyze post-lab questions as a way of understanding student critical thinking in the lab but found the grading of assignments too supportive to use in the research study (all grades were 100's). As a result, we only included the lecture examination results for determination of the laboratory partner impact. In addition, all students were provided information on benefits of joining a study group (see Figure A.1) and were encouraged in both lecture and laboratory sections to find study partners for the General Chemistry I lecture course.

Laboratory sections were randomly designated to partnership group at the beginning of the semester, with variability in day, time, and graduate teaching assistant. Student designated Free Choice sections were allowed to pick their own lab partners on Day 1 of lab. Partners in assigned partner sections were randomly paired before Day 1 and were told of their partner assignment on Day 1 of lab by the GTAs.

2.3.4 Measures

To analyze students' academic performances, student lecture grades from 4 semester exams and the final ACS standardized exam were used. As students had differing instructors and tests administered, all exam grades were converted to z-scores by using respective grade means and standard deviations from each lecture section. For statistical analyses, the average of all five-exam z-score grades were used. Final lecture course letter grades were also used to compare overall course success; letter grades included exam scores and homework assignments for the semester. Student attitudes toward their academic performance, laboratory, and study groups, were collected via end-of-semester surveys. The survey questionnaire is given in appendix (Figure A.2).

2.3.5 Data analysis

To obtain more generalizable samples, all semester data was combined and broken down into four different sets according to laboratory partnership and Pearson's Chi-square was performed to compare gender, ethnicity, and classification of students. Student Math ACT scores (college entrance exam grades) were compared using one-way independent ANOVA. The average z-score of all five examination grades was used in all statistical analyses, with z-score grades being normalized to avoid lecture section bias. To assess the feasibility of bundling these grades into one z-score for the statistical analyses, a Pearson correlation study was conducted. Results are presented in appendix (Table A.2) and discussed in the Results section of this study.

Our first research question, 'Do assigned lab partnerships impact student academic performance through peer-mentoring interactions?' was analyzed via impact of laboratory partnership on student z-score exam performance. Averaged z-score means were compared among the four different lab partnerships using hierarchical regression by controlling for student demographic information (gender, ethnicity, and Math ACT level). In addition, gender, ethnicity, and Math ACT level of lab partner on a particular student's performance was controlled. In the regression analyses, all the variables except student gender were dummy coded and student matrices of ethnicity and Math ACT category, African American and low math ACT category were considered as control groups respectively. Similarly, for the different lab partner grouping profiles of gender, ethnicity, and mathematics performance, male-female, White-White, and low Math ACT-low Math ACT categories were considered as control groups respectively. In addition, the Combined Math ACT group was sub-categorized into the Side-to-Side (SS) and High-Low (HL) categories for additional analysis.

The second research question, 'Do assigned lab partnerships encourage students to find external study partners?' was analyzed between the students who participated (treatment group) in study groups and not participated (control group) and hierarchical regression was performed by controlling for demographic variables that significantly affecting for student chemistry performances (significant outcomes in hierarchical regression; Table A.3) and laboratory partnership type. To evaluate how well study group interactions benefited student learning (for treatment group) or to investigate reasons for not participating in study groups (for control group), survey responses collected at the end of the semester were used.

The third research question, 'Are student attitudes towards general chemistry impacted based on lab partner assignment?' was answered by analyzing survey responses. To investigate the correlation between the two variables, type of laboratory partnership and student opinions about their lab partner interactions (like or dislike their partnership), a Chi square test of independence was used. All data analysis was done by IBM SPSS software (IBM SPSS Statistics 25 version).

2.4 Results and discussion

All four group categories were determined to be similar in ethnicity, gender, and Math ACT score using a Chi-square test for independence. Classification (which is the year enrolled in school) showed statistically significant difference in the assigned partnership groups. Results are presented in Table 2.2. According to the results of one-way independent ANOVA, the students assigned into different laboratory partnerships had equivalent Math ACT score designation prior to the study, $F(4, 1995) = .74$, $p = .56$. Therefore, it can be considered that we have students with considerably similar demography and mathematics performances in each group. The results shown in this section reflect consented students; it is important to note that variability in consent from one group to another might impact results.

Table 2.2 Outcomes of Pearson Chi-square test for the comparison of demographic information of students in different laboratory partnerships

Partnership group format	Variable	Pearson Chi-square statistic	Significance
When comparing the four different laboratory partnerships (FC, RA, CM, and LB)	Gender	6.954	.073
	Ethnicity	12.426	.053
	Classification	17.739	.038*
	Math ACT category	6.640	.675
When comparing the five different laboratory partnerships including the split Math ACT partnership (FC, RA, SS, HL, and LB)	Gender	9.459	.051
	Ethnicity	21.964	.038*
	Classification	24.122	.020*
	Math ACT category	10.072	.610

* Chi-square is significant with $p < .05$ (1-tailed).

In this study, our original intent was to include post-lab questions from laboratory reports in our data analysis, but we found the laboratory grades non-representative of students' actual learning/performances due to the grading done by some teaching assistants. Thus, to evaluate the impact of laboratory partner peer mentoring interactions on students' academic performance, lecture test scores, letter grades, and student survey results were used. All exam grades were converted to z-scores to eliminate bias of individual lecture section.

2.4.1 Research Question 1: Do assigned lab partnerships impact student academic performance through peer-mentoring interactions?

The results of hierarchical regression showed that the change in average z-scores of overall exam grades of students in Side-to-Side, β = .074, p = .002, and High-Low Math ACT group, β = .065, p = .007 are significantly higher than the students in Free Choice group. When considering Side-to-Side and High-Low partnership groups collectively (Combined Math ACT), the results of hierarchical regression showed that the change in average z-scores of overall exam grades of students in Combined Math ACT was significantly higher than the students in Free Choice group, β = .170, p = .001. Outcomes for all the significant variables are given in appendix (Table A.3).

In regression analysis, students' overall exam chemistry performance changed according to their designated laboratory partnership. To investigate the pattern of success, exams 1, 2, 3, 4, and the final ACS exam grades are presented in Figure 2.2.

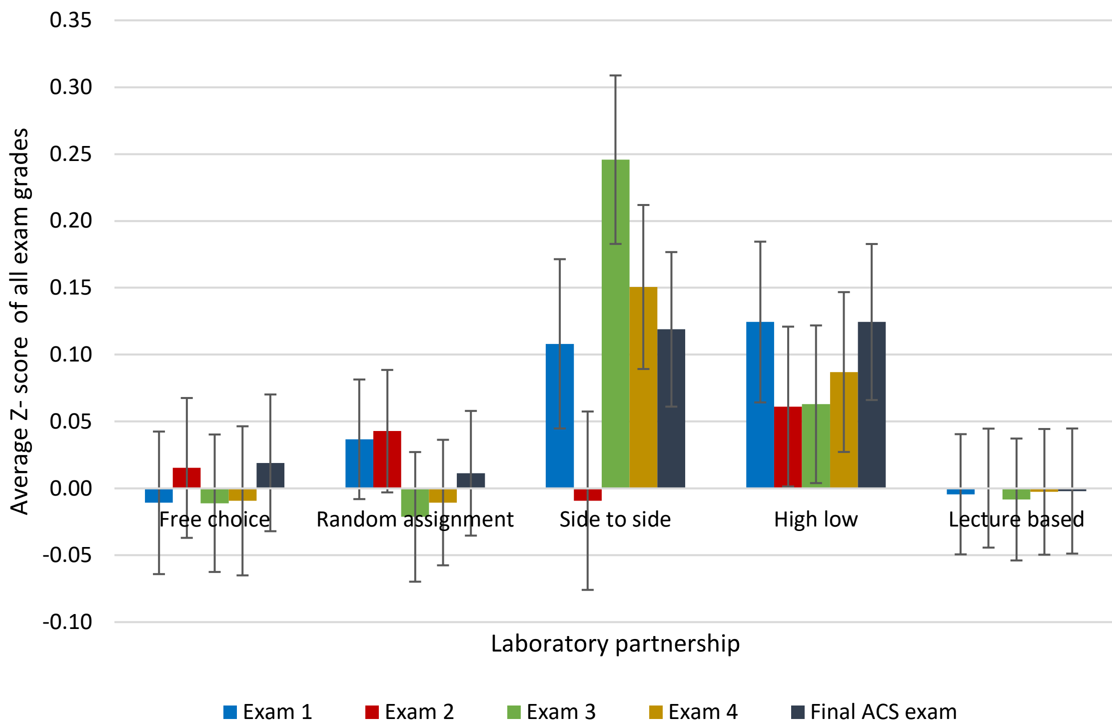


Figure 2.2 Representation of z-score means of exam 1, 2, 3, 4, and final ACS examination grades with different laboratory partnerships. Error bars indicate the standard error of data sets.

As Figure 2.2 shows, students that experienced partnerships based on Math ACT score showed statistically significant higher performance on exams than students of all other categories. SS partnership students had higher scores on exams 1, 3, 4, and the final ACS exam. HL partnership students scored higher than students in other categories for all five examinations. It is important to note for this analysis, that we used z-score comparisons in this research study because each professor used different exams. The variability in exam difficulty is moderated through a comparison of z-scores. However, the Final ACS Exam for all students in all categories of partnership was the identical test and clearly indicates stronger performance by students in the Math ACT designated sections (SS and HL).

To further understand the contribution of Side-to-Side and High-Low partnerships on student success within the General Chemistry I program, student final letter grades are presented versus the laboratory partnership (Figure 2.3). As this figure shows, total A and B letter grade percentages were a little higher in these two partnership categories. Most importantly, both SS and HL partnerships demonstrated lower percentages of failing students (students earning grades of D/F/W) at 27.9% and 26.5% respectively. These failing student percentages are considerably lower than Free Choice and Lecture Based assignment. This finding indicates the importance of having laboratory partners matched based on Math ACT score which can potentially support peer mentor interactions.

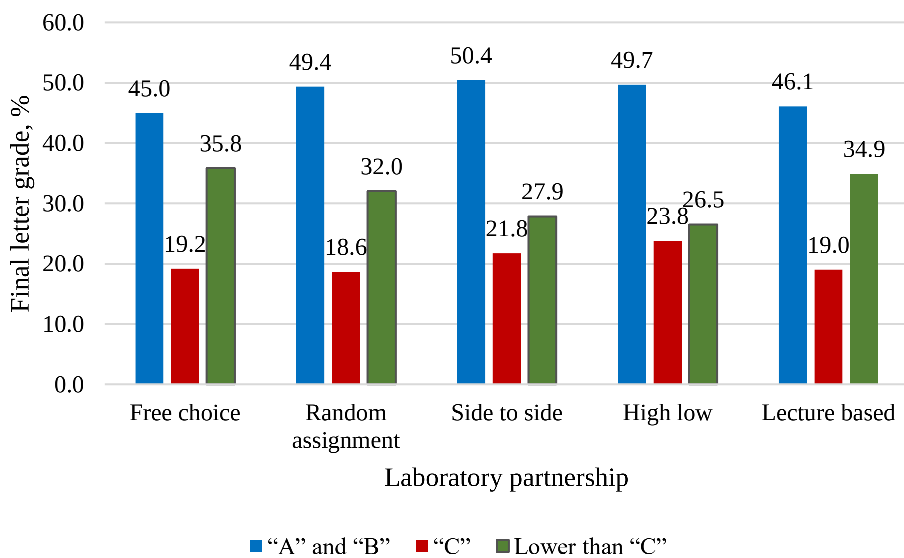


Figure 2.3 Final letter grade percentage of General Chemistry I lecture course grouped by laboratory partnership.

As hierarchical regression outcomes indicate, student academic performance is affected by student ethnicity and Math ACT score, in addition to their laboratory partner's gender and Math ACS score. These factors might have impacted the mentoring relationships developed by student pairs. To study these effects, averaged z-score exam grades were grouped according to partnership and the ethnicity and gender profiles of student pairs.

Figure 2.4 displays the average z-score of students according to their ethnicity pairings. African American students in all three categories (White-AA, AA-AA, and AA-Other) showed improved performance in the Math ACT partner sections (SS and HL) when compared to Free Choice. In addition, the number of African American students choosing partners of same ethnicity in Free Choice was higher than in all other sections (8.0% in Free Choice vs. approximately 2% in other sections). This confirmed our initial observation, that students chose lab partners based on ethnicity patterns and students often performed poorly.

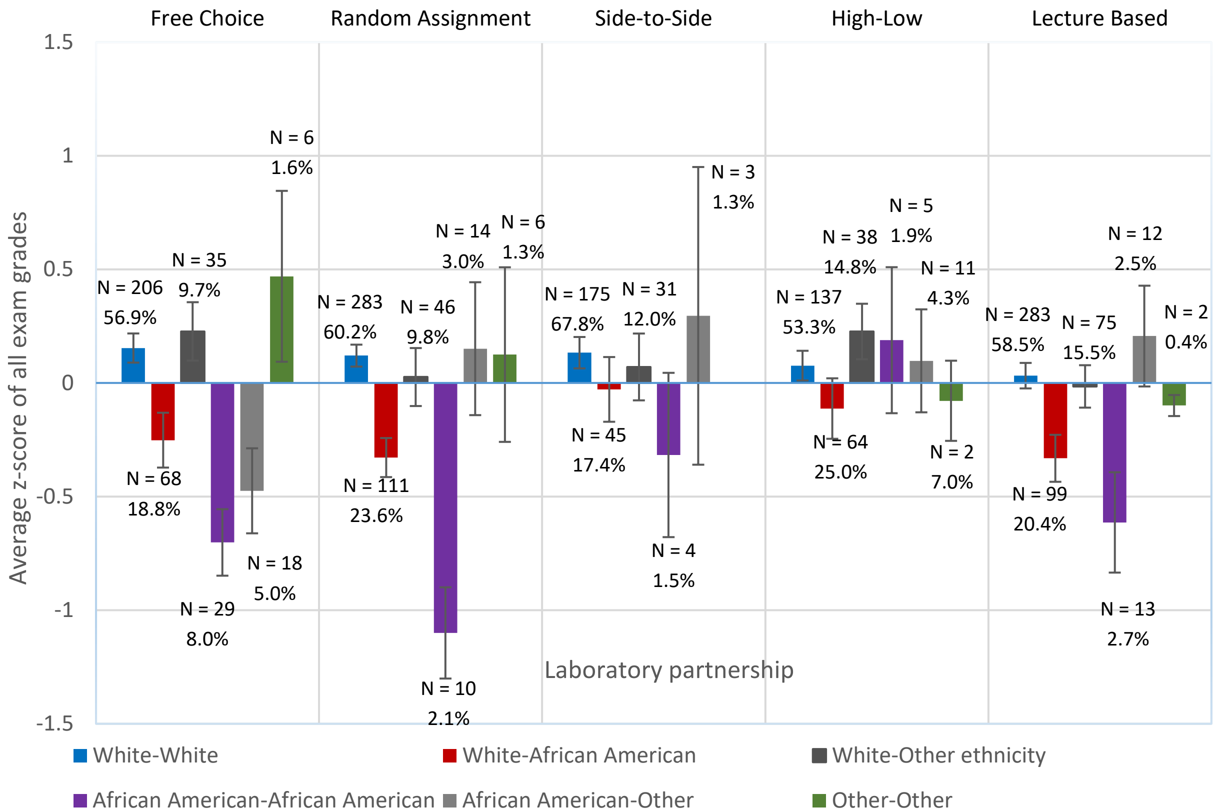


Figure 2.4 Representation of the average z-scores for different ethnicity grouping profiles grouped by the laboratory partnership. Number and percentages of students within each pairing category are presented near each data bar. Error bars indicate the standard error of data sets.

Figure 2.5 shows average z-scores for students paired by gender and laboratory partnership. Results do not show a consistent pattern in student performance among partnership types. In the Free Choice group, Male-Female partnership scored higher exam performances than the other two possible gender grouping pairs. But in SS and HL partnerships students paired to partners with similar gender were more successful than the students paired to a partner with opposite gender. In the Lecture Based partnership none of the profiles showed improved exam performance over the other two partner pairing categories. The z-score comparison among the gender profiles in RA, SS, and HL groups confirm the statistically significant exam performance enhancement in Female-Female partner pairs over Male-Female partner pairs found in the results of hierarchical regression (Table A.3). With our assigned partnerships, each assigned group comprised approximately 30% MM, 20% FF and 50% MF/FM for lab partners. We did note that when students were allowed to choose their own partner, they did not show a gender preference (approximately 33% in each category for FC assignment).

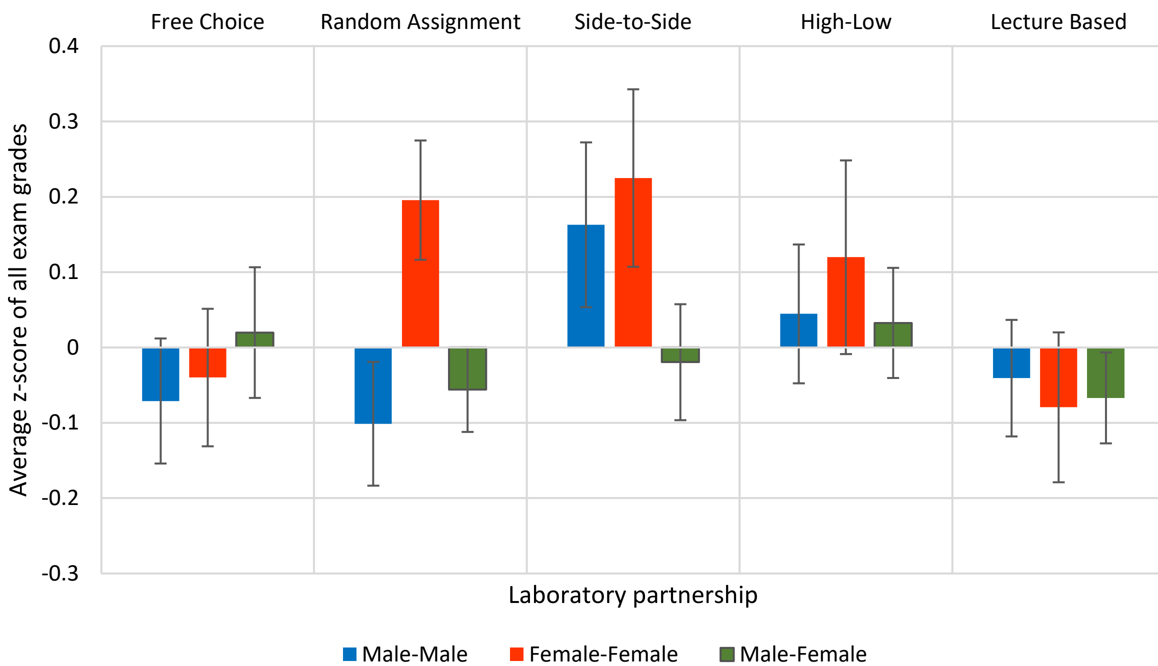


Figure 2.5 Representation of average z-scores of students in different gender grouping profiles grouped by the laboratory partnership. Number and percentages of students in each gender category are presented near each data bar. Error bars indicate standard error of means.

To study Math ACT partnership, average z-scores of exam grades were graphed versus the laboratory partnership (Figure 2.6). Here SS and HL groups were combined into one category so that our groups compare equally (SS lacks low to high Math ACT partnerships) to the other three partnership groups. Partnerships were established based on Math ACT scores, but the variability in score matching changes from SS to HL. Therefore, the combined sample has similar math ACT variability compared to FC, RA, and LB groups. Z-score distribution for all the five groups are given in appendix (Figure A.3).

Among all the Math ACT partnerships, high-high Math ACT partners were found to be most successful, with Free Choice partnerships showing strongest scores. But Free Choice partnership scored lowest z-scores for low-low partners and low-mid partners. The combined Math ACT partnership group (SS and HL) showed stronger z-score averages for partnerships including a low-Math ACT score student, but did not show as strongly for the mid-, or high-Math ACT students.

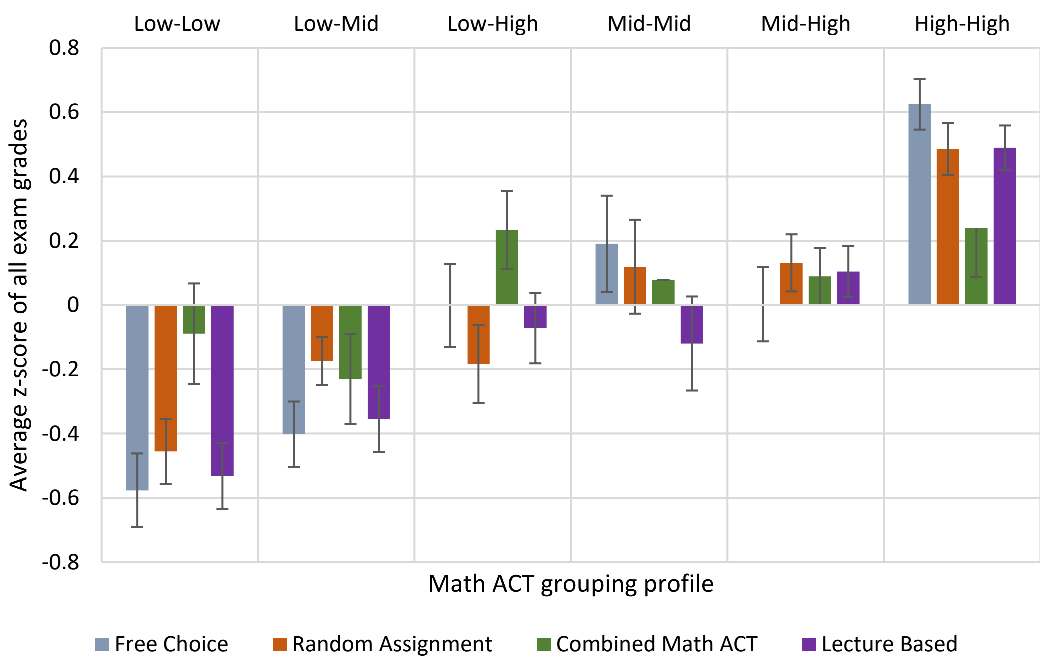
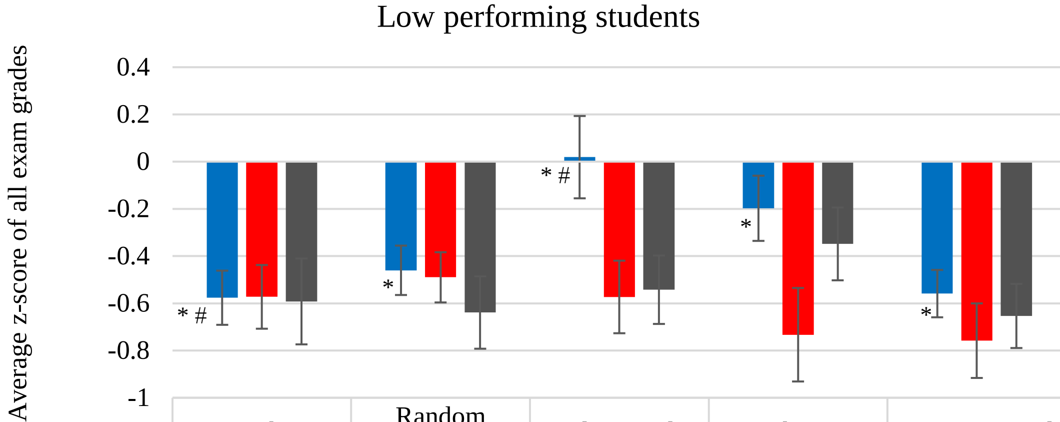


Figure 2.6 Representation of the average z-scores for different Math ACT grouping profiles grouped by the laboratory partnership. Error bars indicate the standard error of data sets.

Since Figure 2.6 represents all students within a partnership, it hides the information on whether one student in a pairing is performing more or less strongly. Therefore, all students were separated into the three categories, low, mid, and high Math ACT score. Each math ACT category was then analyzed with the possible Math ACT student pairs using one-way ANOVA. Bonferroni post hoc pairwise comparison was used to show statistically significant differences among laboratory partnerships. Results of one-way ANOVA and Bonferroni post hoc pairwise comparison are given in the Figures 2.7, 2.8, and 2.9 which are for low, mid, and high math ACT separate categories respectively. Average z-score grades for each Math ACT profile were compared to the grand z-score mean of the respective Math ACT performance category. Grand means and standard deviations for low, mid, and high Math ACT categories were: Mean = -.536, SE = .033; Mean = -.061, SE = .032; and Mean = .499, SE = .024 respectively. With the assumption that the higher math ACT person is the one who mentors the other student, we analyzed low-mid and low-high pairs within the low-performing category, low-mid and mid-high pairs within the mid-performing category, and low-high and mid- high pairs within the high-performing category. Here our goal was to examine whether the higher level (mentoring student) or the lower level (mentored student) benefited more in their partnerships.



	Free Choice	Random Assignment	Side-to-Side	High-Low	Lecture Based
Low-Low	-0.5764	-0.4602	0.019	-0.1975	-0.559
Low-Mid	-0.5727	-0.4899	-0.5731	-0.7331	-0.7583
Low-High	-0.592	-0.639	-0.5424	-0.3484	-0.6537

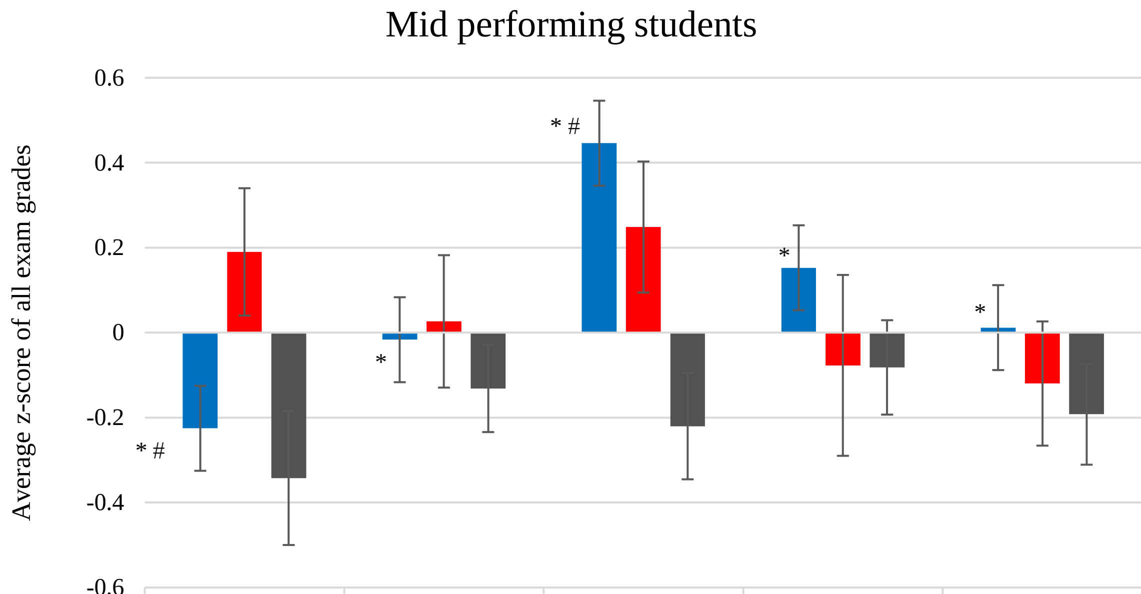
Laboratory partnership

Mean of average z-score of exam grades for all low performing students = -.536

Figure 2.7 Representation of average z-scores of low Math ACT score students grouped by the laboratory partnership.

Circled groups have higher z-score means than the mean of all low performing students (.536). * indicates statistically different Math ACT student pairs, $p < .05$ (Results of one-way ANOVA).

According to Figure 2.7, low-Math ACT students paired to another low Math ACT student showed stronger performance in RA, SS, and HL Groups with the SS students showing much stronger z-score averages. Other student pairings also showed higher z-score means (low-mid in RA, low-high in High-Low). We believe the data presented here shows a subtle interaction where low-low students paired successfully with each other as one (or both) of them have the ability to step into the mentor role. We cannot distinguish the dynamics with this data. In addition, the SS partnership was specifically designed to pair two students close in ability but still establishing a mentor dynamic. The stronger performance of SS students reflects that this partner style worked best.



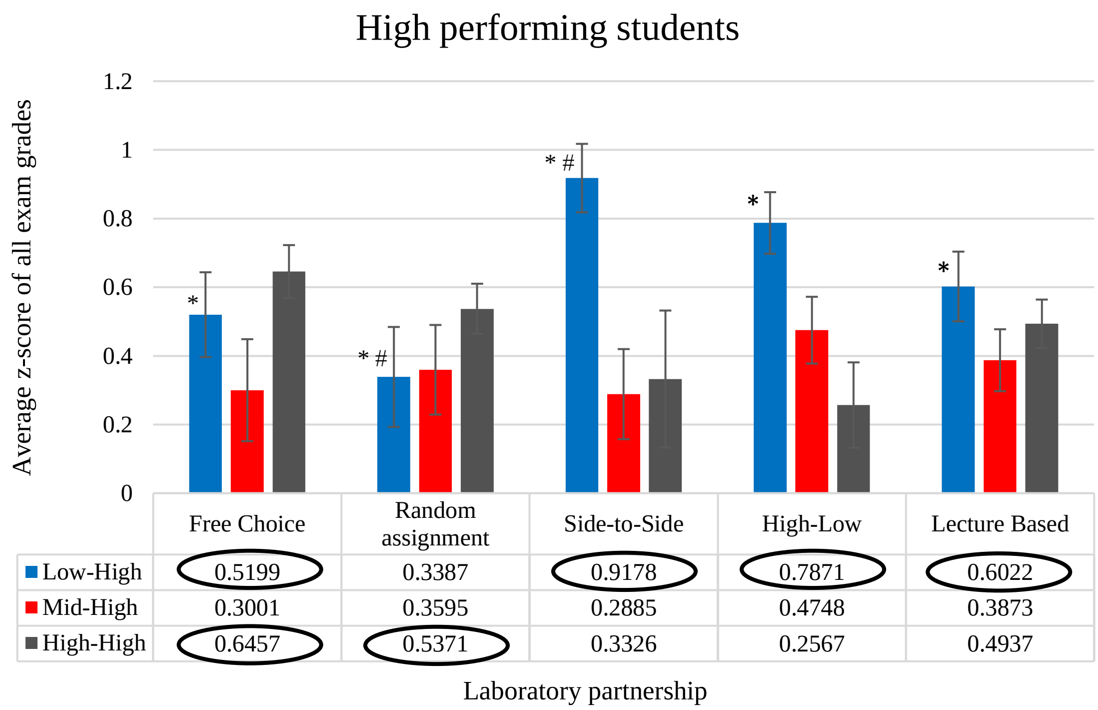
	Free Choice	Random Assignment	Side-to-Side	High-Low	Lecture Based
Low-Mid	-0.2253	-0.0167	0.446	0.1527	0.0118
Mid-Mid	0.1901	0.0265	0.2486	-0.0771	-0.1198
Mid-High	-0.3424	-0.1314	-0.2205	-0.0819	-0.192

Laboratory partnership

Figure 2.8 Representation of average z-scores of mid performing students in different math ACT grouping profiles grouped by the laboratory partnership.

The groups having higher z-score means than the mean of all mid performing students, which is -.061 are circled in the data table. * indicates statistically different Math ACT student pairs, $p < .05$ (Results of one-way ANOVA). [#] indicates statistically different lab partnership groups, $p < .05$ (Results of Bonferroni post hoc pairwise comparison)

According to Figure 2.8, irrespective to the partnership type, Mid Math ACT students paired to another Mid Math ACT student or a Low Math ACT student performed better than the Mid Math ACT students paired to a High Math ACT student. Also, except in FC partnership, Mid Math ACT students paired to Low Math ACT students showed improved academic performances than the overall average of a Mid-performing student. This indicates improved mentoring interactions in Mid Math ACT students as the Mid student could potentially act as "mentor" in all low-mid or mid-mid partnerships. Students paired with High students and having the role of "mentee" did not show improvement. The strongest performance for Mid Math ACT students was partners with similar Math ACT designation (the SS partnership) which created more successful mentoring interactions. This observation replicates the same observation we had with Low Math ACT students in Figure 2.7.



	Free Choice	Random assignment	Side-to-Side	High-Low	Lecture Based
Low-High	0.5199	0.3387	0.9178	0.7871	0.6022
Mid-High	0.3001	0.3595	0.2885	0.4748	0.3873
High-High	0.6457	0.5371	0.3326	0.2567	0.4937

Figure 2.9 Representation of average z-scores of high performing students in different math ACT profiles grouped by the laboratory partnership.

The groups having higher z-score means than the mean of all high performing students, which is .499, are circled in the data table. * indicates statistically different Math ACT student pairs, $p < .05$ (Results of one-way ANOVA). # indicates statistically different lab partnership groups, $p < .05$ (Results of Bonferroni post hoc pairwise comparison).

Overall, the exam performances of High Math ACT students are higher for all students irrespective to their lab partnership. However, the High Math ACT students performed significantly better in the Combined Math ACT groups (SS and HL) when mentored Low Math

ACT students. The definition of z-score comparisons forces us to have a mean of 0 for the students, however the students scored better academically when they were clearly defined in a mentor role.

2.4.2 Research Question 2: Do assigned lab partnerships encourage students to find external study partners?

Overall student participation in outside study groups was found to be very poor. Of those consented students, $N = 2122$, only 384 students stated that they participated in study groups. The expected dynamic that might extend the laboratory partner interactions through to outside study groups was not supported by any laboratory partnerships. Among the different partnership types, High-Low partnership reported the highest percentage of students that participated in study groups (23.1%). Students that participated in study groups that belonged to each laboratory partnership are presented in appendix (Table A.3). The results of hierarchical regression that controlled for laboratory partnership and students' significantly affecting demographic variables showed that study group participation had no significant effect on students' exam performances, $\beta = -.018$, $p = .377$. But the statistical power is very low in this analysis as there was a huge discrepancy between the two sample sizes.

The students who participated in study groups stated that they mostly worked on online lecture homework assignments and test preparation with their group. Survey results indicated students primarily found their study group partners outside of General Chemistry labs and lectures. Percentage responses for given answer choices are listed in Table 2.3. However, 46.6% of students said that their study group was helpful in preparing for General Chemistry I exams and it helped them understand the material better. We could not find any strong reason why students did not choose study group participation as only 18.0% of students said that they have participated in study groups before and they thought it was just a waste of time. The analysis of students' written comments indicated that they think that they do not have enough time to spend on outside studying with their busy schedules.

Table 2.3 Information on the method/place that students found their study group partners

The method that students found their study group partners	Frequency	Percentage, %
From lab	75	19.5
From lecture	153	39.8
From dorm	133	34.6
From another organization sorority/fraternity/other organization	139	36.2
Other	29	7.6

Students were asked to select all options that apply.

2.4.3 Research Question 3: Are student attitudes towards general chemistry impacted based on partner assignment?

Overall, most students in General Chemistry I laboratories rated their partnerships as poor-quality interactions. Survey responses for the survey question, "Did you and your partner talk in lab and help each other understand the materials?" were, "No, Not at all" = 63.2%, "No, Not really" = 8.1%, "Yes, kind of" = 7.6%, "Yes, very much" = 21.2%. The results of Chi-square test of independence showed that the two categorical variables, laboratory partnership type and student opinions about their lab partner interaction (like or dislike their partnership) were significantly correlated (p = .037). Therefore, the percentage survey responses grouped by the laboratory partnership type were graphed for the above survey question and are presented in Figure 2.10. As this figure shows, students in Side-to-Side and High-Low groups had the least positive attitudes about their lab partner interactions. Black-Beard et al had shown that students matched with mentors of their own gender or race reported that having a mentor of their own gender or race was more important as they got more help from their mentors. But in terms of the academic outcomes, matching by gender or race did not show any difference at all.[45] Our student performance and attitudes also aligned with the outcomes of the study performed by Black-Beard et al. (Table A.5), where Free Choice students showed preference for matching with lab partners of same ethnicity and gender, yet academic outcomes and attitudes were not improved.

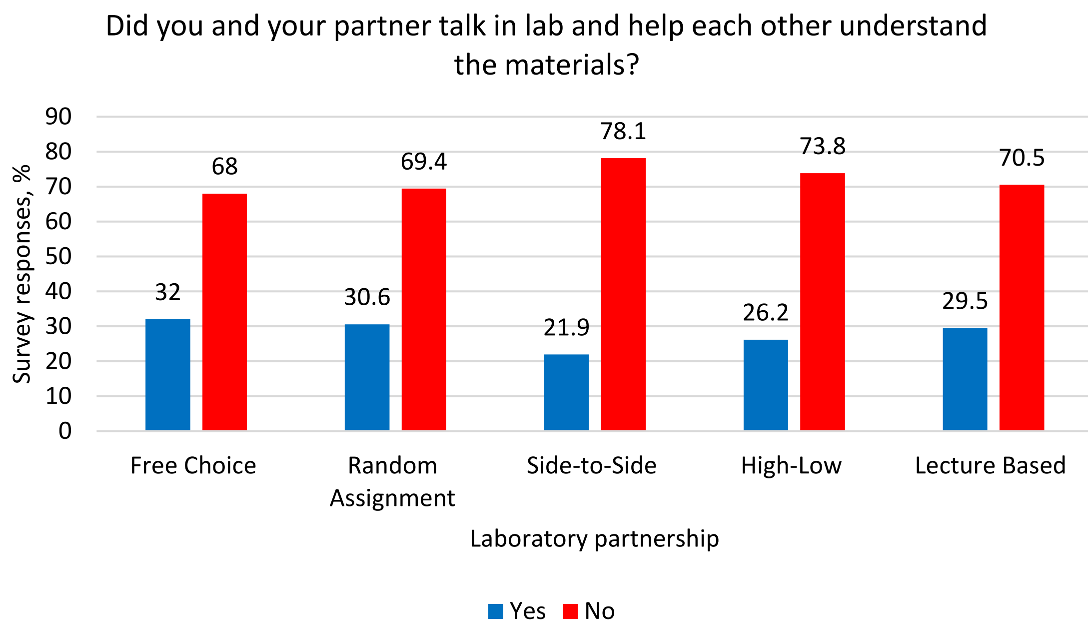


Figure 2.10 Percentage survey responses grouped by the laboratory partnership type for the survey question, 'Did you and your partner talk in lab and help each other understand the materials?'.

At the semester end survey, students' opinions about their performance in General Chemistry I course were collected. Overall, all students showed poor confidence about their final grades and their successfulness in the program. But when we only compare their positive rankings among partnership groups, we found percentages of confident students in each group as FC: 44.7%, RA: 46.8%, SS: 38.5%, HL; 40.5%, and LB: 43.2%. However, when we analyze their thoughts on the overall course performances, based on their attitudes towards their laboratory partners, we found that many students who liked their partnerships had positive attitudes about their final grades/course performances (Figure 2.11). Similarly, most students who did not like their lab partnerships had negative attitudes or confidence about their performance (Figure 2.12). These consistent outcomes were obtained from all kinds of laboratory partnerships indicating the possibility of building confidence in their own learning by improving partner interactions.

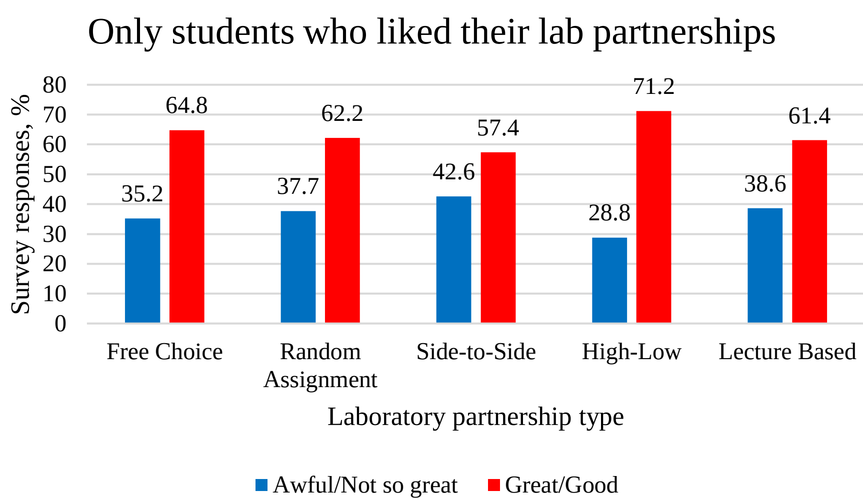


Figure 2.11 Percentage survey responses of students who liked their lab partners (The students who said 'Yes, kind of' and 'Yes, very much' for the survey question, Rank the quality of your lab partner interaction. Did you and your partner talk in lab and help each other understand the materials?), grouped by the laboratory partnership type for the survey question, 'How do you feel about your performance in General Chemistry I?'.

Answer choices, 'Awful', 'Not so great', Good', and 'Great' were combined so that it only represents positive or negative attitude about student performances in General Chemistry.

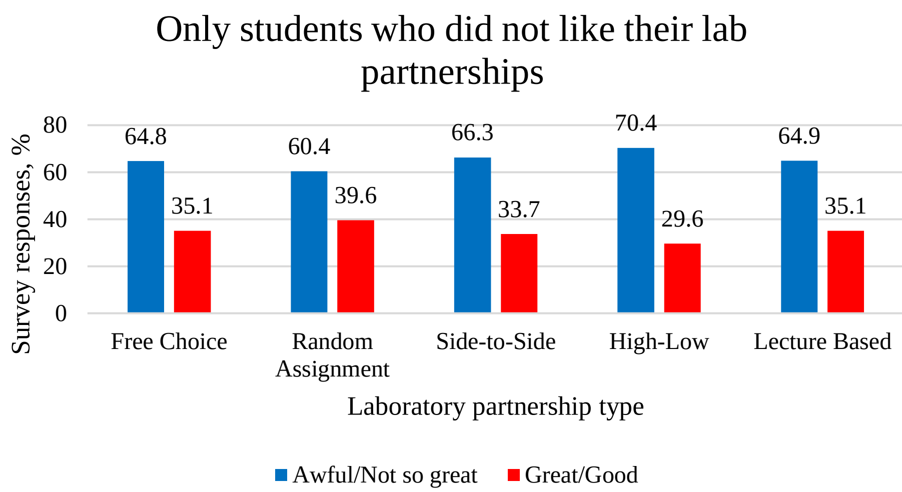


Figure 2.12 Percentage survey responses of students who did not like their lab partners (The students who said 'No, not really' and 'No, not at all' for the survey question, Rank the quality of your lab partner interaction. Did you and your partner talk in lab and help each other understand the materials?), grouped by the laboratory partnership type for the survey question, 'How do you feel about your performance in General Chemistry I?'.

Answer choices, 'Awful', 'Not so great', Good', and 'Great' were combined so that it only represents positive or negative attitude about student performances in General Chemistry.

2.4.4 Limitations

This study has limitation in that it presumes impact on examination grades as a result of peer interactions in the laboratory. As noted in the introduction, we originally tried to include laboratory grades that reflect critical thinking in the classroom to explore the impact of partner mentoring, but we were unable to include that data. Further exploration is needed to determine the impact of laboratory peer mentor interactions.

2.5 Conclusions

This research study explored if we could create effective peer mentoring interactions through strategic lab partnerships. We did see a positive effect from partners established via Math ACT score, and students in these relationships consistently performed better on lecture examination material. It was observed by researchers and Graduate Teaching Assistants that large gaps in Math ACT score made partner dynamics difficult, while narrow gaps in Math ACT score supported more even and pleasant partner interactions. Further research on peer mentoring interactions in the laboratory explore the impacts of students switching partners on a regular rotation so that each person can have benefit of varied interactions and be able to explore different roles in the laboratory. The more we can support students to enjoy their lab-partner interaction and improve science attitudes, the more students can potentially improve their academic performance within the classroom.

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CHAPTER III

PEER REVIEW AND RESPONSE: SUPPORTING IMPROVED WRITING SKILLS IN ENVIRONMENTAL CHEMISTRY

3.1 Introduction

Problem solving or critical thinking, data interpretation, and oral and written communication are some of the most essential skills that undergraduates need to practice. Among these skills, writing is often neglected in a typical STEM curriculum. If students continue on to graduate school or begin careers as scientists they will be expected to write scientific reports (Gragson & Hagen, 2009). STEM undergraduates often have relatively few opportunities to write scientific reports and consequentially the writing of recently graduated students is often poor with a general unawareness of the requirements for clear scientific text (Guilford, 2001; Walker & Sampson, 2013). Moreover, undergraduates might have limited opportunities to review and critique scientific papers, which leads to lower confidence in their writing abilities (Walker & Sampson, 2013). Here we introduce a modified peer assessment approach (Glaser, 2014; Guilford, 2001; Ricker & Whelan, 2016) designed to encourage scientific writing and critical thinking of their writing for undergraduate students. Our study shows that the modified peer assessment process provides a valid mechanism for students to improve their writing and practice critical analysis of their work.

The utilization of peer review, both online and on-campus, has been proven effective for supporting enhanced writing skills for undergraduate students. Many of the peer review formats use online essays to train students to edit critically and understand assignment goals (Boase-Jelinek, Parker, & Herrington, 2013; Dominguez et al., 2015; Gunersel, Simpson, Aufderheide, & Wang, 2012; Miyazoe & Anderson, 2010; Novakovich, 2016; Zwicky & Hands, 2015). When used well, peer review of essays serves several layers of purpose as it allows student reviewers to gain experience editing and providing constructive feedback on a piece of writing, student authors receive comments from diverse perspectives as multiple editors give feedback, and instructors can reduce their grading burden related to editing when working with large enrolment classes (Boase-Jelinek et al., 2013; Guilford, 2001; Huisman, Saab, van Driel, & van den Broek, 2018).

A challenge with the peer review process, however, is supporting students to give quality feedback when providing comments (C. E. Kulkarni, Bernstein, & Klemmer, 2015; C. Kulkarni et al., 2015; Yuan et al., 2016). Poor student edits short-change the process, where authors are less likely to improve their work and may develop a false sense of confidence related to ineffective feedback (Russell, 2004). A number of attempts in the literature have been made to improve the quality of student feedback, which includes providing common feedback phrases for quick use by the editing student (C. Kulkarni et al., 2015), including interactive hints to help students stay on track (Krause et al., 2017), and designing grading rubrics with care (Hicks, Pandey, Fraser, & Klemmer, 2016).

This research study focused on the incorporation of a Response to Reviewer Comments document, which allowed students to critically review student edits received on their writing and determine if the change was warranted to improve their reports. Students have demonstrated that the perceived competence of peer feedback impacts their editing decisions (Berndt, Strijbos, & Fischer, 2018; Strijbos, Narciss, & Dünnebier, 2010). The process of critical evaluation of suggested edits can potentially improve student ownership of their learning and help them develop critical reflection skills in the process (Thomas, Martin, & Pleasants, 2011). We sought to answer the following research questions with this study:

1. Are peer edits and feedback sufficient to improve student writing?
2. Did editing peer reports support students to improve their own writing?
3. Does the Response to Reviewers Comments document encourage students to critically evaluate their own writing?

3.2 Methods

3.2.1 Students and demographics

Peer review writing assignments were incorporated into three sections of Environmental Chemistry at Mississippi State University in the semesters Spring 2015, 2017, and 2018. Most of the students enrolled in the class were Chemistry or Chemical Engineering majors and were typically Junior or Senior level students. The model used was a modification of the published Calibrated Peer Review protocol with the edits and reviews occurring in class instead of in an online format (Chapman, 1999). In addition, the Response to Reviewer Comments document was added to encourage critical thinking of each student toward their writing and suggested peer edits. Table 3.1 lists student demographic information including sample size, gender, major, and academic year.

Table 3.1 Student demographics including sample size, gender, major, and academic year by class

Criteria		**Semester**		
Semester		Spring 2015	Spring 2017	Spring 2018
Sample size, N		31	28	67
Gender	Male	15	11	48
	Female	16	17	19
Major	Chemistry	14	22	9
	Chemical engineering	17	1	54
	Other	-	5	4
Academic year	Junior	-	7	3
	Senior	31	21	64

3.2.2 Data collection

This peer review assignment was implemented in conjunction with a University-wide initiative at Mississippi State University known as the Maroon and Write Quality Enhancement Plan. Maroon and Write is a comprehensive university model instituted in 2014 designed to improve undergraduate student writing through the implementation of writing across the curriculum, the use of write-to-learn strategies and formal writing instruction. This peer review writing assignment supported upper-division writing needs in the chemical sciences.

The peer review assignment was designed as follows:

1. Students had the opportunity to 'train' on essays before writing their own literature review, with examples provided of high quality and low-quality essays. Essays were provided on the class website for review. Also, students were given detailed instructions and examples of peer editing style feedback with discussion on the types of edits and approaches that could be taken.

2. Each student wrote an original literature review of a current topic in environmental science. Sample essays that showed literature review structure and content were available. The literature review was approximately 1500 words and cited at least four original scientific papers. This original assignment was turned in to the instructor for review and grading. This initial grade was not seen by the student and was used only for this research study.
3. Each student reviewed and edited three papers in-class for approximately 20 minutes per peer essay. Papers had names and identifying information removed. Edits and grading rubrics were handwritten on papers and returned to the instructor for redistribution back to the original author. Students were tasked to have the average of their peer reviews not exceed a grade of 85. This was designed to stop students from just giving everyone high grades for their feedback and eliminating critical review.
4. Anonymous peer edits were returned to the original author. After student edits were addressed and deficiencies improved, a final literature review was turned in to the instructor for grading.
5. Each student also turned in a Response to Reviewer Comments document which detailed the important student edits and explained how suggestions were addressed. This review document was intended to have each author critically think about their peer edits and determine if the suggestions were warranted or to provide an explanation if they were not. It was included to improve the critical thinking of each author toward their own writing and to enhance ownership of their writing decisions.

Grades for each student were awarded as 65% from the final literature review turned in after peer edits; 15% from the quality of the student edits they made on other student papers and 20% from their Response to Reviewer Comments document. The Response to Reviewer Comments document was graded with an assessment of how thoroughly students addressed reviewer suggestions and determined edit suitability. Essays and peer editors were assigned anonymously through a numeric system. All identifying information was removed from essays before papers were given to student editors to account for anonymity of review. Student editors were also kept anonymous from each author. All student papers and edit comments were scanned and kept for instructor assessment of the process. Figure 3.1 provides a general overview of the writing assignment implementation.

Each paper was graded using a rubric developed from the ACS Style Guide provided through the American Chemical Society (Coghill & Garson, 2006). Students were tasked to review papers in four categories: appropriate citation of references, correct use of citations in an essay, grammar, spelling, and neatness of work, and the overall content of essay topic. Grades were based on a 100-point total and student marks were recorded for each section. An example peer review grading rubric is included in the appendix.

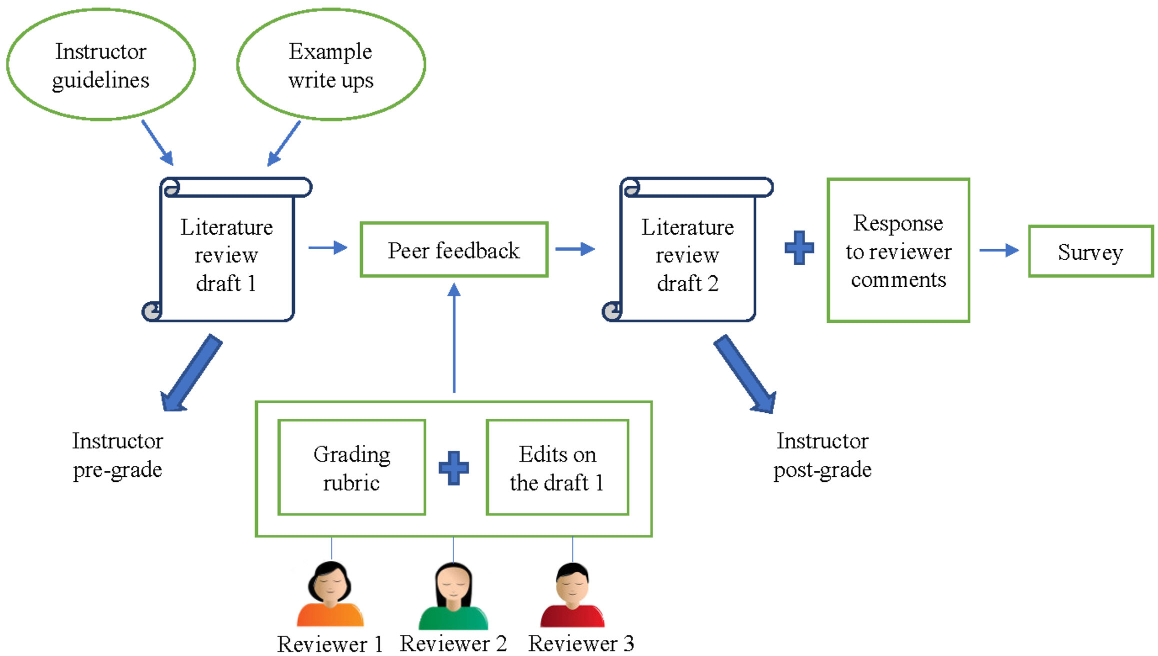

Figure 3.1 General overview of the writing assignment.

3.3 Results and discussion

Our initial research question was to determine if peer edits and feedback are sufficient to improve student writing. In this study, report grades were assigned based on a grading rubric with four evaluation criteria: work cited, using cited works, grammar, spelling, and neatness, and content. Students' pre- and post- total essay grades and the four category rubric grades from the instructor were analysed using paired sample t-test at the 95% confidence interval. Results showed that students' report grades significantly improved after the peer editing process, with total essay grade: $t(91) = -16.3$, $p < .001$, $d = 1.6$; works cited: $t(91) = -11.9$, $p < .001$, $d = 1.2$; using cited works: $t(91) = -9.2$, $p < .001$, $d = 1.0$; grammar, spelling and neatness: $t(91) = -9.0$, $p < .001$, $d = 0.9$; content: $t(91) = -12.3$, $p < .001$, $d = 1.3$. Students' pre- and post- report grade percentages are displayed in Figure 3.2. Students were able to improve their report quality after incorporating peer edits with essay average grades improving from 58% to 70% after student revisions.

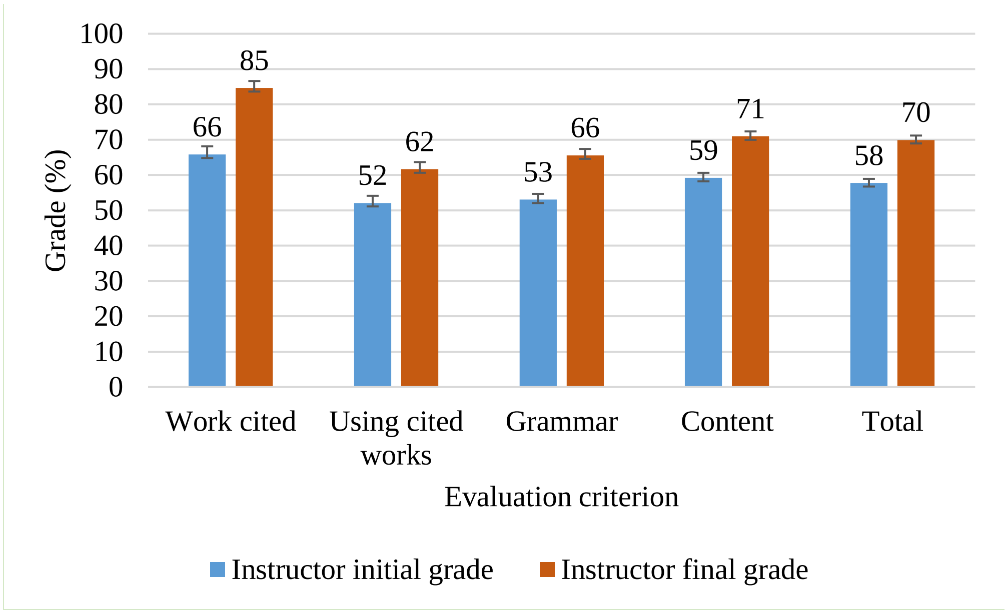

Figure 3.2 Students' pre- and post- total essay grades and category rubric grades in the different evaluation areas, works cited (10 points), using cited works (15 points), grammar, spelling and, neatness (15 points), and content (60 points). Graph is displayed as percentages. Results of paired sample t-test show significant improvement in all these categories. Error bars represent the standard errors.

To further study how successfully students edit or grade their assigned essays, paired sample t-test was conducted among the instructor pre-report grades and the averaged peer report grades. Results showed significant differences between the groups in all the areas, total essay grade: $t(91) = 13.1$, $p < .001$, $d = 1.4$; works cited: $t(91) = 7.0$, $p < .001$, $d = 0.7$; using cited works: $t(91) = 8.1$, $p < .001$, $d = 0.8$; grammar, spelling, and neatness: $t(91) = 4.5$, $p < .001$, $d = 0.5$; content: $t(91) = 13.0$, $p < .001$, $d = 1.4$. The significance in these areas indicates that the peer edits did not correlate well with the instructor pre-grade, and we observed that the peer edits consistently scored higher than the instructor. However, student edits were still sufficient to improve the overall quality of reports. Paired sample correlations are given in Table 3.2. The significant positive correlation of the variables ‘work cited’ and ‘grammar, spelling, and neatness’ indicate that students who got higher points from their peers, also received a higher grade from the instructor. The scatter plot for the initial total report grades from the peers and the instructor is given in Figure 3.3.

Table 3.2 Results of the paired sample correlation of the peer average and instructor initial report grade

Evaluation area	**Paired Sample Correlation**
Works cited	.234**
Using cited works	.094
Grammar, spelling, and neatness	.272*
Content	.188
Total	.196

*means that the correlation is significant with p <.01(2-tailed).
** means that the correlation is significant with p <.05 (2-tailed).

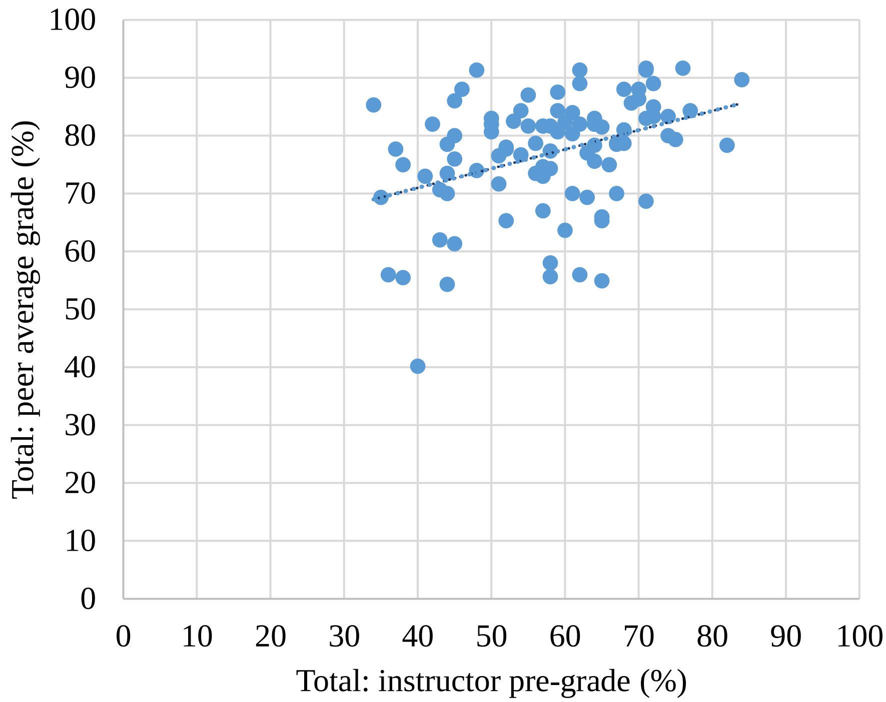

Figure 3.3 Scatter plot for the initial total report grades from student reviewers and the instructor.

To understand the student perception about the essay improvement due to the peer edits, two survey questions, ‘I found the reviewer comments I received helpful’ and ‘I felt my paper improved as a result of the feedback I received’ were analyzed. Survey response percentages are given in Figure 3.4. Likert responses ‘agree’ and ‘strongly agree’ were combined, as well as ‘disagree’ and ‘strongly disagree’ for this analysis.

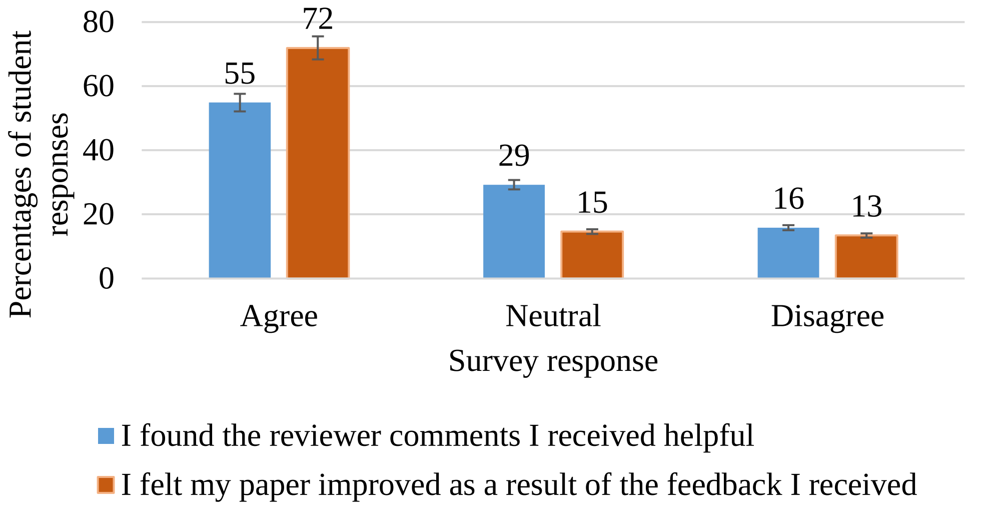

Figure 3.4 Percentage survey responses in three Likert scale categories for the survey questions. Error bars represent the standard errors. In general, students thought the review process improved their papers.

Amongst all students, 55% said that the comments they received from their peers were helpful; only 16% said they were not. Most importantly, 72% of the students thought their papers were improved as a result of the student feedback. To determine if report grades were enhanced for students who thought reviewer comments were helpful, average grade differences (final instructor report grade – initial instructor report grade) were plotted against the three survey response categories of agree, neutral, and disagree. Results are given in Figure 3.5. Grade improvements do not show a significant difference among the groups, as all groups improved.

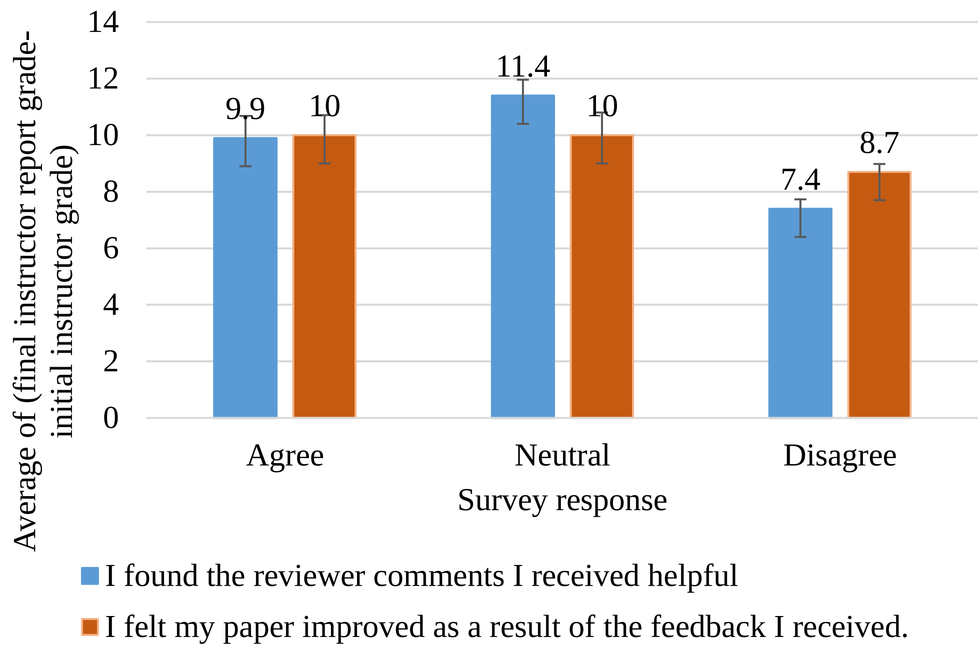

Figure 3.5 Averaged instructor grade improvements for the students who rated their response as agree, neutral, and disagree in the survey questions, ‘I found the reviewer comments I received helpful’ and ‘I felt my paper improved as a result of the feedback I received’. Error bars represent the standard errors.

Our second research question was to determine if the editing of peer reports helped students to improve their own writing. This was addressed by the evaluation of student perception on the two survey questions, ‘Reading other papers helped me understand what the assignment should look like’ and ‘Reading other papers gave me ideas for things I could change in my own paper’. In the analysis, Likert scale items of strongly disagree and disagree and strongly agree and agree were merged and considered as disagree and agree respectively. Survey response percentages are shown in Figure 3.6. Approximately 60% of students thought that reading other student papers helped them understand the assignment and supplied ideas to improve their own report.

Average values of grade differences (final instructor report grade – initial instructor report grade) were plotted against the three survey response categories of agree, neutral, and disagree to evaluate if student writing improved as a result of reading other papers (Figure 3.7). The average grade improvements among the three categories were not significantly different. Students strongly responded to the survey question however and thought the process helped their writing.

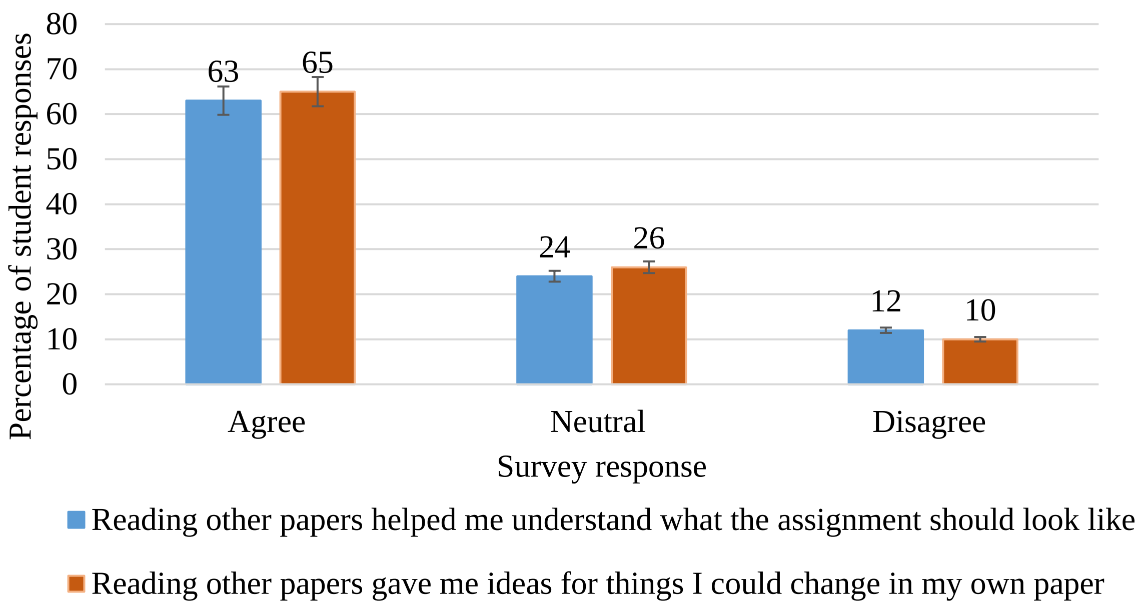

Figure 3.6 Percentage survey responses in three Likert scale categories for the survey questions. In general, students thought the reviewing process improved their understanding about the writing assignment. Error bars represent the standard errors.

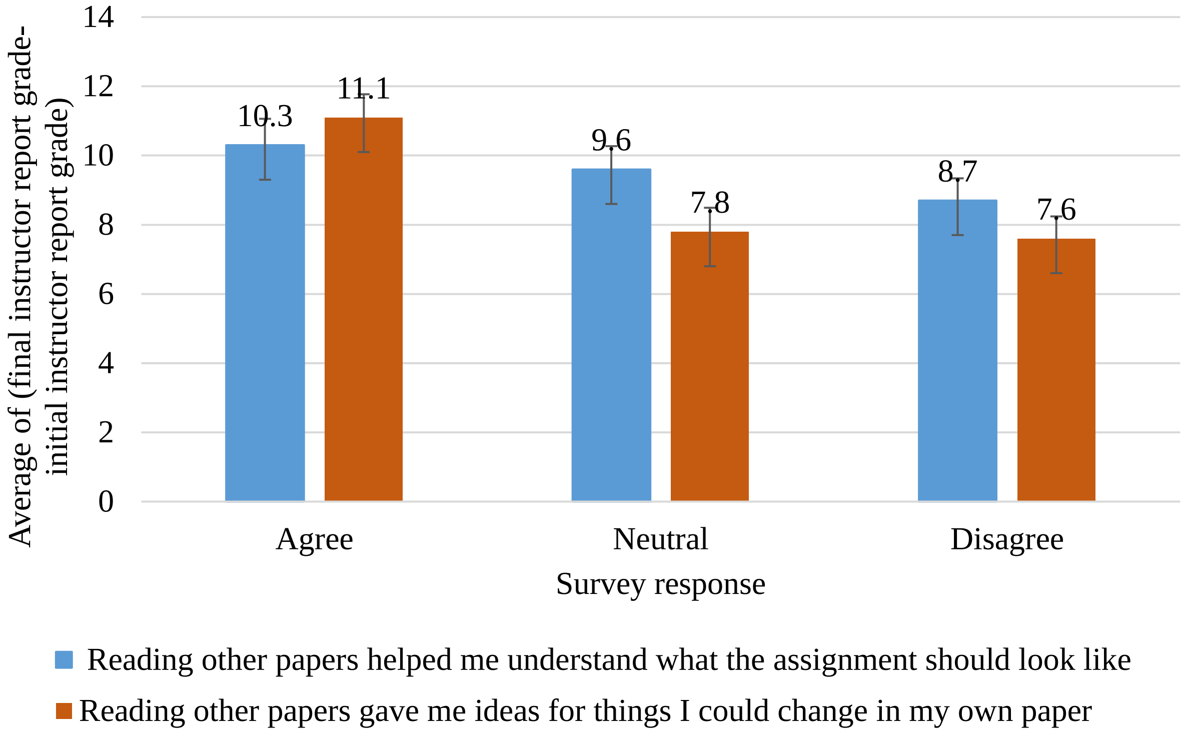

Figure 3.7 Averaged instructor grade improvements for the students who rated their response as agree, neutral, and disagree in the survey questions. Error bars represent the standard errors.

Our third research question was to determine whether the Response to Reviewer Comment document helped students critically evaluate their own work. Overall, the quality of Response to Reviewer Comments documents was poor as students took all peer edit suggestions as changes to be made. Therefore, in our opinion, many students did not really use the Response to Reviewer Comments comments to critically evaluate their own work. Instructor grades for the quality of the Response to Reviewer Comments document correlated with students' final report grades (Pearson correlation .467 with $p < .001$). Students that critically considered the peer edits and addressed comments in detail further improved their essay grade. Results are shown in Figure 3.8. Improved training with the Response to Reviewer Comments document is needed to support students to critically evaluate peer edits and appropriately defend their writing choices. The level of student writing confidence may currently be impacting these choices.

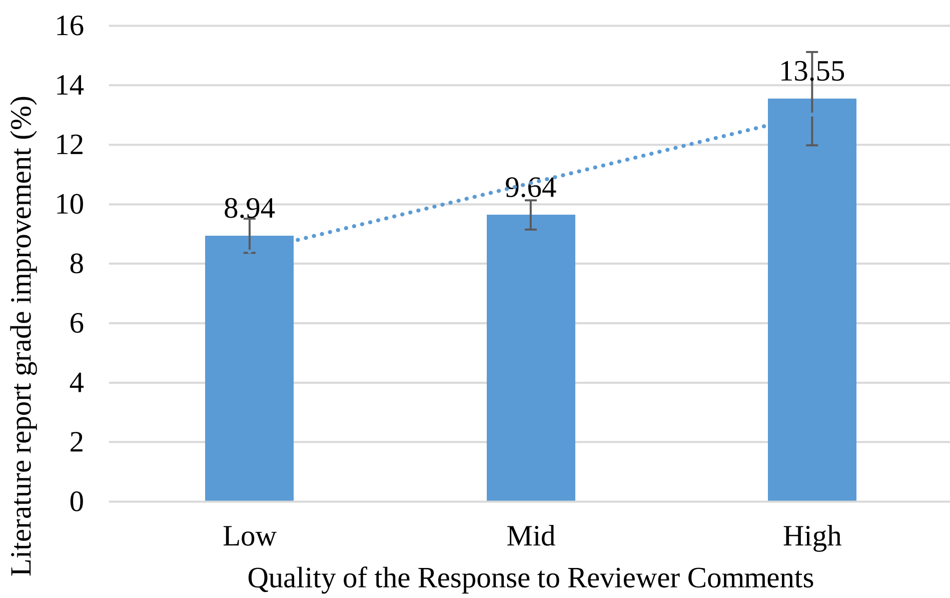

Figure 3.8 Representation of students' report grade improvement related to the quality of their Response to Reviewer Comments document. Student quality was characterized as 'high' if students showed strong engagement with the comments and defended their literary choices; 'mid' if students showed weak engagement; 'low' if students made all suggested corrections with no discussion of literary choices. Error bars represent the standard errors.

3.3.1 Peer feedback and response to peer review assignment perceptions

Student survey responses about the peer editing process are shown in Figure 3.9. Average student responses were found to be supportive of the peer editing approach. In addition, student comments on the peer editing approach are shown in Table 3.3.

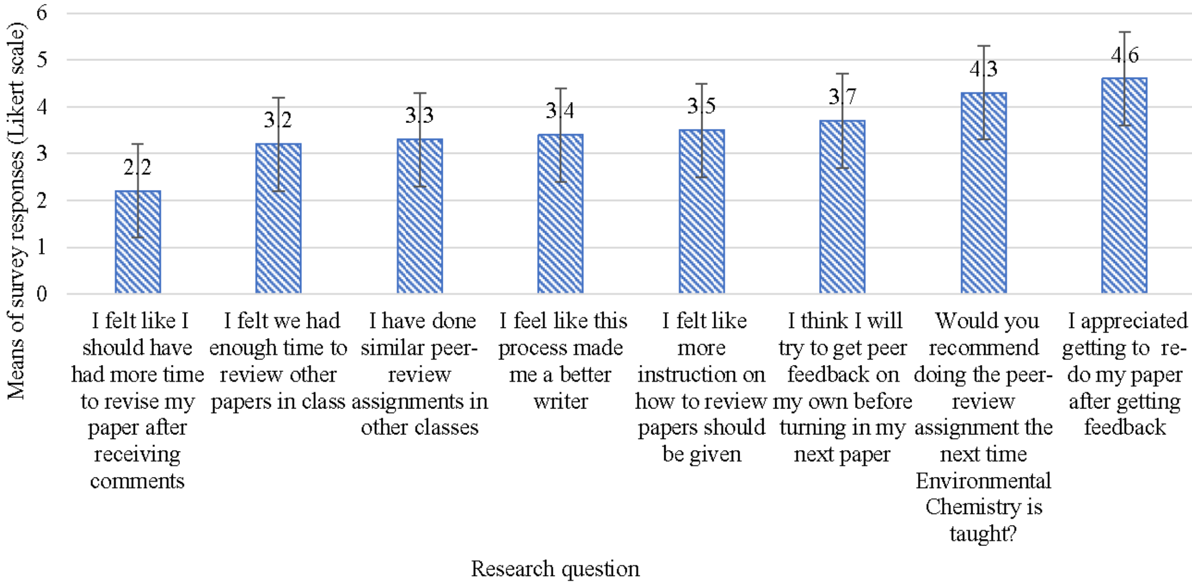

Figure 3.9 Student responses to additional survey questions. A Likert scale was used ranging from 1=Strongly Disagree to 5=Strongly Agree. Error bars represent standard errors.

Table 3.3 Student feedback on survey questions and additional comments

Survey question	Student comments
Please give us additional comments on the in-class peer review process. Was there enough time? Enough instruction on what to do? Enough work to accomplish? What would you do differently?	*The review paper is a good idea and should continue.* *Instructions were clear and enough work to accomplish.* *I thought there was plenty of time-20min/paper was sufficient. Overall, I thought it was a good assignment.* *I liked and appreciated the peer review process.* *I think we were given the right amount of time. I liked going through someone else's paper and helping them grow.* *There was plenty of instruction on what to do, especially with the rubric we were given. I wouldn't do anything differently.* *I think it worked very well.*
Please give us additional feedback on the revision process for your own paper. Were comments helpful? What was most helpful? Was there enough time to revise? What would you differently?	*Time was enough and peer comments were helpful.* *Great idea and interesting to see other new topics.* *Comments were very helpful and helped to improve the paper.* *I thought the revision process was very fair. I liked it!* *For the most part, comments were helpful. They were a little contradicting at times, but mostly they helped me catch my mistakes.*

3.3.2 Limitations

There are some limitations to this study. We focused on one essay written by students within the course which limits our conclusions concerning writing improvement. Multiple assignments would allow us to determine if student writing skills improved over the course of the semester with feedback. In addition, we did not directly compare the student edits made for each assignment with instructor edits. This could lead to further exploration of the impact and efficacy of student edits. However, other researchers have found that student edits from multiple peers carried more weight than one expert opinion in contributing to essay improvement (Cho & MacArthur, 2010).

3.4 Conclusions

This peer editing exercise did improve student writing for the technical writing assignment and encouraged students to evaluate their own writing through peer-edit feedback. Analysis of student edits showed that peer editing was sufficient to improve essay quality and the feedback was appreciated by students undergoing review. Student edits correlated with instructor grades most closely on work cited and grammar edits, with student edits focusing primarily on small, discrete suggestions instead of large, conceptual improvements. Students also valued the ability to read peer essays and maintained that reading other essays improved their own work. The majority of students appreciated the editing exercise and concluded that they would get peer edits on their own before their next writing assignment. The Response to Reviewer Comments document was included with this editing exercise to encourage students to think critically about their own work and analyze if editing suggestions were worthwhile. Overall, most students did not critically defend their writing but instead incorporated all peer edits into their work. The ability to critically think and defend their own writing needs more support for students in this upper-division science course as students were not confident enough in their own essay to defend their writing choices.

Overall, our results support the inclusion of peer edits as part of a writing assignment for students learning technical writing. Instructors can implement peer editing with assignments as a review cycle to improve student performance. The peer edit process reduces instructor-grading load, and results indicate that the students gained as much benefit from reading peer essays as they did in receiving student edits. Further work on this approach includes incorporation of several peer editing cycles to allow students the opportunity to improve overall writing skills. Further focus on critical evaluation of their own work is needed for students to fully utilize the Response to Reviewer Comments document.

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CHAPTER IV

ANALYSIS AND IDENTIFICATION OF MAJOR ORGANIC ACIDS IN WINE AND FRUIT JUICES BY PAPER CHROMATOGRAPHY

(Published in *J. Chem. Educ.* **2018**, 95, 9, 1621-1625)

4.1 Introduction

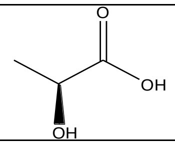
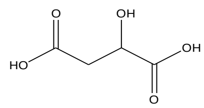
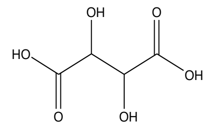
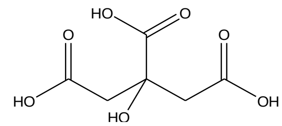
Chromatographic separation is one of the most important concepts introduced to students in introductory laboratories and as such, it is often included as a lab topic for students. The experiment described herein focuses on the separation of four organic acids present in fruit juice and wine samples and introduces a real-world application for chromatography to an introductory organic chemistry survey course. A wide variety of juice or wine samples can be selected for this technique. The colored paper chromatograms are highly visual for the students and allow for easy measurement and comparison of retention factors. In addition, this procedure allows students to learn simple extraction techniques using a separatory funnel during preparation of the mobile phase, introducing basic laboratory skills in the organic survey course.

Paper chromatography experiments are often used in introductory laboratory courses as the experiments can engage students to understand concepts of polarity in addition to basic chromatographic principles. Common experimental applications include the separation of food dyes,[1−3] indicator dyes,[4] amino acids with ninhydrin visualization,[5,6] and tomato extracts.[7] In addition, thin layer chromatography (TLC) laboratory experiments have also focused on separation of food dyes,[8] plant pigments,[9] inks,[10] and other organic compounds.[11−13] The pairing of chemical concepts with tangible real-world examples helps motivate students to accomplish meaningful learning.[14]

This experiment uses a simple and inexpensive paper chromatography technique to introduce chromatographic principles to introductory organic students. Four organic acids, citric, malic, tartaric, and lactic acid, are separated and visualized using a bromocresol green infused mobile phase. Student understanding of relative polarities and pKa values allows for comparison of organic acid strength related to organic structure (Table 4.1). Inclusion of wine samples, with a discussion of malolactic fermentation, appeals to undergraduate students and allows students to relate chromatographic principles to real-world applications. Instructors can choose to use this experiment with fruit juices only if working with younger students or can include a variety of wine samples for further expansion of sample selection.

Learning objectives for this experiment include an introduction to both extraction and chromatographic separations and an analysis of acid behavior based on pKa values. The exploration of organic acid behavior is relevant for students in an introductory organic lab course and lays the foundation for further analysis of functional group modification and organic compound structure. Students are tasked to predict acid behavior based on the pH of solution and describe how functional groups on the acid structure impact pKa value. The laboratory experiment includes a variety of pre- and post-lab questions to reinforce retention factor and pH calculations.

Table 4.1 Molecular formulas, molecular structures, and acid dissociation constants for Lactic, Malic, Tartaric, and Citric acids

Molecular aspects	Lactic	Malic	Tartaric	Citric
Molecular formula	$C_3H_6O_3$	$C_4H_6O_5$	$C_4H_6O_6$	$C_6H_8O_7$
Molecular structure				
Acidity	pK_{a1} = 3.86 pK_{a2}= NA	pK_{a1} = 3.40 pK_{a2} = 5.20	pK_{a1} = 2.89 pK_{a2} = 4.40	pK_{a1} = 3.13 pK_{a2} = 4.74 pK_{a3} = 5.40

4.2 Experimental background

The major organic acids found in wine are tartaric and malic acids, with citric, acetic, lactic, and succinic acids represented in smaller quantities. The organic acid balance in wine determines the overall character and perceived taste.[15] The acid balance and overall acidity impact the quality of finished wine product since pH impacts the aging process and shelf life through physical, biochemical, and microbial stability.[16] In grape wines, the organic acid content varies according to the climate and average temperature of the region where grapes are grown. Thus, wines from warmer regions contain more tartaric acid than malic acid while wines from colder regions are reversed with more malic acid than tartaric acid.[15–17] Excess malic acid in wine produces a harsh taste in the mouth, and therefore, winemakers reduce excess malic acid through a process of deacidification. The most common deacidification process is malolactic fermentation, where lactic acid bacteria in the wine samples convert malic acid into lactic acid.[15]

Wine makers control the organic acid content in wine as it impacts the taste and "mouth feel" of the product.[15,17] Widely used quantitative analytical techniques to determine the organic acids in wine include capillary electrophoresis (CE) with direct[18,19] and indirect[20–22] injection, high performance liquid chromatography in various modes,[23–28] enzymatic flow injection analysis,[29,30] and titrimetric techniques.[31,32] Additionally, to get a rough estimation of the acid profiles, wine makers use several commercially available kits[4,33,34] which utilize thin layer or paper chromatography.

Organic acids available in fruits vary with the fruit type. The main acids present in natural and commercial fruit juices are tartaric, malic, citric, and ascorbic acids.[35] Acid components in fruit juices are important as they are used to monitor microbiological alterations[18] or for authenticity testing[36] in fruit juices. As a result, the separation, identification, and quantification of organic acids in fruit juices are important to the quality of product and process control within the juice industry.[17,35]

4.3 Materials and methods

4.3.1 Chemicals

All chemicals and solvents used for this experiment were reagent grade or lab grade chemicals and were used without purification. Malic, citric, tartaric, and lactic acids and bromocresol green indicator were purchased from VWR (Randor, PA). 1-Butanol was purchased from Alfa Aesar (Ward Hill, MA), and formic acid and chromatography papers were purchased from Sigma-Aldrich (St. Louis, MO). All chemicals and standards were prepared and distributed among students as described in the appendix. The mobile phase was prepared according to a method modified from a commercial wine analysis.[36] The mobile phase solvent used for all the experiments described herein is a mixture of 10 mL of freshly extracted solution of 1-butanol with bromocresol green and formic acid, 4 mL of acetone, and 6 mL of ethanol.

Fruit juice and red carbonated beverage samples for analysis purchased at local grocery stores (Walmart, Kroger) and included a variety of brands and juice composition. Both red and white grape wine samples were purchased from a local wine store.

4.3.2 Equipment

Separatory funnels (250 mL) were used by students to extract their mobile phase. Developing chambers were created using 1 L beakers and watch glass covers. Whatman 200 × 200 mm chromatography papers were used as the stationary phase for these experiments, and drying time was shortened by using either a conventional chemical fume hood with exhaust or a standard 1100 W hairdryer.

4.3.3 Experimental procedure

A total of 20 students in our Survey of Elementary Organic course were divided into groups of 2 to perform this experiment. Each group extracted and prepared their own mobile phase. Four standard samples were spotted onto the chromatography paper in addition to four wine or fruit juice samples selected from a variety of prepared options. Students prepared their chromatographic chamber and poured 20 mL of mobile phase solution in before placing chromatographic sheets within the chamber. Papers were allowed to develop approximately 75 min and were dried to visualize the organic acid components. Students measured retention factors and compared selected samples to standards for compound identification.

4.4 Safety hazards

Students should wear goggles and gloves throughout the experiment to prevent chemical contact with skin or eyes. Formic acid is slightly hazardous in the case of inhalation, so mobile phase preparation should occur in a fume hood. Used mobile phase solution must be disposed in a hazardous organic waste container.

4.5 Results and discussion

In the present study, 15 grape wine samples, including 4 white wines and 11 red wines, and 27 fruit juice samples, including 5 carbonated beverages, 12 natural single fruit juices, and 10 natural mixed fruit juices, were analyzed using the described experimental approach. The majority of analyzed wine samples contained three acid components, malic, tartaric, and lactic acids, with a few only showing tartaric and lactic acid (see Figure 4.1 for examples).

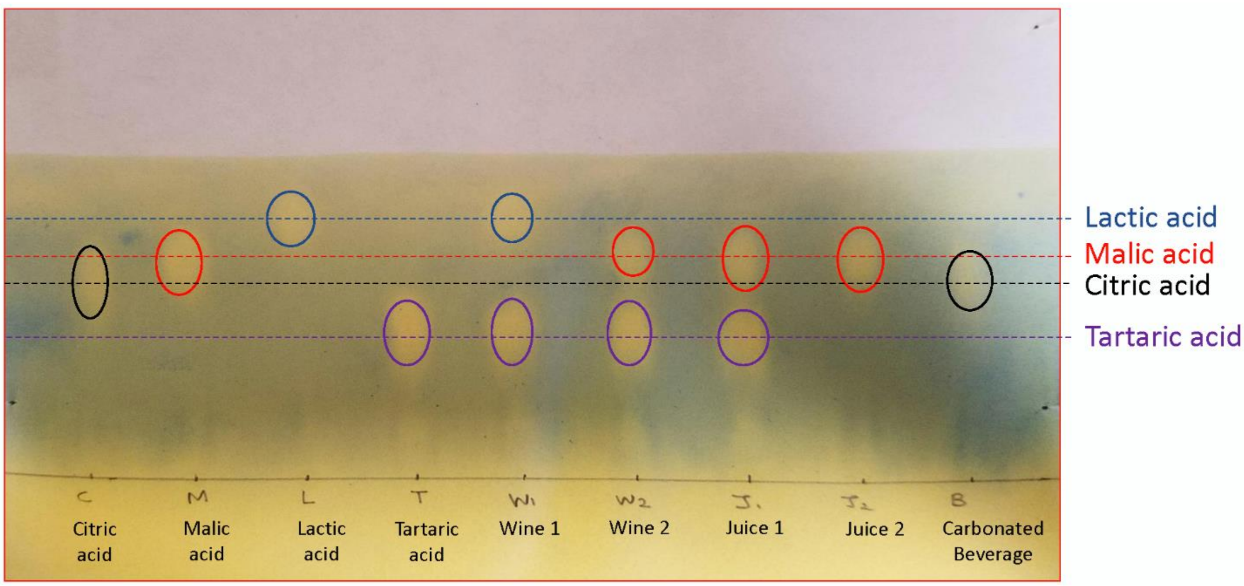


Figure 4.1 Sample chromatogram showing standards and selected samples.

All of the wine samples were successfully separated using this method, but we could identify no reliable pattern between white and red varietals. The single fruit juices, orange, apple, red grapefruit, grape, cranberry, lime, and lemon were analyzed, and among those, apple (contains malic), red grapefruit (contains malic and citric), grape (contains malic and tartaric), and cranberry (contains malic) could be separated successfully with clear and well-separated spots irrespective of their brand. It is best to purchase varieties that do not contain added vitamin

C as high amounts of ascorbic acid can merge with the citric acid spot and impact the Rf value. Orange, lemon, and lime juice plus mixed juices containing the same produced larger spots on the chromatogram which overlapped citric and malic acid spots. The 1 h separation may not be sufficient enough to observe well-separated spots for those juices, so we avoided using those citrus juices as samples for the teaching laboratory. Mixed fruit juices of apple, pineapple, cranberry, grape, pear, passionfruit, strawberry, and raspberry were also successfully separated and showed either a citric/malic pattern or a citric/malic/tartaric pattern. Specific results for juice varieties are included in the appendix.

A 75 minute separation time worked well for good spot resolution. The initial color development of the chromatogram begins around 45 min with fume hood drying. To observe clear acid spots, 1 h of drying is adequate, and at that time, the paper is dry enough to circle the spots and measure distances. Students often found that the backside of the chromatography paper afforded clearer visualization of the spots. A standard hairdryer can be used to dry the chromatography paper faster if time is of the essence. Students successfully calculated retention factors that correlated well with our values and were successful in correctly identifying sample components.

4.6 Evaluation of learning outcomes

This experiment was performed with two separate laboratory groups of undergraduate students in the elementary organic laboratory program at Mississippi State University (20 students in total). The learning objectives for this experiment are for students to

1. Understand the experimental and theoretical background of paper chromatography
2. Recognize the relationship between acid dissociation constants (pKa) and relative polarity of chemical compounds

3. Practice correct separatory funnel technique related to extraction and defined sample application for chromatographic separations

As referenced in Table 4.2, student average R_f values for the four acid standards were close to our experimental R_f values (the maximum difference found was 0.059). Students correctly identified acid spots in selected wine and fruit juice samples (84% correct), and correctly answered multiple questions related to chromatography theory in the post-lab questions (90% correct). Student understanding of the relationship between acid dissociation constant (pKa) and relative polarity of the organic acids was found to be 75%. Almost all students correctly described the correlation between polarity and movement within the polar chromatographic plate, and the minimum worksheet grade reported was 80%.

Table 4.2 Comparison between our reported R_f values and student average R_f values

R_f value	**Citric**	**Malic**	**Tartaric**	**Lactic**
Our experimental R_f values	0.623(±0.026)	0.671(±0.026)	0.391(±0.020)	0.824(±0.015)
Student's R_f averages	0.645(±0.050)	0.709(±0.036)	0.450(±0.029)	0.829(±0.044)
Difference	± 0.022	± 0.038	± 0.059	± 0.005

Student survey responses of the developed experiment indicated that they felt “the experiment worked well so that they got good results” (average of 4.0 “agree” on a Likert scale of 1-5): they found “the experiment interesting to perform” (4.0 out of 5); and they would “recommend others to do the lab” (4.1 out of 5).

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CHAPTER V

DETERMINATION OF XYLITOL IN SUGAR-FREE GUM BY GC-MS WITH DIRECT AQUEOUS INJECTION: A LABORATORY EXPERIMENT FOR CHEMISTRY STUDENTS

(Published in *J. Chem. Educ.* **2018**, 95, 11, 2017-2022)

5.1 Introduction

Xylitol is a sugar alcohol, commonly used as an artificial sweetener or sugar substitute in many "reduced-calorie" foods (Figure 5.1). Xylitol is extensively utilized in chewing gum because it helps prevent dental caries.[1–5] Although xylitol consumption has proven beneficial to humans, it is toxic to dogs. Xylitol ingestion by dogs causes vomiting, ataxia, seizures, hypoglycemia, and hepatotoxicity in the animal.[6–10]

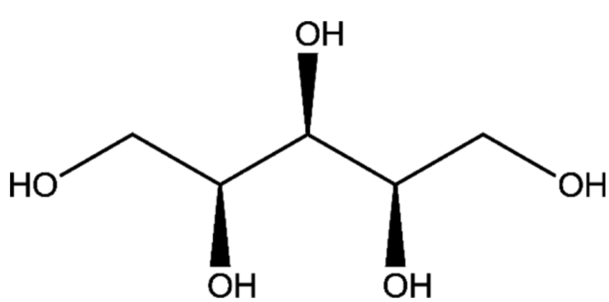

Figure 5.1 Xylitol

Ingestion of xylitol containing products such as chewing gum can result in xylitol poisoning for dogs if enough product is consumed (Table 5.1).[7,11,12] This undergraduate experiment uses a reliable low-cost method to determine amounts of xylitol in sugar-free gum sticks to predict dangerous exposure levels for dogs. An aqueous extraction technique and GC-MS analysis method using water as a solvent allow students to calculate levels of hazardous xylitol in selected gum samples.

Table 5.1 Comparison of xylitol amounts from chewing gum that would cause hypoglycemia in dogs[a]

Dog Breed	Typical Dog Size, kg	Dose, 0.1 g of Xylitol/ kg of Dog, g	Amount of Xylitol That Would Cause Hypoglycemia in Dogs, by Sample Breeds: Required Pieces of Fresh Chewing Gum — Ice Breakers: 1.5 g of Xylitol/Piece	Stride: 0.2 g of Xylitol/Piece	Trident: 0.2 g of Xylitol/Piece
Chihuahua	2	0.2	1	1	1
Yorkie	4	0.4	1	2	2
Jack Russell Terrier	6	0.6	1	3	3
Border Collie	12	1.2	1	6	6
Golden Retriever	25	2.5	2	12	12

[a]See references *7, 11,* and *12.*

Learning objectives for this experiment include sample injection techniques, quantification of xylitol using GC-MS, and a comparison of external versus internal standard techniques while allowing students to explore a topic that has direct impact on animal safety. Previous undergraduate laboratory experiments have been developed which utilize GC-MS to analyze and quantify components of diverse samples including gasoline, plasticizers, food, water, urine, perfume, beverages, and others.[13–27] GC-MS experiments have also been utilized within the organic chemistry curriculum, since it provides a great opportunity for students to analyze organic reactions such as nucleophilic substitution[28] and elimination reactions.[29] This experiment is designed for upper-level undergraduate students enrolled in organic or instrumental analysis courses. Fundamental theoretical principles and practical quantification techniques underlying this experiment present opportunities for undergraduates to apply textbook information to a real-world situation.

5.2 Experimental overview

The procedure of this experiment has four parts, including preparation of mixtures of D,L-threitol (used as an internal standard) and xylitol standard solutions to generate a calibration curve; extractions of xylitol from fresh, 1 min chewed, and 5 min chewed gum sticks; sample preparation of each extract for GC-MS analysis; and analysis of samples (Figure 5.2). Students are directed to chew gum sticks outside of the laboratory environment due to safety concerns. Multiple extractions are performed for each gum sample by grinding chewed or fresh gum pieces with 10 mL of deionized (DI)-water for 5 min, three times, using a mortar and pestle. The three extractions (which have been shown to remove approximately 99% of the xylitol) from a single gum sample are pooled.[30] All pooled extractions are then centrifuged to remove any particulates before preparation of solutions for GC-MS analysis. Both fresh and 1 min chewed gum samples contain a large quantity of xylitol, so sample preconcentration before the analysis is not required. The 5 min chewed gum samples contain very small amounts of xylitol and are concentrated via rotary evaporation before GC-MS analysis. Sample preparation includes addition of an internal standard (D,L-threitol) for quantification of xylitol.

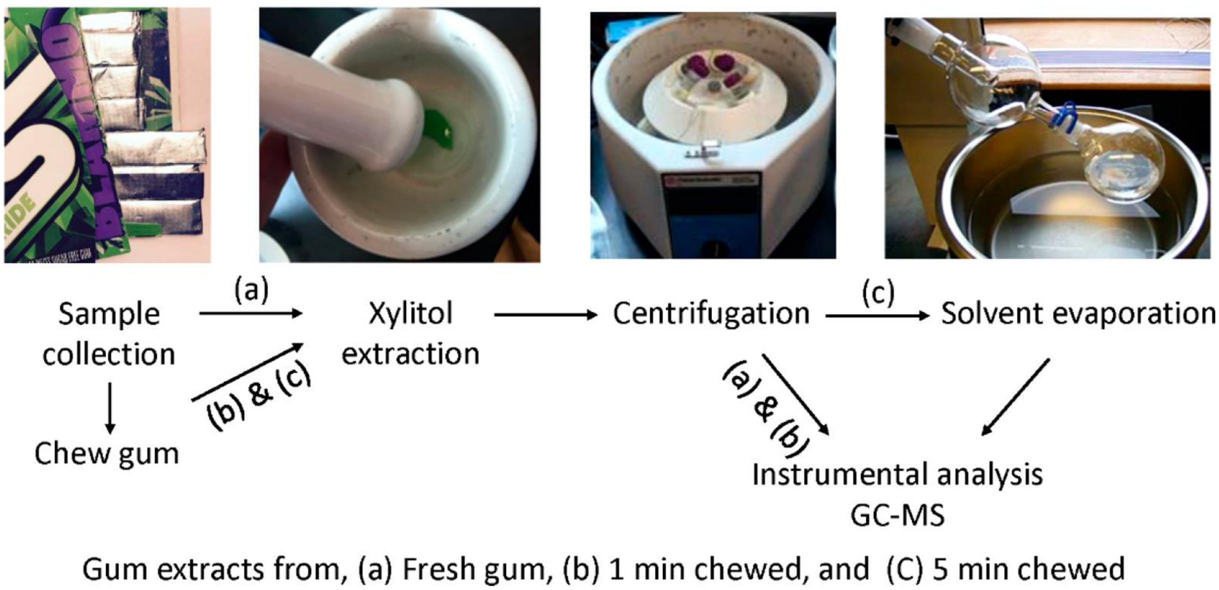

Figure 5.2 Overview of xylitol analysis laboratory procedure: (a) Fresh gum and 1 min chewed gum extractions require no sample preconcentration, (b) 5 min chewed gum extractions require preconcentration.

Students are given detailed instructions on GC-MS including instrument operation and proper injection technique. The instrument, an Agilent 7890A-5975C gas chromatograph with a quadrupole mass analyzer (GC-MS) and helium carrier gas, is used with a water resistant 60 m × 0.32 mm × 1 µm, 100% dimethylpolysiloxane column. Student instruction includes a brief tutorial indicating that compound identification is done using retention times (and fragmentation patterns when using a MS) and quantification is performed using chromatogram peak areas. This can be accomplished using total ion counts or specific ion count depending on the GC detector. Our experiments utilize a mass spectrometry detector. With this approach, inspection of fragmentation analysis can confirm compound identity; however, GCs with other detectors can be used that rely primarily on retention times for compound identification.

Water is often considered to be a poor solvent in GC analysis for a variety of reasons including backflash and chemical reactivity; however, steady advances in the field have provided solutions to most common issues. Typical GC solvents such as hexane, ethyl acetate, acetone, and dichloromethane have vapor-to-liquid volume ratios between 100 and 300.[31] However, the water vapor-to-liquid volume ratio is 1000. Hence, injecting 1 μL of liquid water into the GC liner creates 1000 μL of water vapor.[31] A typical volume of a liner is between 200 and 900 μL; solvent vapor that expands beyond the liner volume results in backflash, which can cause both sample and solvent to contaminate purge lines and the GC inlet. For best results with aqueous injections, small injection volumes and a suitable GC inlet should be used. For example, a laminar cup splitter is suitable for large volume injections of low-volatility compounds. With a laminar cup inlet, liquid can trap at the liner base until it is vaporized, ensuring complete vaporization. Maintaining a stable vacuum can also be a concern with water injections; therefore, the best results are obtained with high-capacity pumps.[32]

Chemical damage to the stationary phase is another problem associated with water injection GC. However, it was shown that immobilized and cross-linked nonpolar liquid film columns are stable with water injections.[33] In order to avoid stationary phase degradation and enable high-temperature analysis, a water resistant, 100% dimethylpolysiloxane, Agilent J&W DB-1, low-bleed, cross-linked, and water rinsable column (or similar) is recommended for this experiment.[31]

5.3 Safety hazards

Gum sticks are weighed on a food scale in a clean, nonchemical environment prior to the lab experiment. Gum chewing should occur outside the laboratory environment before putting on any personal protective equipment or gloves. Students should wash hands with soap and water and carefully transfer chewed gum pieces back to the gum wrapper for transfer into the laboratory or use a clean weighing boat to retain chewed samples. DI water is used for extraction in this experiment. Discarded gum pieces can be safely disposed in the trash can.

5.4 Results and discussion

A Trident gum sample has three polyols in large quantities, glycerol, xylitol, and sorbitol. Figure 5.3 illustrates a total ion current chromatogram of Trident gum extraction after adding the internal standard D,L-threitol for the analysis.

A GC coupled to a mass analyzer operating under electron impact (EI) mode produces a fragmentation pattern that plays a key role in compound identification. Glycerol, threitol, xylitol, and sorbitol are members of a series of compounds in which any two members in a sequence differ by one carbon atom, two hydrogen atoms, and one oxygen atom (CH−OH unit) (Figure 5.3). Because of these similarities, they have similar fragmentation patterns.[34] Glycerol has a base peak of m/z 61 resulting from the loss of CH and H_2O. Loss of hydrogen atoms and H_2O molecules and C−C bond cleavages can result in peaks at m/z 61, 91, 103, and 117 which are common for threitol, xylitol, and sorbitol, while peaks m/z 129 and 147 are common for both xylitol and sorbitol (example xylitol mass spectrum shown in Figure 5.4).[35] The molecular ion peaks of these polyols are extremely weak or not visible. Cleavage of a C−C bond and rearrangement processes associated with hydrogen, formaldehyde, ethylene, or water elimination are common fragmentation pathways for sugar alcohols. In addition, hydrogen atoms and formaldehyde and hydroxyl groups can be captured at different positions.[36] Students can use both retention time and mass spectra when identifying components of the gum extractions. This lab was written for a GC-MS; however, many different quantifying detectors could be used where identification is made using retention time alone.

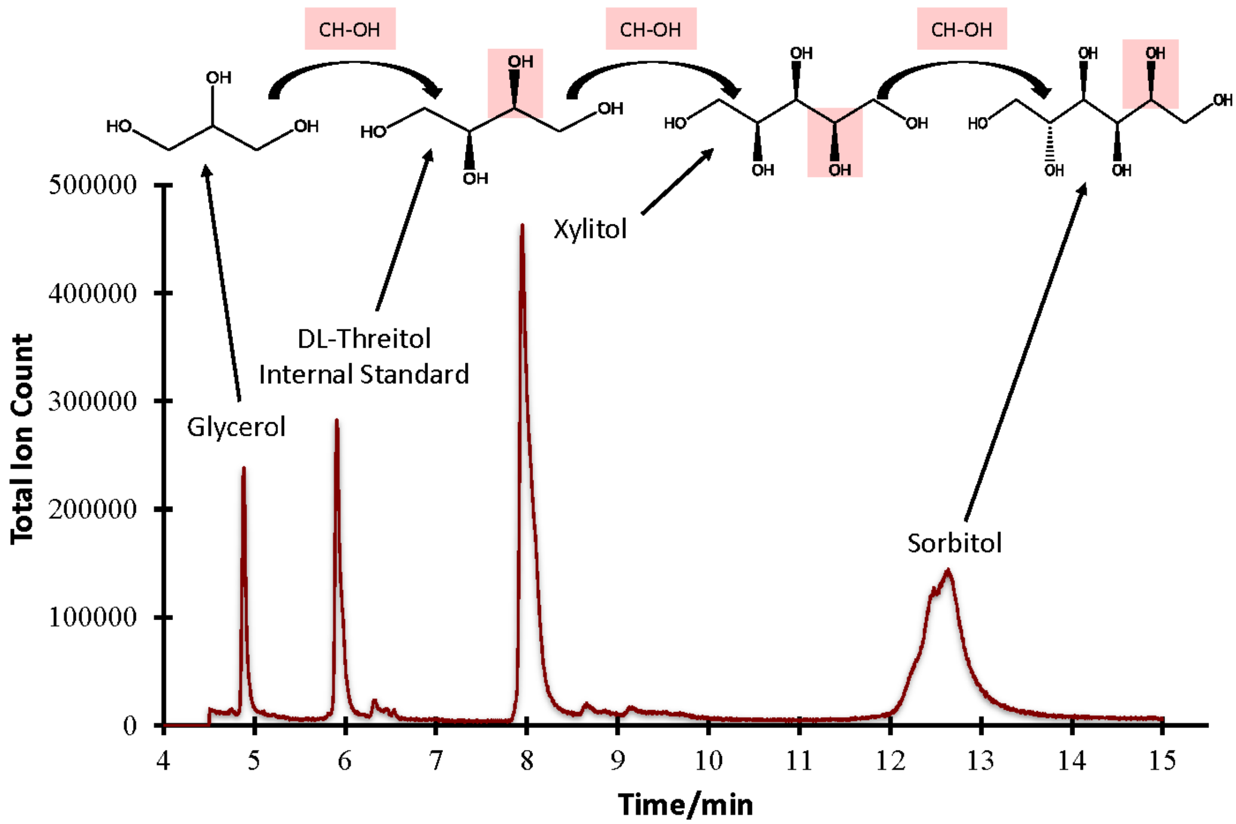

Figure 5.3 Total ion current chromatogram (TIC) of Trident gum extraction with internal standard.

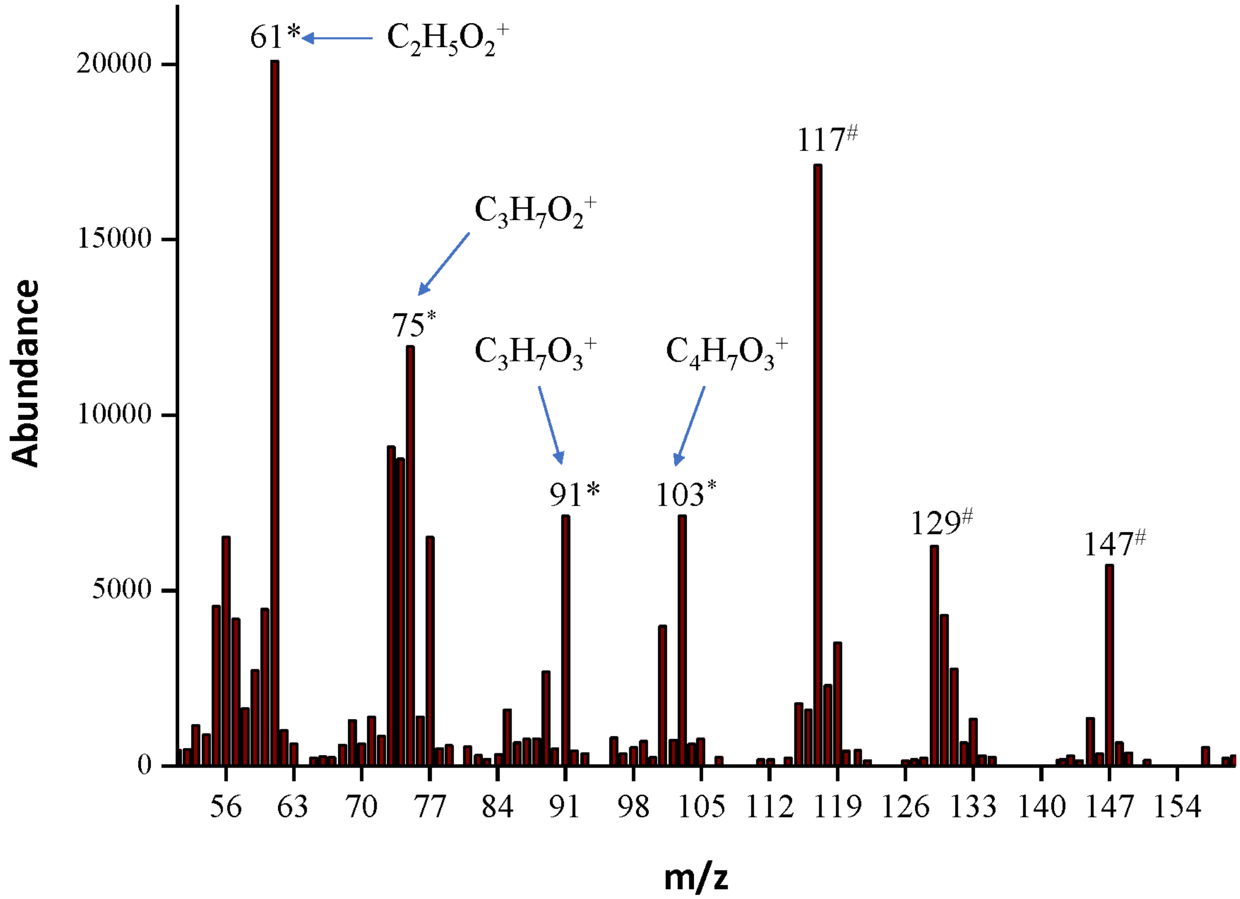

Figure 5.4 Low resolution mass spectrum collected from GC-MS for the peak at 8 min, xylitol. * indicates ions with m/z found in xylitol NIST library mass spectrum.[37] # indicates ions with m/z found in xylitol mass spectrum when silanes are present.[34–36]

Calibration methods can improve the accuracy and precision of GC-MS results. External standard calibration is commonly used to establish a linear relationship between signal magnitude and sample concentration. However, this method does not account for sample matrix chemicals, inconsistent injection volumes, or instrument drift. An internal standard calibration method can be used to reduce these potential sources of error. When using an internal standard, a known substance is added to both gum samples and calibration standards, and a calibration curve is produced by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration.

In this experiment, data from the standard xylitol samples is provided to the students to generate two calibration curves. One graph is produced according to the external calibration method, and another is created using the internal standard method. Students are tasked to compare the square of the correlation coefficient (R^2) for each method in order to determine the best calibration curve to analyze xylitol in the gum samples. A nearly perfect linear calibration curve is often obtained using the internal standard method (R^2 = 0.9992) (Figure 5.5). Conversely, poor linearity (Figure 5.6) is often observed with the external standard calibration (R^2 = 0.9808). Upon quantification of xylitol in samples, students calculate the xylitol concentration that causes hypoglycemia in dogs, with emphasis on determining the quantity of gum sticks that would cause toxicity for dogs of varying weights. Example results along with student experiment and instructor keys are available with the appendix for this experiment.

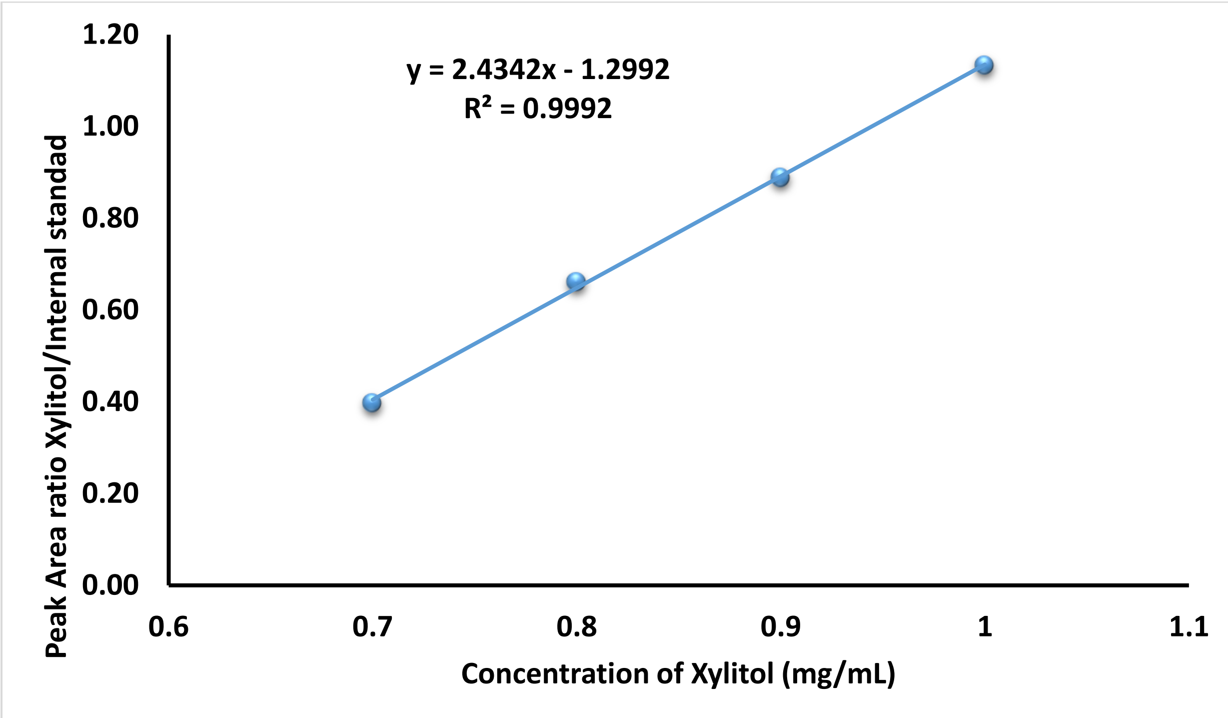

Figure 5.5 Calibration plot with internal standard.

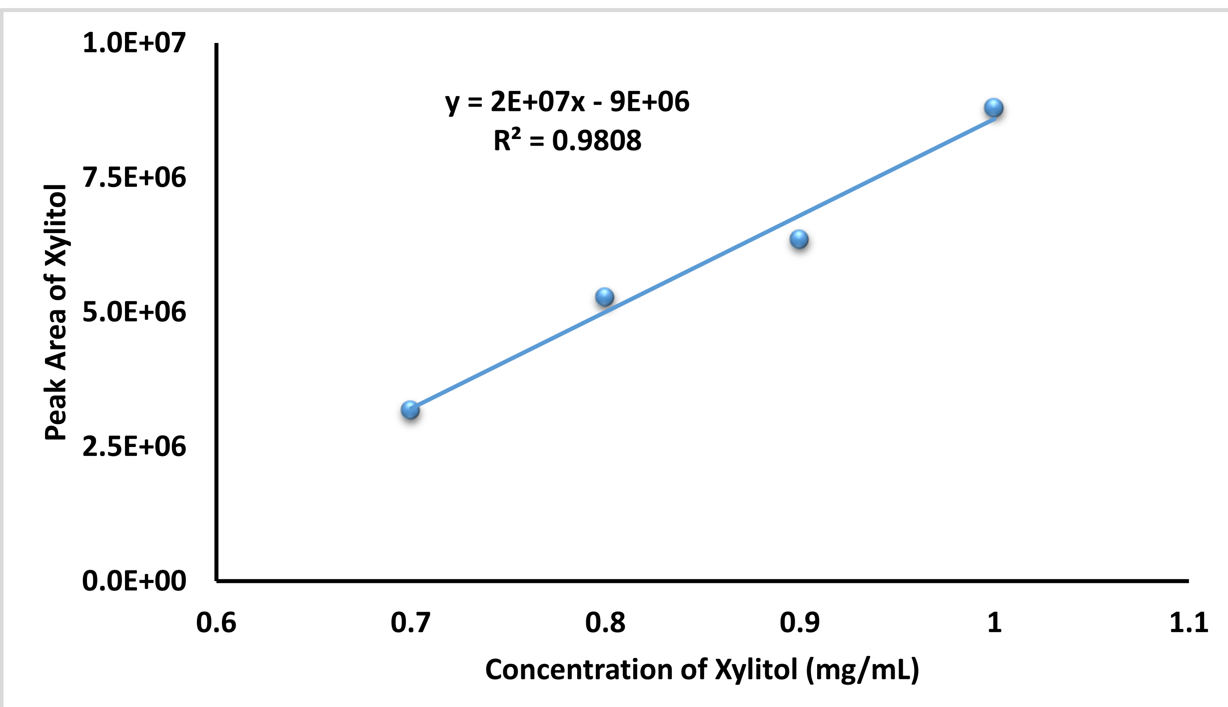

Figure 5.6 Calibration plot without internal standard.

5.5 Evaluation of learning outcomes

This laboratory exercise was initially placed in front of 31 students in a second-semester organic laboratory course. Students were excited to find gum chewing a part of the planned exercise and enthusiastically engaged in the extraction and analysis of xylitol from samples. As part of an end of semester survey which asked students to pick their favorite lab experiment of the semester, over 77% of surveyed students identified "Xylitol Chewing Gum" as their favorite laboratory experiment of the semester. Learning objectives for this experiment include the comparison of internal versus external calibration techniques and the correct calculation of xylitol concentrations within each gum sample extract. We found that only 55% of our students could correctly calculate xylitol concentrations. A revised experimental protocol for the student procedure allowed us to clarify the extraction volumes for students to fix this difficulty. The revised procedure supported a second group of students (9 students; instrumental analysis course) to successfully calculate the xylitol concentrations (89%). Interpretation of "number of gum sticks" for each category of sample (unchewed, 1 min chewed, and 5 min chewed) was also evaluated with all students correctly identifying the toxic amount of sample.

Laboratory reports for the experiment encourage students to practice mass spectral interpretation for peak identification in each chromatogram and support students in understanding fragmentation patterns in MS. The data analysis for each chromatogram hones student skills in critical thinking and supports their knowledge in spectral interpretation. However, sugar alcohols have complex fragmentation, elimination, and capture patterns when analyzed using EI MS. Therefore, matching fragmentation patterns with knowns may also be appropriate. Students enjoyed the real-world application of identifying toxic concentrations of xylitol in sugar-free gums. In addition, the laboratory exercise allowed for analysis of organic compounds using water as the only extraction solvent and a method that avoided derivatives for GC-MS analysis. The laboratory experiment supports several of the 12 Principles of Green Chemistry,[38] including the use of safer solvents, the reduction of derivatives, and safer chemistry for accident prevention initiatives.

5.6 Conclusions

This laboratory experiment is an excellent vehicle to explore the topics of extraction, solution preparation, calibration, and identification of components by gas chromatography-mass spectrometry. The topic allows students to apply textbook knowledge as they work to address a real-world situation. Challenging the students to choose a suitable calibration method for the analysis helps develop critical thinking while supporting a safe and green chemistry approach in the laboratory.

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APPENDIX A

IMPLEMENTATION AND EVALUATION OF DESIGNATED LABORATORY PARTNERSHIPS IN AN UNDERGRADUATE CHEMISTRY LABORATORY

Table A.1 Comparative demographic information of students in different laboratory partnerships grouped by the semester

Demographic categories and variables		Student population in different semester, % (Number of students)			
		Fall 2012	Spring 2013	Fall 2013	Spring 2014
Gender	Male	59.6 (376)	52.1 (198)	56.1 (393)	46.1 (187)
	Female	40.4 (255)	47.9 (182)	43.9 (307)	53.9 (219)
Ethnicity	White	73.5 (464)	76.5 (284)	80.1 (561)	71.4 (290)
	African American	17.4 (110)	16.4 (61)	11.4 (80)	19.5 (79)
	Other ethnicities	9.0 (57)	7.1 (26)	8.4 (59)	9.1 (37)
Year in school	Freshman	77.5 (489)	70.1 (260)	83.6 (585)	67.2 (273)
	Junior	6.2 (39)	7.0 (26)	4.3 (30)	7.4 (30)
	Sophomore	13.6 (86)	20.5 (76)	11.3 (79)	19.2 (78)
	Senior	2.7 (17)	2.4 (9)	.9 (6)	6.2 (25)
Competency level (according to the college entrance exam grades)	Low, Math ACT < 24	23.6 (139)	55.2 (196)	17.2 (116)	59.3 (228)
	Mid, Math ACT = 24 -26	30.6 (180)	24.2 (86)	32.3 (218)	20.6 (79)
	High, Math ACT > 26	45.8 (268)	20.6 (73)	50.5 (340)	20.1 (77)

Table A.2 Pearson Correlations between exam grades

Correlations

		Exam 1 z-score	Exam 2 z-score	Exam 3 z-score	Exam 4 z-score	ACS exam z-score
Exam 1 z-score	Pearson Correlation	1	.640**	.569**	.537**	.621**
	Sig. (2-tailed)		.000	.000	.000	.000
	N	1872	1843	1815	1772	1782
Exam 2 z-score	Pearson Correlation	.640**	1	.598**	.555**	.643**
	Sig. (2-tailed)	.000		.000	.000	.000
	N	1843	1855	1815	1774	1782
Exam 3 z-score	Pearson Correlation	.569**	.598**	1	.586**	.641**
	Sig. (2-tailed)	.000	.000		.000	.000
	N	1815	1815	1826	1769	1774
Exam 4 z-score	Pearson Correlation	.537**	.555**	.586**	1	.607**
	Sig. (2-tailed)	.000	.000	.000		.000
	N	1772	1774	1769	1782	1752
ACS exam z-score	Pearson Correlation	.621**	.643**	.641**	.607**	1
	Sig. (2-tailed)	.000	.000	.000	.000	
	N	1782	1782	1774	1752	1794

**. Correlation is significant at the 0.01 level (2-tailed).

Table A.3 Significant hierarchical regression outcomes for the research question 1

Variable	*B*	*SEB*	*β*	*t*	*P*
Step 1					
(Constant)	-.723	.071		-10.199	< .001
African American vs White	.327	.063	.165	5.148	< .001
African American vs Other	.381	.096	.124	3.988	< .001
Low vs Mid math ACT	.443	.053	.234	8.416	< .001
Low vs High math ACT	.963	.051	.543	18.750	< .001
Male-Female vs Female-Female	.132	.052	.061	2.550	.011
Low-Low vs Mid-High math ACT	-.228	.059	-.103	-3.875	< .001
Step 2					
(Constant)	-.783	.077		-10.142	< .001
African American vs White	.328	.063	.165	5.181	< .001
African American vs Other	.378	.095	.123	3.965	< .001
Low vs Mid math ACT	.443	.053	.234	8.439	< .001
Low vs High math ACT	.967	.051	.545	18.873	< .001
Male-Female vs Female-Female	.140	.052	.065	2.707	.007
Low-Low vs Mid-High math ACT	-.241	.059	-.109	-4.102	< .001
Free choice Vs Random assignment	.035	.051	.018	.691	.489
Free choice Vs Side to side	.180	.059	.074	3.075	.002
Free choice Vs High low	.160	.059	.065	2.699	.007
Free choice Vs Lecture based	-.002	.050	-.001	-.040	.968

$R^2 =$ .27, Adjusted $R^2 =$ .26 for step 1 ($p <$ .001); Δ $R^2 =$ .01 for step 2 ($p =$ .003)

Table A.4 Amounts of students participating in external study groups from each laboratory partnership type

Students participated in Study group for Chemistry outside of class	Free Choice (FA)	Random Assignment (RA)	Side-to-Side (SS)	High-Low (HL)	Lecture Based (LB)
Sample size	86	100	46	63	89
Percentage, %	20.9	21.9	16.9	23.1	17.9

Table A.5 Academic performances and attitudes of students in Free Choice group according to the gender and ethnicity profiles

Variable	Profile	Sample size	Z-score (SE)	Positive attitude about their partner, %
Gender	Male-Male	125	-.7100 (.0830)	36.1
	Female-Female	120	-.0399 (.0912)	31.7
	Male- Female	120	.0198 (.0867)	29.6
Ethnicity	White-White	172	.1536 (.0642)	31.3
	White-African American	61	-.2518 (.1207)	40.0
	White-Other	34	.2268 (.1285)	32.3
	African American-African American	29	-.7020 (.1463)	34.6
	African American-Other	17	-.4748 (.1871)	27.8
	Other-Other	6	.4693 (.3760)	-

Joining a Study Group in General Chemistry

We strongly encourage you to join/find a study group for General Chemistry as *PART* of your plan to learn Chemistry.

Studies have shown that students involved in peer study groups……

- -achieve higher grades
- -learn at a deeper level
- -retain information longer
- -acquire greater communication and teamwork skills, and
- -gain a better understanding of the course and the material

Why do study groups work? Group study tends to encourage positive behaviors by

- -reducing procrastination,
- -changing ineffective patterns of thinking,
- -increasing self-confidence on the material and
- -increasing understanding through explaining ideas out loud.

How to Find a Study Group-

-talk to other students in your laboratory or lecture section. Try to find other students with common goals or schedules so you can easily overlap

-communicate with other students in your dorm or Greek organization. Many times you can find study partners through mutual friends.

-put the word out that you are interested in finding a group through Facebook or message boards. Online study groups can work too and you don't even have to be on campus together.

-don't only rely on a study group only for your preparation. It is just as important to study on your own and get practice doing problems with no outside help.

Study Group Tips

- For an effective study group, **limit group size to 3-6 members**. If the group gets too large, have several smaller groups meet and then rearrange into different pairings. That way no one feels left out and you can take advantage of different people's strengths.
- Establish a regular meeting time/place with goals at each meeting (i.e. review Chapter 3 or go over practice test). The library offers study group rooms for use; larger groups get priority for room use (let the librarian know you need a room and they will clear one for you.)
- Exchange contact information. Students should exchange email addresses, Facebook info, and phone numbers, so everyone can be contacted to help the others.
- At your first meeting, encourage each member to talk about his/her strengths that will help the group. Talk about your goals and the format for your study group.
- For effective studying, predict test questions and quiz each other. Have each student come prepared with a sample question. Practice doing problems on your own before comparing answers so one member doesn't do all the work.
- Above all, don't wait until the last minute to prepare for an exam! Below is a link with suggestions on how to study for an exam 6 days away. http://testprep.about.com/od/tipsfortesting/a/Study_Schedule.htm

Figure A.1 Information on study group participation.

Reference

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Survey questions for laboratory partner research study:

Name________________________________NetId_________

Circle the most Appropriate Response Below:

1a. Rank the quality of your lab partner interaction. Did you and your partner talk in lab and help each other understand the material?

_4_Yes, very much _3_Yes, kind of _2_No, not really _1_No, not at all

1b. If you answered "yes, very much" or "yes, kind of" above, choose the situation that best describes your interaction.

______3_ I did most of the work and my lab partner kind of followed along.
______2_ My lab partner and I each did work equally and helped each other.
______1_My lab partner helped to explain things and finished some of the answers first.

2. How would you describe the overall experience with your lab partner? Was the experience enjoyable?

___4__Yes, we worked great together.
___3__Yes, it was ok.
___2__No, it was ok but could have been better.
___1__No, the experience wasn't enjoyable at all.

3. Did you participate in a regular study group(s) for Chemistry I (CH 1211/CH 1221) outside of class? If you participated in more than one, base your responses upon the group that seemed most effective in helping you.

_____1______Yes _____0______No

If you answered "Yes", continue to Question #4. If you answered "No", skip to Question #8.

4. If you answered "Yes" to question #3: How often did your study group typically meet?

____3___Often; two or more times per week
____2___Regularly; typically, once a week
____1___Occasionally; we would meet just before exams or other assignments

5. If you answered "Yes" to question #3: what was the typical activity that you and your study group worked on? Check all that apply.

1=Y;0=N

________ lab assignments: we worked on lab homework or studied for lab quizzes

________ online lecture homework: we worked on online homework assignments

________ test preparation: we studied for tests

________ other. Please explain:

____

6. How did you find your study partners? Check all that apply below.

1=Y;0=N

_______ from lab; I studied with my lab partner or others I met in lab

_______ from lecture; I studied with other students I met in my lecture section

_______ from my dorm; I studied with others that I met through my dorm or housing

_______ from another organization; I studied with others from my sorority/fraternity/other organization

_______other: Please explain:

____

7. Did you find your study group helpful in preparing for Chemistry? What seemed helpful about it? Check all that apply.

1=Y;0=N

________ it helped me understand the material better

________ it helped me get through the assignments faster since we shared the task

________ it forced me to study when I might not have on my own

________ it wasn't very helpful, and it didn't help me understand better

________ my study group was a total waste of time

If you Answered "Yes" to Question #3 and have responded to the Study Group Questions, please skip to Question #10.

If you answered "No" to Question #3, please answer questions #8 and #9.

8. If you answered "No" to question #3, you did not participate in a study group outside of class. Why not? Check all that apply.

1=Y;0=N

__________ I have participated in study groups before and they are just a waste of time

__________ I wasn't able to connect with anyone to form a study group with

__________ I have never participated in a study group before and didn't think I'd want to

__________ I tried to meet with a study group, but I couldn't find a time that would work

__________ I didn't realize until too late that I should have met with a study group

__________ other.

Please explain: ____

9. If you did not participate in a study group, what do you think would have been helpful for you? Please check all that apply:

1=Y;0=N

_________ I needed an easier way to connect with interested people.

(please explain how you would want to connect

____)

_________ I needed more information about how study groups could help me learn

_________ I needed more information about the difficulty of the Chemistry course, so I knew what to expect for exams.

_________ I needed more information on how to study for Chemistry

_________ I needed more information on how to study for all my courses

10. How do you feel about your performance in General Chemistry I (CH 1211/CH 1221)?

___4______ Great: I got the grade I wanted, and I feel good about my performance

___3______ Good: I didn't get the grade I wanted but still did just fine

___2______ Not so great: My grade is a little lower than I wanted, and I really feel I could have done better

___1______ Awful: My grade is way lower than I wanted

Written Comments: Please expand or explain on any of the questions above to give us your feedback. We appreciate your willingness to help us improve!

Figure A.2 Survey questionnaire.

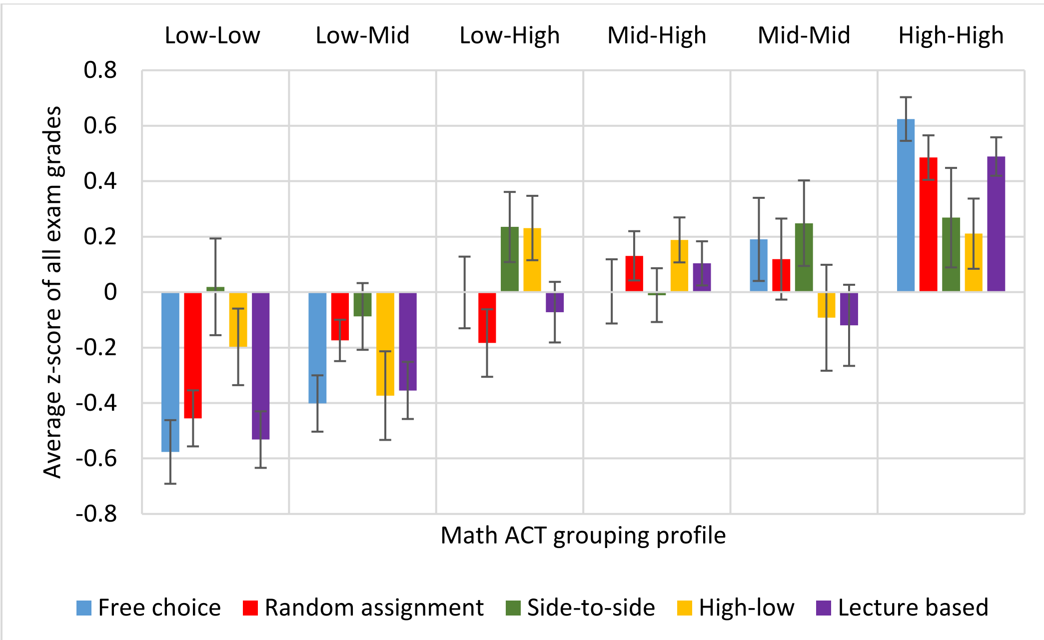


Figure A.3 Representation of the average z-scores for different math ACT grouping profiles grouped by the laboratory partnership. Error bars indicate the standard error of data sets.

APPENDIX B

GRADING RUBRIC

Table B.1 Environmental Chemistry literature review rubric

Area Evaluated	***Excellent (10 pts)***	***Good (6-9 pts)***	***Fair (2-5 pts)***	***Poor / Missing (0-1)***
Works Cited Page	• ACS format correct • includes at least 4 sources cited • correct source categories • alphabetical order	• ACS format with minor errors • includes 3 cited sources • alphabetical order • missing a source category	• ACS format with major errors • includes 2 cited sources • no alphabetical order • missing more than one source category	• ACS format not attempted or not present • results in honor code violation
Area Evaluated	***Excellent (9-15 pts)***	***Good (7-8 pts)***	***Fair (5-6 pts)***	***Poor / Missing (1-3)***
Using Cited Work	• valuable information used from each source • works cited correctly • proper ACS format	• half of the sources used • proper ACS format or minor citation errors	• most info taken from 1 source • incorrect format or major errors	• No info from sources • Minimal or no citations
Area Evaluated	***Excellent (9 – 15 pts)***	***Good (7 – 8 pts)***	***Fair (5 - 6 pts)***	***Poor / Missing (1-4 pts)***
Grammar, Spelling, Neatness	• proper grammar usage with no errors • No spelling errors • written in 3rd person • neat, organized • appropriate images/charts • title page is well-designed and has appropriate image	• 1-3 grammar errors • 1-2 words misspelled • written in 3rd person • mostly neat and organized • title page is well designed, but image may not be appropriate to subject being discussed	• 4-6 grammar errors • 3-6 misspelled words • includes 1st or 2nd person • lacking in neatness and/or organization • title page is neat, but not well-designed, image may be inappropriate or missing	• more than 6 grammar errors • more than 6 misspelled words • includes 1st or 2nd person • lacking in neatness and/or organization • title page is missing or hand-written, image missing or inappropriate
Area Evaluated	***Excellent (43 –60 pts)***	***Good (35 - 42 pts)***	***Fair (17 - 34 pts)***	***Poor/Missing (1-16 pts)***

Appendix Table 5.1 (continued)

Content	• includes introduction and conclusion • subject thoroughly discussed • Logical progression • writing style clear and concise • original question or problem is thoroughly discussed • avenues for future research well discussed • greater than minimum length	• introduction and/or conclusion somewhat brief or weak • discussion of subject is good • progression mostly logical • writing style mostly clear/concise • original question or problem is somewhat discussed • future research somewhat discussed • minimum length	• introduction and/or conclusion missing or very weak • inadequate discussion of subject • progression weak • writing style is not clear and/or concise • original question or problem is briefly answered • future research is poorly related to material discussed • less than minimum length	• introduction and conclusion missing • very inadequate discussion of subject • progression illogical • writing style not clear or concise • original question or problem is not addressed • no avenues for future research provided • significantly shorter than minimum length

APPENDIX C

ANALYSIS AND IDENTIFICATION OF MAJOR ORGANIC ACIDS IN WINE AND FRUIT JUICES BY PAPER CHROMATOGRAPHY: AN ORGANIC EXPERIMENT FOR UNDERGRADUATE LABORATORY

(Published in *J. Chem. Educ.* **2018**, 95, 9, 1621-1625)

C.1 Student laboratory experiment

"Analysis and identification of major organic acids in wine and fruit juices by paper chromatography"

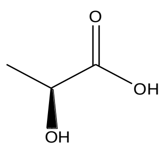
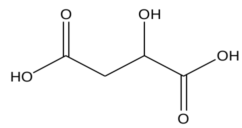
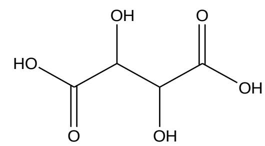
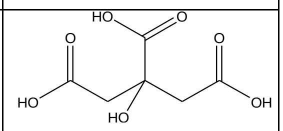
Overview

Mixtures can be divided into two major classes – **homogeneous** and **heterogeneous**. A **homogeneous mixture** is composed of two or more pure substances that when mixed together have the physical appearance of uniformity. Grape juice is an example of a homogeneous mixture. A **heterogeneous mixture** contains two or more pure substances but lacks the uniformity described above. A chocolate chip cookie is an example of a heterogeneous mixture. The pure substances that form mixtures can be separated from one another through techniques that exploit both their physical and chemical properties. "Extraction" separates compounds between two liquids that are not miscible with each other. "Chromatography" separates compounds by passing a liquid or gaseous solution through a stationary phase. In this experiment, both techniques are used where extraction is used to prepare an organic liquid mobile phase that is used with paper chromatography to separate the organic acid components from juice and wine samples and visualize the location of each acid component.

Organic acids found in wine and fruit juice

There are three primary acids found in grapes or wine samples; tartaric, malic, and lactic acid (see Table C.1). Citric acid is often found in other juices as well, and the combination of these four organic acids contribute both to the "tart" taste of fruit juices and wines and also impact the overall pH value of the samples.

Table C.1 Common organic acids found in many fruit juices and wines

Molecular aspects	Lactic	Malic	Tartaric	Citric
Molecular formula	$C_3H_6O_3$	$C_4H_6O_5$	$C_4H_6O_6$	$C_6H_8O_7$
Molecular structure				
Acidity	pK_{a1} = 3.86 pK_{a2}= NA	pK_{a1} = 3.40 pK_{a2} = 5.20	pK_{a1} = 2.89 pK_{a2} = 4.40	pK_{a1} = 3.13 pK_{a2} = 4.74 pK_{a3} = 5.40

Tartaric acid is considered the most important acid type in wine as it maintains the chemical stability, color, and taste of the finished wine. Malic acid and tartaric acid are the two principal organic acids found in grape wines. Grapes that are grown in cool climates or grapes that are used to form robust, red wines often have higher levels of acidity. These grapes require de-acidification via Malolactic Fermentation (MLF) which reduces the overall acid taste and softens the wine's flavor. This conversion takes place through bacteria in the wine and, since this conversion is accompanied by the production of carbon dioxide, this process is called fermentation. Lactic acid has a less sour taste and higher "mouth-feel softness" than malic acid, so higher lactic acid content enhances the body and flavor of the wine. For acidic, red wines to be ready for bottling the vast majority of malic acid must undergo this Malolactic Fermentation. Therefore, a wine that shows a strong presence of malic acid should not be bottled, but if malic acid has been converted to lactic acid, the wine is ready to bottle and sell.

When organic acids are present in fruit juices, they influence the growth of microorganisms and therefore affect the quality of the product. Malic acid is present in many sour/tart foods and fruit juices. Fruit juices of apple, cherry, cranberry, and peach have malic acid as the primary organic acid, and both grape and pineapple juices have malic as a secondary acid. However, the malic acid level in fruits can vary according to the fruit variety, growing region, fruit maturity, and juice extraction conditions. Grape juices are rich in both tartaric acid and malic acid. And with very tart juices such as lemon, lime, passionfruit, and pomegranate, we often see citric acid, a fourth organic acid. The role of citric acid in fruit juices is to improve taste, flavor, antioxidant content, and to maintain stability.

This laboratory experiment uses paper chromatography, a separation technique, to identify the presence of the four organic acids (malic, lactic, tartaric, and citric) in wine or juice samples. A pH indicator, bromocresol green, helps visualize the acid components after separation. Bromocresol green undergoes a color change from yellow to blue in the pH range of 3.8-5.4. The organic acids lose protons below the 4.0 pH value and show as yellow spots on the blue background of chromatography paper. The bromocresol green indicator is extracted with organic solvents to function as the mobile phase for chromatography.

Chromatography is a process in which components of a mixture can be separated based on their interactions with their environment. In this experiment, chromatography paper is spotted with wine and fruit juice mixtures and is then placed in a sealed container that contains an organic solvent. The organic solvent can move up the chromatography paper via capillary action and can potentially carry some of the components up the paper as well. We define the chromatography paper as the **stationary phase** since it stays in one spot. The organic solvent that moves up the paper is defined as the **mobile phase**. Any compound that prefers to spend time in the organic mobile phase will move up the paper with the solvent.

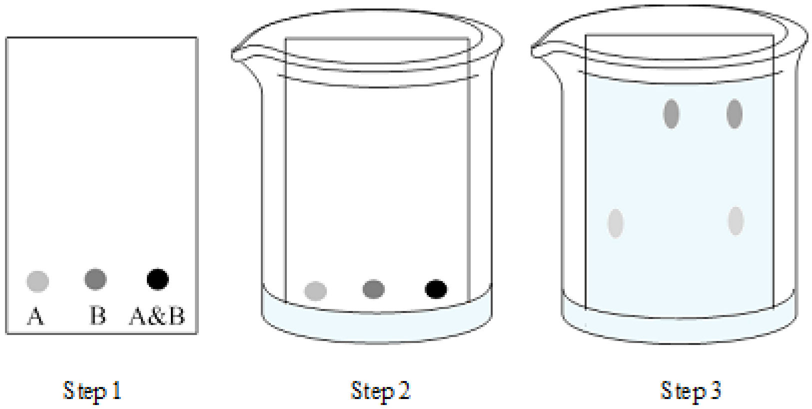


Figure C.1 Sample chromatography plates showing spot separation.

Usually, two components in a mixture will not have the same affinity for both phases. The extent of separation depends on each component's time in the mobile phase – the longer a component is in the mobile phase, the farther it will travel along the plate. In Figure C.1 shown above, pure A, pure B and a mixture of A & B are spotted at the bottom of the stationary phase in Step 1. The mobile phase is allowed to pass through the components in Step 2 and carry them up the stationary phase. The stationary phase is withdrawn in Step 3 and the positions of A and B are located – note the separation between A and B that occurred in the third lane. The compounds separate because of the "polarity" of the stationary phase and the mobile phase; the stationary phase is polar in this case, and the mobile phase is nonpolar.

The **retention factor** value (R_f) is a number that corresponds to how far a component travels versus how far the solvent travels.

$$R_f = \frac{distance\ travelled\ by\ component}{distance\ travelled\ by\ solvent\ front} \quad (C.1)$$

The R_f value can be calculated either from the most intense point of the component spot OR from the first edge of the component spot. For the purposes of this lab, we recommend that you use the most intense, middle of the spot for your calculations. An R_f value of 0 indicates that the sample remained completely in the stationary phase and was immobile. An R_f value of 1 indicates that the sample was very soluble in the mobile phase and traveled with the edge of the solvent front.

The R_f value for each organic acid is also related to the polarity of each molecule. Polar bonds are created due to unequal sharing of electrons and the overall polarity of a molecule is related to the contributions of each individual dipole moment. The unequal sharing of electrons is also related to the pK_a of a bond. The pK_a for an acid is a representation of acid strength, where a low pK_a value means the compound is a stronger acid (a high Ka value); a high pK_a value means the compound is a weaker acid (a low Ka value, see Equation 1).

$$pK_a = -\log[K_a] \quad \text{Eqn. 1}$$

Since organic acid molecules contain more than one acidic hydrogen, we can report more than one pK_a value for the molecule. Acidic hydrogen atoms in an organic acid with lower pK_a values are highly polarized and thus, more polar. In this experiment, the stationary chromatography paper is polar, so more polar compounds will move slowly along the paper. The organic solvent mobile phase is nonpolar, so nonpolar compounds will prefer the mobile phase and will travel quickly along the paper.

This experiment allows students to prepare their mobile phase organic solvent using extraction techniques in a separatory funnel. Wine or fruit juice samples are then applied to chromatographic paper and eluted with the mobile phase to separate and identify the four organic acids of interest. You will be provided organic acid standards so that the R_f values of known compounds can be compared to spots present in complex samples.

Reagents

Bromocresol green indicator

Formic acid

1-Butanol

Ethanol

Acetone

Acid standards (citric, malic, lactic, tartaric)

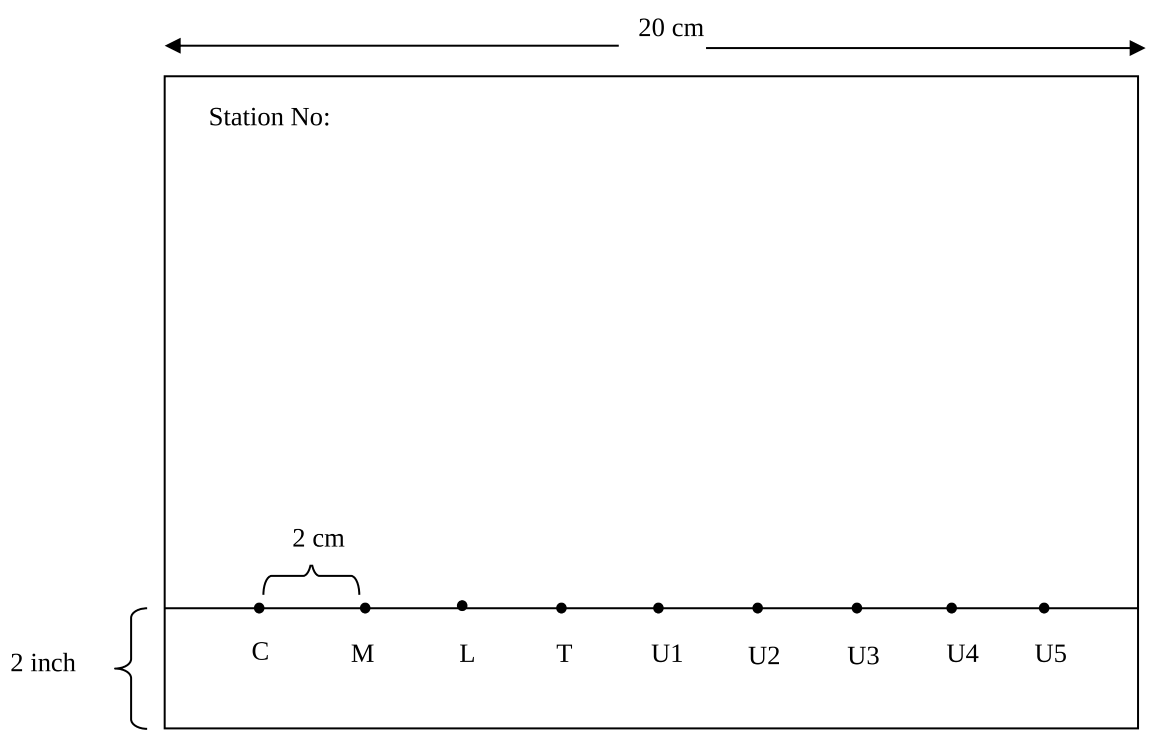
Safety

Make sure to wear gloves, safety goggles, and lab coat through the experiment. Do not inhale the mobile phase solvent!!!

Procedure

1. In a 100 mL beaker, dissolve 0.0375 g of bromocresol green in 7.5 mL of distilled water. Then add 25 mL distilled water and 25 mL of 1-butanol.
2. Using a micropipette, add 2.6 mL of formic acid to the above solution.
3. Transfer the mixture into a 250-mL separatory funnel with a closed-stopcock. Stopper the funnel and hold the top with several of your fingers to secure the top. Mix the reagents thoroughly by tilting the separatory funnel back and forth. Periodically vent gases through the stopcock valve; make sure the stopcock valve is not pointing at any persons when venting. Your TA will provide a demonstration of proper separatory funnel technique.

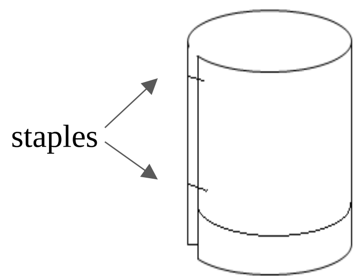
4. Allow the mixture to settle for 5 minutes to separate the phases. Discard the aqueous (lower) phase into a hazardous organic waste container. Collect the orange-colored organic phase into a clean beaker. (You should extract about 30 mL portion of the solution).
5. Prepare the chromatographic chamber by pouring 10 mL orange-colored solution, 6 mL of ethanol, and 4 mL of acetone into a 1000 mL beaker. Cover the beaker with a large watch glass.
6. On a sheet of chromatography paper, draw a pencil line about 2 inches from the bottom edge of the paper (see figure below). Draw 9 evenly spaced dot marks along the pencil line and label them as in the figure below.



7. Write your name or station number in the top left corner of the chromatography paper.
8. Lay the chromatography paper onto a paper towel. Dip one end of a toothpick into each provided solution (standards, wine and/or fruit juice samples) and spot a small amount onto a

tick mark. Repeat for each sample/ tick mark. Use only the toothpick provided for each sample as cross-contamination will impact your results. As you spot each sample avoid making the spot very large – it should be about 0.2-0.3 cm in diameter at most. (Do not spot multiple times. Wine and fruit juices contain high levels of organic acids.)

9. Allow the spots to dry for about 5 minutes. Carefully curve the chromatography paper into a cylinder and staple the top edge and bottom edge together so that it forms a cylinder. Be careful NOT to overlap the edges of the paper (see figure below)



10. Place the rolled chromatographic papers in the chromatographic chamber. **Do not allow the paper to touch the walls of the container**. Close the chamber and allow the compounds to travel for approximately 1 hour and 15 minutes until the solvent front has moved to within an inch of the cylinder top. Do NOT move, shift or shake the beaker while the mobile phase is moving up the paper.

11. Once the solvent front has reached 1 inch from the top, remove the paper cylinder from the chamber and place in a fume hood for drying. Place a funnel inside the cylinder to keep the paper upright.

12. After approximately 45 minutes of drying, remove the chromatography paper from the fume hood. A hairdryer can be used to dry the paper faster.

13. Carefully pull out the staples and flatten the chromatography paper. Using a pencil, draw a line to mark the solvent front (the line where the solvent ends on the paper) and lightly circle each spot. Mark each spot middle point with a dot or an "x". Use a ruler to calculate R_f for each standard spot and each unidentified spot. *You might find it easier to look at the backside of the paper- check if spots look clearer on the back.*

Datasheet

Part A

Determine the R_f value of each spot on your chromatogram and identify the organic acids present in your samples by comparing the R_f values to standards. Record all data on your datasheet. Keep all spot measurements consistent, measuring from the center of the spot. Identify each spot by comparing R_f value to standards.

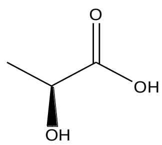
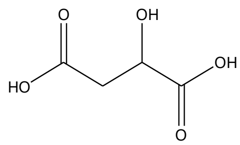
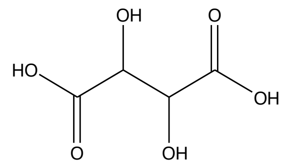
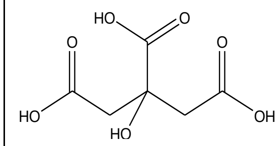
Distance to the solvent front from the bottom line: ______________ cm.

Spot	Distance to the spot from the bottom line (cm)	Retention factor, R_f	Identity of Organic acid
Citric standard			
Malic standard			
Lactic standard			
Tartaric standard			
Sample 1			
Sample 2			
Sample 3			
Sample 4			
Sample 5			

Could any of your samples have completed MLF fermentation? Explain.

What is the most common acid present in your samples?

Part B

Molecular aspects	Lactic	Malic	Tartaric	Citric
Molecular formula	$C_3H_6O_3$	$C_4H_6O_5$	$C_4H_6O_6$	$C_6H_8O_7$
Molecular structure				
Acidity	pK_{a1} = 3.86 pK_{a2}= NA	pK_{a1} = 3.40 pK_{a2} = 5.20	pK_{a1} = 2.89 pK_{a2} = 4.40	pK_{a1} = 3.13 pK_{a2} = 4.74 pK_{a3} = 5.40

Based on the R_f information you determined, rank the four organic acids from most polar to least polar.

How does this ranking correspond to the pK_a values given for these acids?

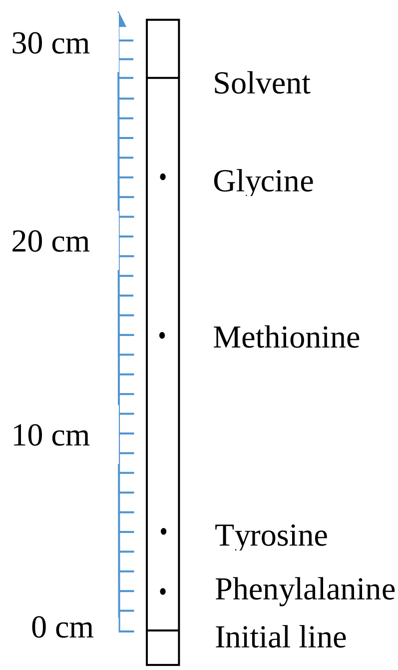
Post lab questions

1. In a paper chromatography experiment, why is it necessary to apply the sample spots above the level of the solvent?
2. Why should you use separate toothpicks for each sample? What would happen if you used the same toothpick?
3. Why is it necessary to open the stopcock periodically when shaking the separatory funnel?

Worksheet

1. Butanoic acid, the substance responsible for the order of rancid butter, has pK_a = 4.82. What is its K_a?
2. Formic acid, HCO_2H, has pK_a = 3.75, and picric acid, $C_6H_3N_3O_7$, has pK_a = 0.38.
   a) What is the K_a of each?
   b) Which is a stronger acid, formic or picric acid?
3. A sample containing a mixture of amino acids was separated using the ascending paper chromatography technique.

   The results of the experiment are shown below.



Component	Distance traveled
Solvent	
Glycine	
Methionine	
Tyrosine	
Phenylalaninie	

Component	Retention factor, R_f
Glycine	
Methionine	
Tyrosine	
Phenylalaninie	

a) Calculate the retention factor for each of the amino acids in the mixture.

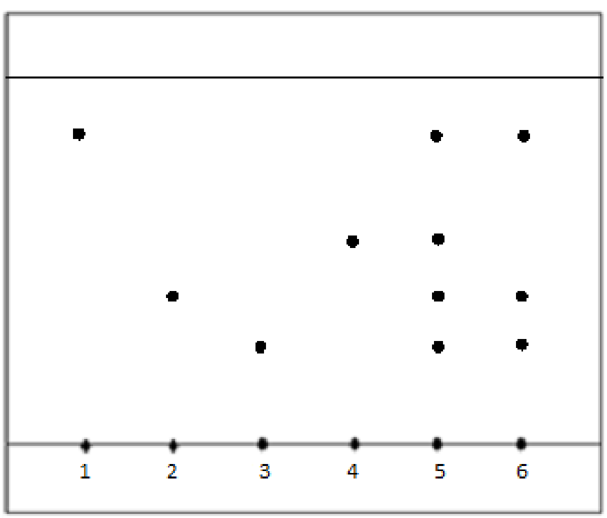
b) Name the least and the most soluble components in the stationary phase.

Least soluble:

Most soluble:

c) Based on the R_f information you determined, rank the above amino acids from most polar to least polar.

4. An example paper chromatogram for the separation of color pigments from Red and Green leaves is given below.



1. Carotene
2. Clorophyll a
3. Clorophyll b
4. Xanthophyll
5. Red leaves
6. Green leaves

What are the available color pigments in each leaf extract?

Red leaves:

Green leaves:

C.2 Instructor answer key

Datasheet

Part A

Determine the R_f value of each spot on your chromatogram and identify the organic acids present in your samples by comparing the R_f values. Record all data on your data sheet.

a) Distance to the solvent front from the bottom line: 12.0 cm

Spot	*Distance to the midpoint of the spot from the bottom line (cm)*	*Retention factor, R_f*
Citric standard	7.3	0.61
Malic standard	8.3	0.69
Lactic standard	10.0	0.83
Tartaric standard	5.4	0.45
Wine 1	5.1, 8.5	0.43, 0.71
Wine 2	5.3, 10.3	0.44, 0.86
Wine 3	5.0, 8.4, 10.4	0.42, 0.7, 0.87
Fruit Juice 1	5.0, 8.4	0.42, 0.7
Fruit Juice 2	7.1	0.59

a) *What is the identity of the spots seen in your fruit juice and wine samples? Explain your reasoning.*

- Wine 1 has tartaric and malic- R_f = 0.43 is very close to the R_f value of tartaric standard (0.45), and R_f =0.71 is very close to the R_f value of malic (0.69)

- Wine 2 has tartaric and lactic- R_f = 0.44 is very close to the R_f value of tartaric standard (0.45), and R_f =0.86 is very close to the R_f value of lactic (0.83)
- Wine 3 has tartaric, malic, and lactic-R_f = 0.42 is close to the R_f value of tartaric standard (0.45), R_f = 0.70 is close to the R_f value of malic standard (0.69) and R_f =0.87 is close to the R_f value of lactic (0.83)
- Fruit juice 1 has tartaric and malic- R_f = 0.42 is close to the R_f value of tartaric standard (0.45), and R_f =0.70 is very close to the R_f value of malic (0.69)
- Fruit juice 2 has citric – R_f = 0.59 is very close to the R_f value of citric standard (0.61)

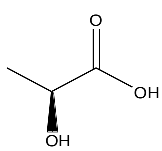
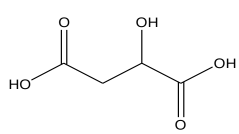
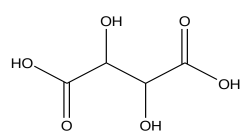
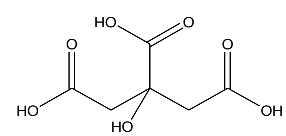
b) *Have your wines completed MLF fermentation? Explain.*

Only wine 2 has completed the malolactic fermentation (no malic acid spot). Both wine 1 and wine 3 have malic acid.

What is the most common acid present in your samples?

This answer could change depending on sample selection.

Part B

Molecular aspects	*Lactic*	*Malic*	*Tartaric*	*Citric*
Molecular formula	$C_3H_6O_3$	$C_4H_6O_5$	$C_4H_6O_6$	$C_6H_8O_7$
Molecular structure				
Acidity	pK_{a1} = 3.86 pK_{a2}= NA	pK_{a1} = 3.40 pK_{a2} = 5.20	pK_{a1} = 2.89 pK_{a2} = 4.40	pK_{a1} = 3.13 pK_{a2} = 4.74 pK_{a3} = 5.40

Based on the R_f information you determined, rank the given four organic acids from most polar to least polar. How does this ranking correspond to the pK_a values given for these acids?

Tartaric, citric, malic, and lactic

From tartaric to lactic, pK_a values are increased (2.89, 3.13, 3.4, and 3.86). The acidity is decreased with decreasing polarity. Acidic hydrogen atoms in an organic acid with lower pK_a values are highly polarized and the acid is more polar.

Post lab questions

1. *In a paper chromatography experiment, why is it necessary to apply the sample spots above the level of the solvent?*

   It is important to keep the sample spots above the solvent level because if the spots are submerged in the solvent, the spots would dissolve into the solvent preventing them from separating out and no measurements or observations could be made.

2. *Why should you use separate toothpicks for each sample? What would happen if you used the same toothpick?*

   Sample spots can be contaminated with organic acids present in other samples. This can lead to incorrect determinations for the compositions of the selected samples.

3. *Why is it necessary to open the stopcock periodically when shaking the separatory funnel?*

   Gases can build up when shaking the solutions and can cause the separatory funnel to gain too much pressure (and explode). Venting the gases through the stopcock relieves that built-up pressure.

Worksheet

1) *Butanoic acid, the substance responsible for the order of rancid butter, has pK_a = 4.82. What is its K_a?*

$$pK_a = -\log K_a,\ K_a = 10^{-pKa},\ K_a = 10^{-4.82},\ K_a = 1.5\times10^{-5}$$

2) *Formic acid, HCO_2H, has pK_a = 3.75, and picric acid, $C_6H_3N_3O_7$, has pK_a = 0.38.*

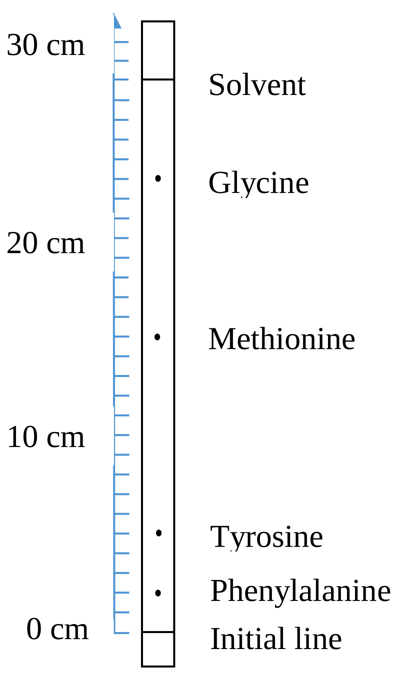
   a) *What is the K_a of each?*

   Formic acid $K_a = 1.8\times10^{-4}$

   Picric acid $K_a = 4.2\times10^{-1}$ or 0.42

   b) *Which is stronger, formic or picric acid?* Picric acid

3) *A sample containing a mixture of amino acids was separated using the ascending paper chromatography technique.*



The results of the experiment are shown below.

a) *Calculate the retention factor, for each of the amino acids in the mixture.*

Component	***Distance travelled***
Solvent	28 cm
Glycine	23 cm
Methionine	15 cm
Tyrosine	5 cm
Phenylalaninie	2 cm

Component	***Retention factor, R_f***
Glycine	0.82
Methionine	0.54
Tyrosine	0.18
Phenylalaninie	0.07

b) *Name the least and the most soluble components in the stationary phase*

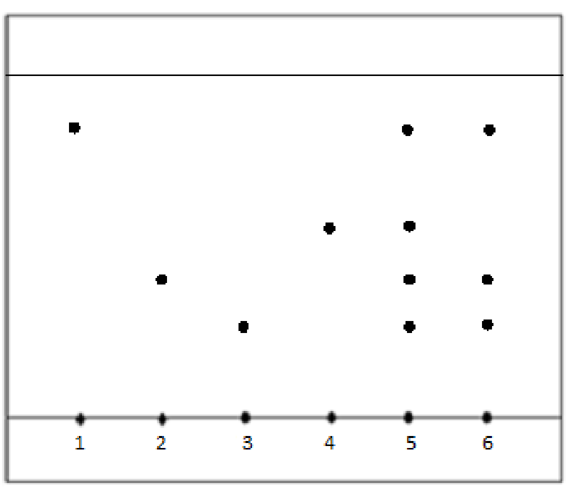
Least soluble: Glycine

Most soluble: Phenylalanine

c) *Based on the R_f information you determined, rank the above amino acids from most polar to least polar.*

Phenylalanine, Tyrosine, Methionine, Glycine

4) *An example paper chromatogram for the separation of color pigments from Red and Green leaves is given below.*



1. Carotene
2. Clorophyll a
3. Clorophyll b
4. Xanthophyll
5. Red leaves
6. Green leaves

What are the available color pigments in each leaf extract?

Red leaves: Carotene, Chlorophyll a, Chlorophyll b, Xanthophyll

Green leaves: Carotene, Chlorophyll a, Chlorophyll b

C.3 Note for instructors

This laboratory experiment is designed for the first year, survey organic chemistry students. During the pre-lab lecture, the importance of avoiding possible contamination, and spotting too much sample onto the stationary phase needs to be emphasized. This laboratory exercise is designed for groups of 2 or 3 students.

Several fruit juice or wine samples can be selected for separation. Below is a partial list of possible sample choices.

Table C.2 List of potential fruit juices to use as samples.

Sample	Brand tested	Organic acid components
Apple	Tropicana, Simply, Minute maid	Malic
Grape	Tropicana	Malic, Tartaric
Cranberry	Simply (Cranberry cocktail), Tropicana	Malic
Red grape fruit	Minute maid	Malic, Citric
Carbonated beverages	Ginger ale, Fanta	Citric

During the chromatography separation time, students may work on an additional worksheet (provided), which emphasizes basic chromatography theory and simple pH calculations. Students should receive instructions for chemical safety and hazardous material handling (refer to MSDS for each chemical). Appropriate protective gear of safety goggles and gloves should be worn when performing the experiment. A freshly prepared mobile phase solution is needed for experiments and works best if used within 24 hours. Additionally, when the mobile phase solution is made, proper care must be taken to extract only the organic phase from the aqueous phase as water can ruin the spots on the paper. All the liquid waste should be properly disposed of in a hazardous waste container labeled as hazardous organic waste.

If separatory funnels are not available for mobile phase preparation, the following alternate procedure can be used:

Alternate procedure for mobile phase preparation:

1. In a 100 mL beaker, dissolve 0.0375 g of bromocresol green in 7.5 mL of distilled water. Then add 25 mL distilled water and 25 mL of 1-butanol.
2. Using a micro pipette, add 2.6 mL of formic acid to the above solution.
3. Mix the reagents thoroughly with a glass stir rod. Transfer solution to a large test tube or narrow flask. Gently break any bubbles on top with the stir rod.
4. Allow the mixture to settle for 5 minutes to separate the phases. With a pipet, carefully remove the orange-colored organic phase and transfer it into a clean beaker for preparation of the mobile phase.

C.4 Notes for stockroom preparation

Timeline

These times are approximate but are intended to give instructors an estimate of how long each stage of the experiment takes for a typical student group of 2 or 3.

Activity	Time (min)
Overview of the experiment	15
Preparation of the mobile phase	20
Drawing pencil marks on the paper	5
Spotting the samples; put the chromatogram into the chamber	10
Developing the chromatogram (can do the worksheet during this time)	75
Drying the chromatogram (glassware cleaning during this time)	45
Total time	**2.50 hours**

Chemicals and other materials used in this experiment were purchased from the following chemical suppliers: Malic, Citric, Tartaric, and Lactic acids and Bromocresol green from VWR (Randor, PA), 1-butanol from Alfa Aesar (Ward Hill, MA), Formic acid and chromatography papers from Sigma-Aldrich (St. Louis, MO)

Chemicals	CAS No
Bromocresol green	76-60-8
Formic acid	64-18-6
1-butanol	71-36-3
Ethanol	64-17-5
Acetone	67-64-1
L- Malic acid	97-67-6
L- Lactic acid	79-33-4
Citric acid	77-92-9
Tartaric acid	87-69-4

Materials and supplies

Item	Per group	Comment
Chromatography paper	**1**	
Pencil	**1**	
Ruler	**1**	
Beakers (1000 mL, 100 mL, 50 mL)	**3**	1000 mL- as the chamber, 100 mL- to mix reagents, 50 mL- to collect the organic extract
Funnels (a small one and a large one)	**2**	Small one- to transfer the solution mixture into the separatory funnel, large one- to hold the chromatography paper during drying
Watch glasses (a small one and a large one)	**2**	Small one- to weigh bromocresol green, large one- to cover the chamber
25 mL graduated cylinder	**2**	To measure reagents

Common materials

Toothpicks

Micro pipette (1000 μL)

Stapler

Preparation of standard solutions

0.3% (w/v) solutions of citric, malic, tartaric, and lactic acid solutions

To prepare 100 mL portions of above 0.3% acid solutions, weigh 0.300 g of acids and transfer them into 100 mL volumetric flasks with small amounts of distilled water. Swirl flasks to dissolve solid. Add distilled water to make solutions up to 100 mL.

Preparation of the mobile phase solution

Dissolve 0.0750 g of bromocresol green in 15 mL of distilled water. Then add 50 mL distilled water, 50 mL of 1-butanol and 5.3 mL of formic acid. Transfer the mixture into a separatory funnel and mix the reagents thoroughly. Allow the mixture about 5 minutes to separate the phases.

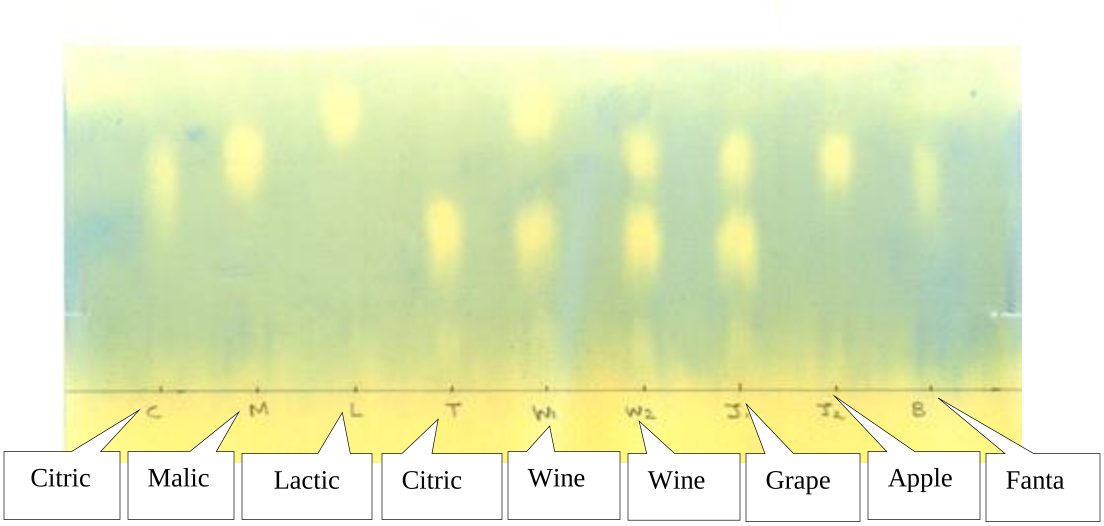
Discard the aqueous (lower) phase into a hazardous organic waste container. Collect the orange color organic phase into a clean reagent bottle. (You should extract about 60 mL of the solution).

Preparation of the stationary phase

Take a piece of chromatographic paper and trim the edge so that it does not exceed the height of the beaker used as a chromatography chamber. (For a 1000 mL tall beaker, 1 inch removed will be enough.)

Selection of wine and fruit juice samples

Any kind of red or white wine can be used for the separation, including cooking wines. With fruit juices, apple (contains malic acid), red grape (contains malic and tartaric acids), and cranberry (contains citric, malic and tartaric acids) work best for this separation. Other fruit juices or "carbonated fruit drinks" except orange and lemon can also be separated successfully. Orange and lemon juices containing higher amounts of citric acid should be avoided as they can produce stretched or overlapped spots with malic acid.



C.5 References

1. McMurry, J.; Simanek, E., Fundamentals of Organic Chemistry. Cengage Learning: 2007.

2. The Winemaking Home Page. http://winemaking.jackkeller.net/acid.asp. (accessed April 15, 2017)

APPENDIX D

DETERMINATION OF XYLITOL IN SUGAR FREE GUM BY GC-MS WITH DIRECT AQUEOUS INJECTION: A LABORATORY EXPERIMENT FOR CHEMISTRY STUDENTS

(Published in *J. Chem. Educ.* **2018**, 95, 11, 2017-2022)

D.1 Note for instructors

It is recommended that instructors provide detailed instructions about GC-MS for students, including the basic theory of chromatography. We also recommend providing information about mass fragmentation patterns when the mass detector is operated with electron impact mode. Students must also understand dilution calculations.

D.2 Safety and hazards

To minimize laboratory accidents, students should be instructed in chemical safety, personal protective equipment (PPE), and the proper handling of glassware and instruments prior to beginning of the experiment. Laboratory goggles, coats, and gloves must be worn at all times to prevent any accidental chemical exposure. Students should be instructed to remove gloves and wash their hands before they handle a gum stick for chewing. Always use fresh paper or the gum wrapper to weigh a gum stick on a food scale that has not been exposed to chemicals. Never use a gum stick for chewing if it was measured on the weighing pan without having a fresh filter paper or gum wrapper placed under it.

D.3 Materials and method

D.3.1 Reagents and materials

Xylitol (CAS-87-99-0, assay 99%, MW- 152.15 g mol^{-1}) and DL-threitol (CAS-7493-90-5, assay-97%, MW- 122.12 g mol^{-1}), were purchased from Sigma Aldrich. Xylitol containing Trident sugar free gum was purchased from Walmart. DI-water was used to prepare all samples and standard stock solutions.

D.3.2 GC-MS analysis

Agilent 7890A-5975C gas chromatograph with a mass detector (GC-MS) was used with a water resistant 60 m x 0.32 mm x 1 µm, 100 % dimethylpolysiloxane, Agilent *J&W DB-1* column. The GC oven was programmed to heat as follows; temperature at injection was 216 ºC, followed by heating from 216 to 230 ºC at 1 ºC min^{-1}, from 230 to 290 ºC at 30 ºC min^{-1}, and then holding at 290 ºC for 3 min. The total program time was 20 min. The carrier gas was He at a pressure of 60 kPa. Using a 10 µL syringe, 1 µL injections were done in split mode (30:1) at 280 ºC. The Agilent 5975C mass spectrometer was operated under scan mode with an electron impact ion source operated at 70 eV. The ion source temperature was 250 ºC and the interface temperature was 280 ºC. The analytes were characterized by full-scan acquisition from 35-350 atomic mass unit (amu). Library matching identified chromatographic peaks to the reference spectra (NISTT05a.L, Agilent Technologies, Inc.).

Instructor Note 1: Instructors can choose to run this lab on a GC and identify alcohol peaks via retention times if a Mass Spectrometer unit is not available. Retention times for alcohols under described ramping conditions are: glycerol (5 minutes), DL-threitol (6 minutes), xylitol (8 minutes), and sorbitol (12.5 minutes).

Instructor Note 2: This laboratory can be operated in two different ways: 1) For instrument intensive labs we will often have 3 experiments operating simultaneously. With this approach, each student gets hands-on experience with instrumentation. Using this approach, student groups are able to complete the experiment within the 3-hour period if they are provided calibration data. Larger sections of students, (such as organic or analytical students; 12 groups of 2) are able to prepare calibration standards, chew gum, extract the xylitol, concentrate the sample and then add in the internal standard within the 3-hour lab period. The TA then collects the samples for GC-MS analysis. This is best done with an auto-sampler and takes 1 hour per group to analyze plus at least 1 set of calibration standards. Resulting data, including integrated GC trace and xylitol fragmentation pattern, is then supplied to the students to complete their reports.

Instructor Note 3: Student instruction includes a brief tutorial on GC-MS operation. Compound identification is accomplished using retention time and MS fragmentation pattern. Quantification is performed using chromatogram peak areas. This can be accomplished using total ion counts or specific ion count depending on the GC detector. Our experiments utilized a mass spec detector. With this approach inspection of fragmentation analysis can confirm compound identity, however GCs with other detectors can also be used that rely primarily on retention times for compound identification.

Instructor Note 4: The instructors should be aware of a few precautions when using water in GC-MS. Additional student instruction may include a brief overview of backflash and other issues associated with using water as a solvent. Water is often considered to be a poor solvent in GC analysis for a variety of reasons including backflash and chemical reactivity however, steady advances in the field have provided solutions to most common issues.

Typical GC solvents such as hexane, ethyl acetate, acetone, and dichloromethane have vapor-to-liquid volume ratios between 100-300.[1] However, the water vapor-to-liquid volume ratio is 1000. Hence, injecting 1 µL of liquid water into the GC liner creates 1000 µL of water vapor.[1, 36] A typical volume of a liner is between 200-900 µL; solvent vapor that expands beyond the liner volume results in backflash, which can cause both sample and solvent to contaminate purge lines and the GC inlet. For best results with aqueous injections, small injection volumes and a suitable GC inlet should be used. For example, a laminar cup splitter is suitable for large volume injections of low volatile compounds. With a laminar cup inlet, liquid can trap at the liner base until vaporized ensuring complete vaporization. Maintaining a stable vacuum can also be a concern with water injections – therefore best results are obtained with high capacity pumps.[2]

Chemical damage to the stationary phase is another problem associated with water injection GC. However, it has been shown that immobilized and crosslinked non-polar liquid film columns are stable with water injections.[3] In order to avoid stationary phase degradation and enable high temperature analysis, a water resistant, 100 % dimethylpolysiloxane, Agilent *J&W DB-1*, low-bleed, cross-linked, and water rinsable column (or similar) is recommended for this experiment.[1, 36]

Instructor Note 5: The instructors should emphasize the importance of quantitative transfer. All flasks, tubes, mortars and pestle should be rinsed, and the washings pooled to ensure complete transfer of the compounds of interest. The three pooled extractions has been shown to remove approximately 99% of the xylitol.[4]

Table D.1 Required lab items

Items	Per group	Comment
10 mL volumetric flask	2	Pre-cleaned with DI water
25 mL volumetric flask	1	Pre-cleaned with DI water
50 mL volumetric flask	2	Pre-cleaned with DI water
100 mL volumetric flask	2	Pre-cleaned with DI water
10 mL graduate cylinder	1	To measure 10 mL of DI water
20.0 mL bulb pipette	2	To measure 20.0 mL from gum extract in part 03
5.0 mL bulb pipette	2	To measure 5.0 mL of internal standard
1.0 mL bulb pipette	1	To measure 1.0 mL of internal standard
15 mL centrifuge tubes	8	To centrifuge gum extractions
Centrifuge machine	1	
Mortar and pestle	1	To crush gum samples
10 µL or 5 µL syringe	1	To inject 1 µL to GC
3 mL bulb pipette	1	To measure 3.0 mL from gum extract in part 04
Food Scale		For weighing gum samples
Rotovap		For sample concentration

D.4 Preparation of standard solution for calibration

D.4.1 Preparation of 5.0 mg/mL xylitol stock solution

Dissolve 505 mg of xylitol with deionized water in a 100 mL volumetric flask to prepare 5.05 mg/mL xylitol stock solution.

D.4.2 Preparation of 5.0 mg/mL DL-Threitol (internal standard) stock solution

Dissolve 515 mg of DL-Threitol with deionized water in a 100 mL volumetric flask to prepare a 5.15 mg/mL DL-Threitol stock solution.

D.4.3 Preparation of standard solutions for calibration

Use the $C_1V_1 = C_2V_2$ formula to prepare standard solutions for the calibration curve. Prepare standard solutions in 50 mL volumetric flasks by measuring the required volume from the 5.0 mg/mL Xylitol stock solution and 5.0 mg/mL DL-Threitol stock solution. Use deionized water to top up to the mark on the flask. All calibration solutions are to be prepared by the teaching assistant or instructor.

Table D.2 Volumes needed to make stock solutions

Volume of 5.0 mg/mL DL Threitol (mL)	Volume of 5.0 mg/mL Xylitol (mL)	Final Volume (mL)	Concentration of Xylitol (mg mL^{-1})
5.0	7.0	50.0	0.7
5.0	10.0	50.0	1.0
5.0	13.0	50.0	1.3
5.0	16.0	50.0	1.6
5.0	20.0	50.0	2.0

For 3 h experiments, students are provided calibration data.

D.5 Lab manual

D.5.1 Introduction

Xylitol is a sugar alcohol, commonly used as an artificial sweetener or sugar substitute in many “reduced-calorie” foods (Figure D.1). Not only do sugar alcohols provide a sweet taste, they also influence product texture, preservation, moisture maintenance, and the cooling sensations experienced in the mouth upon consumption.[5] Consumers respond to sugar-free gums because of perceived reduction in energy intake resulting in weight loss.[6,7] Xylitol is good for diabetics because it stimulates much less insulin release than a comparable quantity of table sugar.[5]

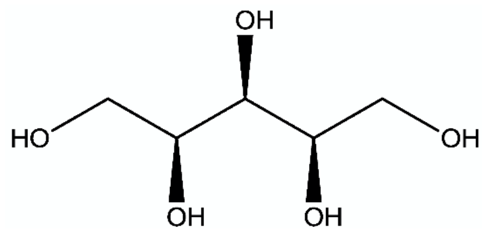


Figure D.1 Xylitol

Xylitol is extensively utilized in chewing gum because it helps prevent dental caries.[8–12] Although xylitol consumption has proven beneficial to humans, it is toxic to dogs. Xylitol ingestion by dogs causes vomiting, ataxia, seizures, hypoglycemia, and hepatotoxicity in the animal.[13–18] Ingestion of xylitol containing products such as chewing gum can result in xylitol poisoning for dogs if enough product is consumed (Table D.3).[14,19,20] Xylitol's presence in chewing gum and other consumer products makes it readily available to dogs with consequent detrimental effects. In this laboratory experiment, students will determine the amount of xylitol in fresh and chewed gum samples in order to analyze how much gum is toxic to a dog.

Table D.3 Comparison of xylitol amounts from chewing gum that would cause Hypoglycemia in dogs

		Amount of Xylitol That Would Cause Hypoglycemia in Dogs, by Sample Breeds			
Dog Breed	Typical Dog Size, kg	Dose, 0.1 g of Xylitol/ kg of Dog, g	Required Pieces of Fresh Chewing Gum		
			Ice Breakers: 1.5 g of Xylitol/Piece	Stride: 0.2 g of Xylitol/Piece	Trident: 0.2 g of Xylitol/Piece
Chihuahua	2	0.2	1	1	1
Yorkie	4	0.4	1	2	2
Jack Russell Terrier	6	0.6	1	3	3
Border Collie	12	1.2	1	6	6
Golden Retriever	25	2.5	2	12	12

PRE-LAB QUESTIONS

1. Name four different polyols used in a sugar free gum and draw their chemical structures.
2. List the boiling points of the polyols you listed above.

D.5.2 Procedure

Extraction of xylitol from a gum stick chewed for 5 minutes (Part 1)

1. Weigh a stick of gum (Trident spearmint) accurately. (Be sure that the scale has been rigorously cleaned. Do not use a chemical balance to measure xylitol gum sticks). Do not put the gum on the weighing pan without using clean filter paper or gum wrapping. Do not let the gum touch any chemical. (You are going to chew it).
2. Leave the lab space and chew the gum for 5 min.
3. Crush the chewed gum using a mortar and pestle with 10 mL of deionized water for 5 min to extract xylitol and transfer the extraction into a 15 mL centrifuge tube. Repeat this step (10 mL x 3 for a total of 30 mL) and pool the extractions. Wash the mortar and pestle using 5 mL of deionized water and add to the centrifuge tube. Split the extractions into multiple centrifuge tubes as needed. Centrifuge extractions for 5 min (3400 RPM) to remove particulates.
4. After centrifugation, carefully decant the extraction into a 100 mL evaporation flask. Wash the tube with 5 mL of deionized water and add to the flask.
5. Use a rotary evaporator (rotovap) to remove water under reduced pressure and to concentrate the xylitol extraction approximately to 5 mL or to dryness. (You can leave the flask on the rotovap because it takes 15-20 min to evaporate water and start part 2).
6. After evaporating water in step 5, transfer the extraction to a 10 mL volumetric flask and add 1.0 mL from the 5.0 mg/mL DL-Threitol standard solution into the same volumetric flask. (If

your rotovap flask does not have any liquid, add 5 mL of DI water to dissolve the residue and then transfer to a 10 mL volumetric flask). Top up to the mark on the volumetric flask with deionized water and analyze by GC-MS. Use 10 µL syringe to inject 1 µL of your solution to the GC-MS.

Extraction of xylitol from a fresh gum stick (Part 2)

1. Weigh gum sticks accurately (Trident spearmint).
2. Crush gum piece using mortar and pestle with 10 mL of deionized water for 5 min to extract xylitol and transfer the extraction into a 15 mL centrifuge tube. Repeat this step (10 mL x 3 for a total of 30 mL) and pool the extractions. Wash the mortar and pestle using 5 mL of deionized water and add to the centrifuge tube. Split the extractions into multiple centrifuge tubes as needed. Centrifuge extraction for 5 min.
3. After centrifugation, carefully decant the extract to a 100 mL volumetric flask. Wash the tube using 5 mL of deionized water and add to the flask. Top up to the mark of the flask with DI-water.
4. Measure 20.0 mL from the gum extract you prepared in step 3, into a 50 mL volumetric flask. Measure 5.0 mL from the 5.0 mg/mL D,L-Threitol stock solution into the same 50 mL flask and top up to the mark using deionized water. Analyze samples by GC-MS. Use a 10 µL syringe to inject 1 µL of your solution into the GC-MS.
5. While the GC is running, repeat these steps with another gum flavor or another brand.

   Note: Check your rotovap flask part 1, step 5. If the liquid is less than 5.0 mL, go to part 1 and finish step 6 of part 1. If the rotovap is not done, start part 3.

Extraction of xylitol from a gum stick chewed for 1 minute (Part 3)

1. Weigh a gum stick accurately (Trident spearmint). (Be sure that the scale has been rigorously cleaned. Do not put the gum on the weighing pan without using clean filter paper or gum wrapping. Do not let the gum touch any chemical because you are going to chew it).
2. Leave the lab space and chew the gum piece for 1 min.
3. Crush the chewed gum piece using mortar and pestle with 10 mL of deionized water for 5 min to extract xylitol and transfer the extraction into centrifuge tube. Repeat this step (10 mL x 3 for a total of 30 mL) and pool the extractions. Wash the mortar and pestle using 5 mL of deionized water and add to the centrifuge tube. Split the extractions into multiple centrifuge tubes as needed. Centrifuge extractions for 5 min.
4. After centrifugation, carefully decant the extraction to a 100 mL volumetric flask, then wash the tube using 5 mL of deionized water and add to the flask. Measure 10.0 mL from the 5.0 mg/mL DL-Threitol stock solution into the same flask and top up to the mark of the flask with DI-water. Analyze the sample by GC-MS. Use a 10 µL syringe to inject 1 µL of your solution into the GC-MS.

POST LAB QUESTIONS AND CALCULATIONS:

You must show all your calculation for full credit.

01. Calculate the concentration of the internal standard in,

    a. The standard solution prepared for calibration. Note: 5.0 mL of 5.0 mg mL-1 DL-Threitol solution was added to the 50 mL volumetric flask during the calibration.
    b. The extraction prepared for the analysis in part 1.
    c. The extraction prepared for the analysis in part 2.

d. The extraction prepared for the analysis in part 3.

02. Plot two calibration curves based on provided GC-MS data:

   a. X-axis concentration of xylitol and Y-axis peak area of the xylitol.

   b. X-axis concentration of xylitol and Y-axis the ratio of peak area (PA) of xylitol and peak area (PA) of internal standard (PA-xylitol/PA-internal standard).

   c. Based on your data, discuss the importance of the internal standard in GC analysis and explain which calibration curve you will be using for calculations (Hint: linear regression).

03. Calculate xylitol concentration in two different types of flavors or two types of fresh gum samples. Which one is more toxic to a dog?

04. Calculate the percentage of xylitol remaining in a gum stick chewed for 1 min.

05. Calculate the percentage of xylitol remaining in a gum stick chewed for 5 min.

06. If the level of xylitol which causes hypoglycemia in dogs is 0.1 g xylitol per kilogram of dog, fill in the blanks of the table below.

Comment on the fragmentation pattern for xylitol in your mass spectrum. Did you get a parent peak? Can you identify a fragment?

Size of dog	Quantity of xylitol to cause illness/mg (dosage 0.1g/kg)	# of fresh gum sticks to cause illness	# of 1 min. chewed gum sticks to cause illness	# of 5 min. chewed gum sticks to cause illness
2 kg (Chihuahua)				
4 kg (Yorkie)				
6 kg (Jack Russell Terrier)				
12 kg (Border Collie)				
25 kg (Golden Retriever)				

D.6 Instructor answer key

Question No 1.

Calculating concentration of internal standard

To calculate concentration of internal standard and solution prepared for calibration, use equation 1.

$C_1V_1 = C_2V_2$ Eq.-1

Where; C_1= concentration stock solution, V_1= volume measured from the stock solution, C_2= concentration final solution, V_2= final volume of solution.

Example calculation for internal standard in calibration solutions using equation 1, where, C_1= 5.0 mg/mL V_1=5.0 mL C_2=? V_2=50 mL.

$C_1V_1 = C_2V_2$; $5.0 \text{ mg mL}^{-1} \times 5.0 \text{ mL} = C_2 \times 50.0 \text{ mL} \rightarrow C_2 = \mathbf{0.5} \text{ mg mL}^{-1}$

Calculating concentration of internal standard in solution prepared in part 1;

Using equation 1 for the calculation.

$C_1 = 5.0 \text{ mg mL}^{-1}, V_1 = 1.0 \text{ mL, and } V_2 = 10.0 \text{ mL}$

$C_1V_1 = C_2V_2$; $5.0 \text{ mg mL}^{-1} \times 1.0 \text{ mL} = C_2 \times 10.0 \text{ mL} \rightarrow C_2 = \mathbf{0.5} \text{ mg mL}^{-1}$

Calculating concentration of internal standard in solution prepared in part 2;

Using equation 1 for the calculation.

$C_1 = 5.0 \text{ mg mL}^{-1}, V_1 = 5.0 \text{ mL, and } V_2 = 50.0 \text{ mL}$

$C_1V_1 = C_2V_2$; $5.0 \text{ mg mL}^{-1} \times 5.0 \text{ mL} = C_2 \times 50.0 \text{ mL} \rightarrow C_2 = \mathbf{0.5} \text{ mg mL}^{-1}$

Calculating concentration of internal standard in solution prepared in part 3;

Using equation 1 for the calculation.

Where; C_1= concentration stock solution, V_1= volume measure from the stock solution, C_2= concentration final solution, V_2= final volume of solution.

$C_1 = 5.0 \text{ mg mL}^{-1}, V_1 10.0 \text{ mL, and } V_2 = 100.0 \text{ mL}$

$C_1V_1 = C_2V_2$; $5.0 \text{ mg mL}^{-1} \times 10.0 \text{ mL} = C_2 \times 100.0 \text{ mL} \rightarrow C_2 = \mathbf{0.5} \text{ mg mL}^{-1}$

Question No 2.

Choosing a correct internal standard (IS) can improve a method's accuracy and precision. Method development for GC-MS often utilizes an internal standard to account for routine variation of the instrument response and injection volumes. An internal standard should be chemically similar to the analyte, but it should not be naturally present in any of the samples analyzed.

Calibration methods can improve the accuracy and precision of GC-MS results. External standard calibration is commonly used to establish a linear relationship between signal magnitude and sample concentration. However, this method does not account for sample matrix chemicals, inconsistent injection volumes or instrument drift. An internal standard calibration method can be used to reduce these potential sources of error. When using an internal standard, a known substance is added to both gum samples and calibration standards, and a calibration curve is produced by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration. In this experiment, data from the standard xylitol samples is provided to the students to generate two calibration curves. One graph is produced according to the external calibration method and another created using the internal standard method. Students are tasked to compare the square of the correlation coefficient (R^2) for each method in order to determine the best calibration curve to analyze xylitol in the gum samples. A near perfect linear calibration curve is often obtained using the internal standard method (R^2=0.9992) (Figure D.2). Conversely, poor linearity (Figure D.3) is often observed with the external standard calibration (R^2=0.9808). Upon quantification of xylitol in samples, students calculate the xylitol concentration that causes hypoglycemia in dogs, with emphasis on determining the quantity of gum stick that would cause toxicity for dogs of varying weights.

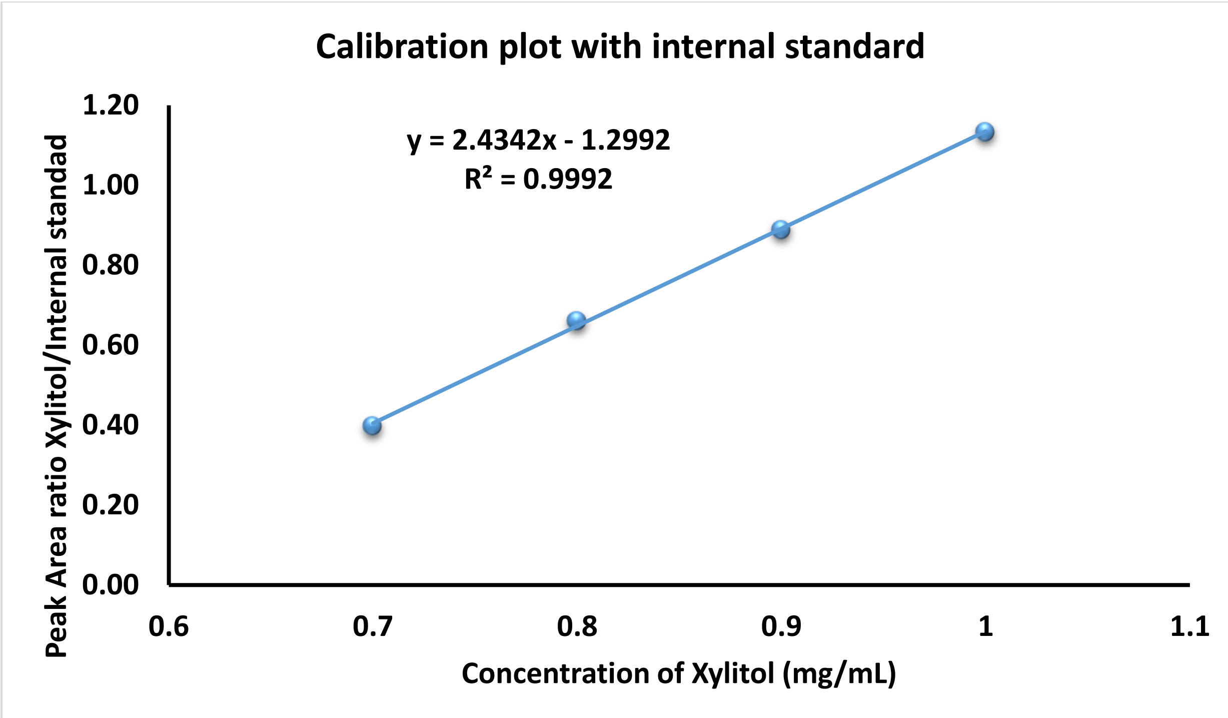


Figure D.2 Calibration plot with internal standard.

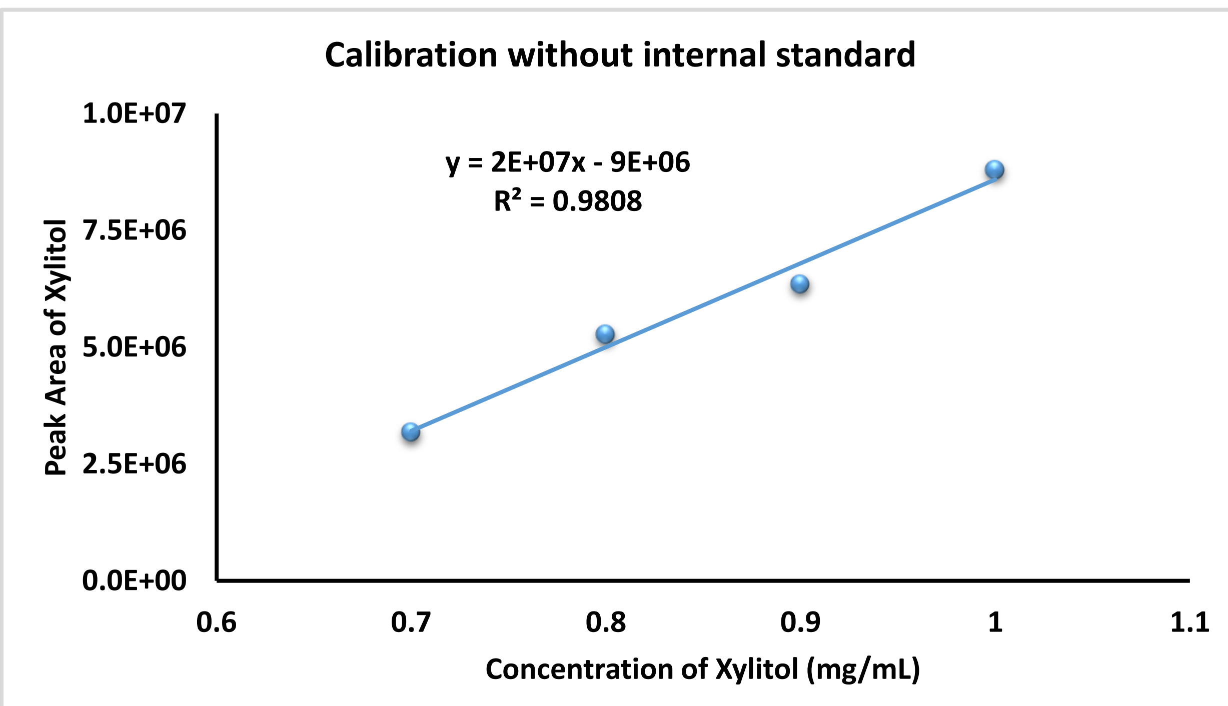


Figure D.3 Calibration plot without internal standard.

Question No 3.

Use linear regression equation for the internal standard calibration curve,

Where, the linear regression equation from 0.7-1.0 mg mL^{-1} was found to be

y = 2.4342x – 1.2992 with (R^2 = 0.9992)

$y = \frac{Peak\ area\ of\ Xylitol}{Peak\ area\ of\ Internal\ Standard}$

If y = 1 for the ratio of xylitol to internal standard, then

1 = 2.4342x – 1.2992

x = 1+1.2992/2.4342

x = 0.94 mg mL^{-1}

Concentration of the solution analyzed was 0.94 mg mL^{-1} for a fresh gum sample.

If the concentration of the final solution was 0.94 mg mL^{-1}, then concentration of original 100 mL solution was 2.4 mg mL^{-1}. Total mg of xylitol in sample was 240 mg.

$$Concentration\ of\ the\ fresh\ gum\ sample\ 100\ mL = \frac{0.94\ mg/mL \times 50\ mL}{20\ mL}$$

$Concentration\ of\ the\ fresh\ gum\ sample\ 100\ mL =$ 2.4 mg mL^{-1}

$Amount\ of\ Xylitol\ in\ a\ gum\ sample = 2.4\ mg/mL \times 100\ mL$ = 240 mg per fresh gum sample.

This can also be represented as 0.24 g per fresh gum sample to allow for comparison in the dog weight table. With this data, 1 stick of unchewed gum can cause toxicity in a 2 kg Chihuahua.

Interpretation of MS data

A GC coupled to a mass analyzer operating under **electron impact** (EI) mode, produces a fragmentation pattern that plays a key role in compound identification. Glycerol, threitol, xylitol, and sorbitol are members of a series of compounds in which any two members in a sequence differ by one carbon atom, two hydrogen atoms, and one oxygen atom (CH–OH unit) (Figure 5.3). Because of these similarities, they have similar fragmentation patterns.[21] Glycerol has a base peak of *m/z* 61 resulting from the loss of CH and H_2O (Figure D.4). Loss of hydrogen atoms from hydroxyl groups, loss of multiple H_2O molecules, and multiple C–C bond cleavages result in peaks at *m/z* 61, 91, 103, and 117 common for threitol, xylitol, and sorbitol, while peaks *m/z* 129 and 147 are common for both xylitol and sorbitol (Figures D.5-D.7).[22] The molecular ion peaks of these polyols are extremely weak or not visible. Cleavage of a C–C bond, rearrangement processes associated with hydrogen, formaldehyde, ethylene, or water elimination are common fragmentation pathways. The detachment of the hydrogen atom from the molecular ion with water molecule formation occurs via a four-member transition state.[22] In addition, hydrogen atom, formaldehyde, and hydroxyl groups can be captured at different positions.[23] Ethylene and water molecules can also be eliminated. This lab was written for a GC-MS; however, many different quantifying detectors could be used where identification is done using retention time alone.

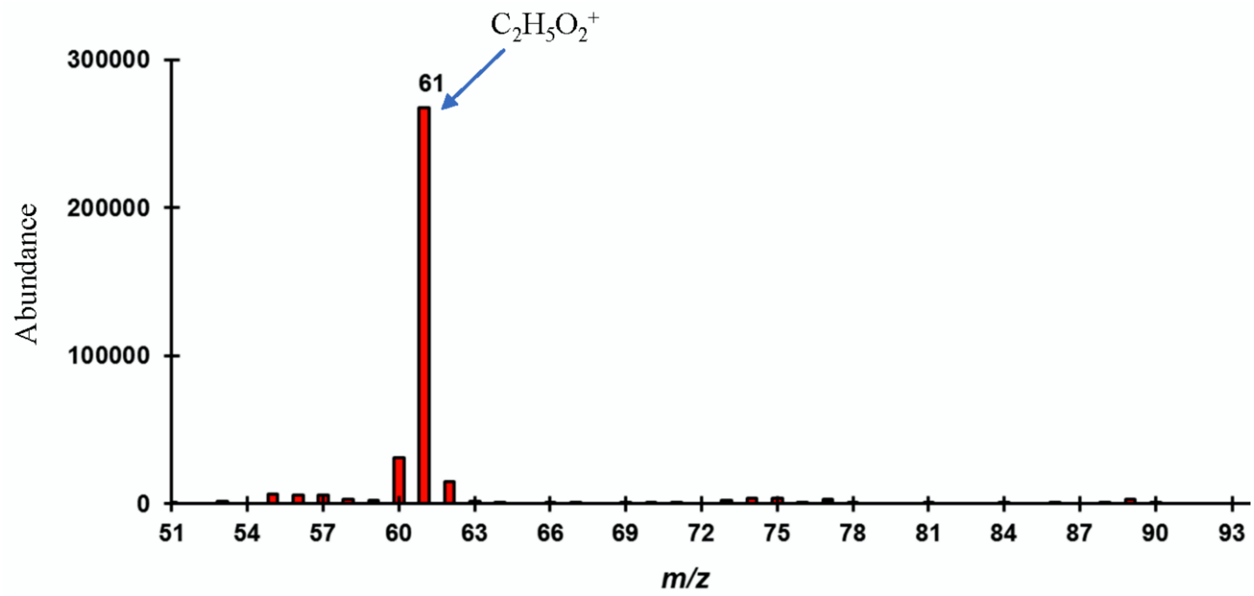


Figure D.4 Low resolution mass spectrum collected from GC-MS for peak at 5 min, glycerol.

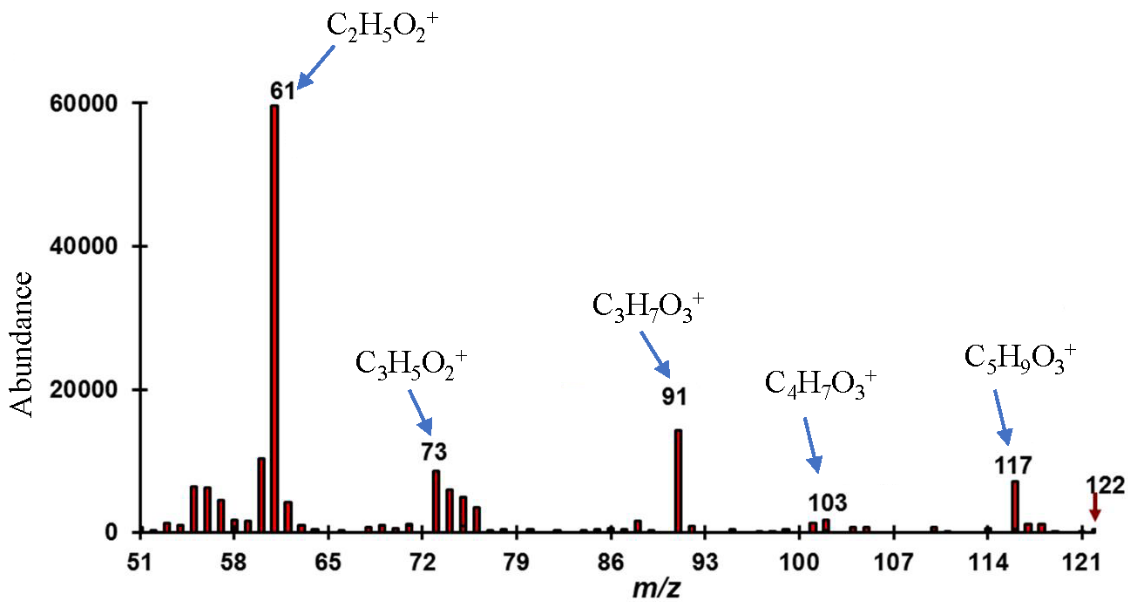


Figure D.5 Low resolution mass spectrum collected from GC-MS for peak at 6 min, DL-threitol.

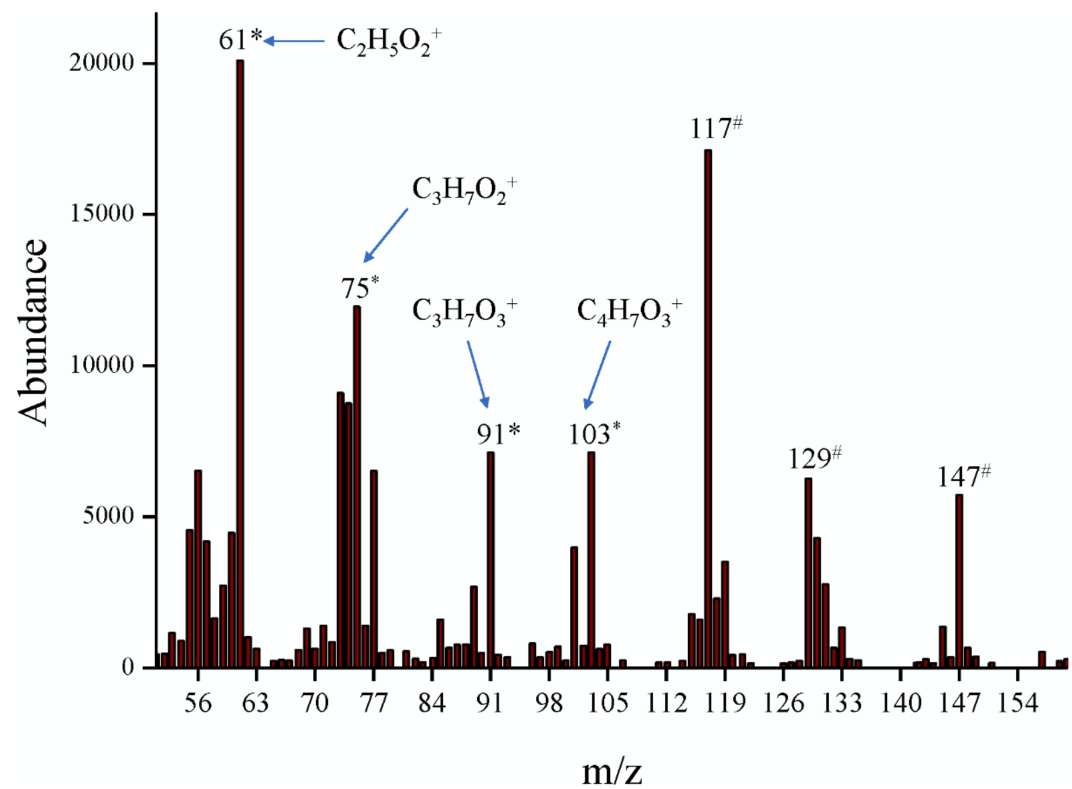


Figure D.6 Low resolution mass spectrum collected from GC-MS for peak at 8 min, xylitol. *Ions with m/z found in xylitol NIST library mass spectrum.[24] #Ions with m/z found in xylitol mass spectrum when silanes are present.[21-23]

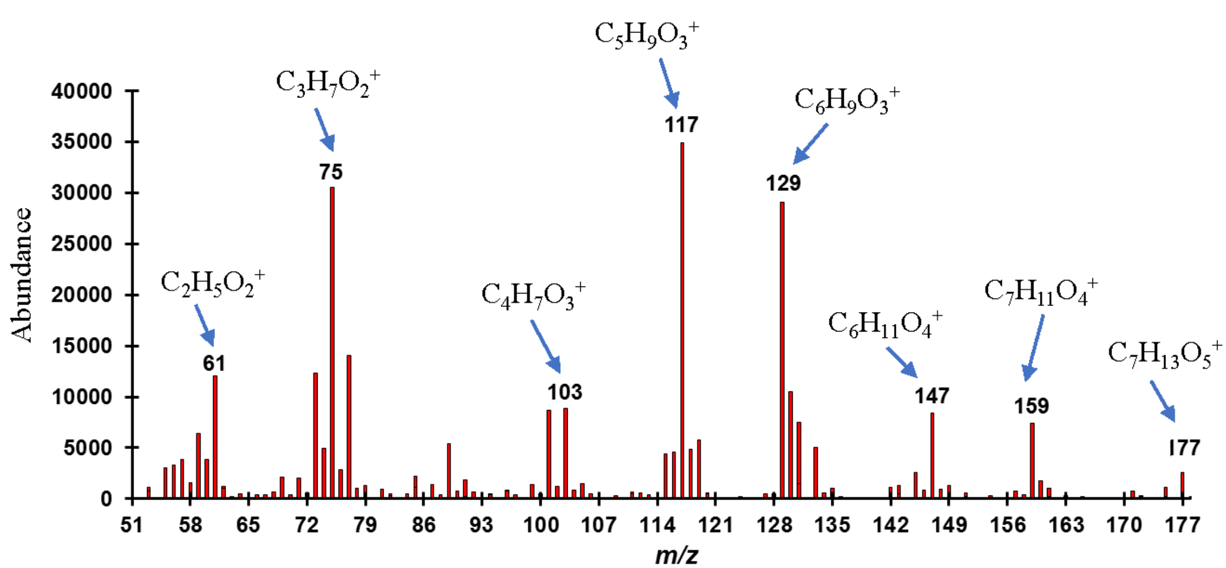


Figure D.7 Low resolution mass spectrum collected from GC-MS for peak at 12.5 min, sorbitol.

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