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### Revision of Introductory Chemistry Lab Curriculum to Incorporate Inquiry-Based Experiments to Enhance Student Learning

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Revision of Introductory Chemistry Lab Curriculum to Incorporate Inquiry-Based Experiments  
to Enhance Student Learning

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Submitted in Partial Completion of the  
Requirements for Commonwealth Honors in Chemical Sciences

Bridgewater State University

May 15, 2020

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## **ABSTRACT**

The Chemistry Department at BSU is restructuring its curriculum to integrate and scaffold research into the four-year curriculum as part of a CUR-NSF grant. The overall goal of this Honors Thesis was to contribute to the Introductory Chemistry laboratory (CHEM 141L) curriculum development by selecting experiments that move away from traditional “cookbook” laboratory experiments to inquiry-based labs to better engage students and maximize their learning. Three skills identified by chemistry faculty as essential for the restructured CHEM 141L are: preparation of solutions; use of standard curves in quantitative analysis; and graphical analysis of data using Excel. The specific goals of this honors thesis were: (1) to select inquiry-based lab experiments (IBLs) that address two to three of those essential skills; (2) test, modify and refine the experiments to ensure that their level is appropriate for introductory chemistry lab and based on resources available in the department; and (3) write a CHEM141 laboratory handout for one to two IBLs that include background information, prelab questions to guide students in their inquiry, experimental procedure, and post-lab questions to ensure students understand the main purpose of the experiment. These goals were accomplished by searching for Journal of Chemical Education articles for IBLs that address 2-3 of the aforementioned skills and narrowing down the selection from an initial pool of 17 to three. Each of the selected IBLs were tested by following the experimental procedure, then the procedure was modified and finalized to ensure that they are level-appropriate for CHEM 141 laboratory curriculum. Of the three IBLs, the “Determination of Percent Cranberry in Cranberry-Apple Juice Blends” was selected for adoption because it was the most cost effective and the safest to perform. Finally, a CHEM 141 lab handout was written for this selected IBL.

## **INTRODUCTION**

While reading the literature on chemistry laboratory instructions, it occurred to me that many academic institutions have developed, or are still developing, laboratory curriculum geared towards improving student learning. More than fifteen years ago, Hofstein and Lunetta (2004) stressed that “cookbook” style laboratory instruction, where students follow stepwise directions without thinking of the greater purpose of the experiment, was still a major hindrance to learning. What appears to be a common goal behind reforming laboratory curricula is to provide students a venue to appreciate chemistry as an investigational science rather than something that is hypothetical (Hofstein and Lunetta, 2004). In other words, the primary focus behind reformed laboratory curricula should be teaching students how to do science. *But how exactly is science practiced?* The Framework for K12 Science Education (NRC 2011) established eight scientific practices that extends to college education: (1) asking questions; (2) developing and using models; (3) planning investigations; (4) analyzing and interpreting data; (5) using mathematics and computational thinking; (6) constructing explanations; (7) engaging in argumentation from evidence; and (8) obtaining, evaluating, and communicating information. This set of practices is very similar to what we know as the scientific method followed by scientists when conducting their own research investigation (NRC, 2011). Knowing the merits of incorporating research into the curriculum, the Department of Chemical Sciences at BSU is currently engaged in curriculum revisions to integrate and scaffold research into the four-year curriculum as part of a four year CUR-NSF grant. Most of the restructuring will take place in the laboratory curriculum, especially at the introductory levels. Currently, only a handful of upper level chemistry labs are research-based. This semester, the department is pilot testing a research-based sophomore-level organic chemistry lab (CHEM 244L). Introductory chemistry labs, CHEM 141L and CHEM

142L, are in dire need of a restructuring because they utilize decade-old cookbook style lab experiments that, as stated above, do not effectively engage students in the learning process.

The current CHEM141L curriculum consists of 10 to 11 weekly experiments that students perform, typically in groups of two, in one semester. The handout provided per experiment contains a very brief background information to describe what the experiment is about. The goals of the experiment are either implied in the background information or, in many cases, not provided at all. The background is followed by a detailed, step-by-step procedure on how to execute the experiment and collect and treat data, often accompanied by appropriate tables and boxes to organize collected information. Figure 1 shows sections of a current CHEM 141 lab experiment titled “Determination of an Empirical Formula”. This experiment is typically performed during the tenth week of classes with expectations that students’ math skills are better towards the end of the semester. Although the background of the experiment defines terms related to the activity, the goals are not provided and students are not clear on the purpose of experimentation. After the experiment, students are asked to answer post-lab questions designed to assess learning. A majority of CHEM 141L experiments do not provide any excitement because there is no real-world significance to the students. Additionally, the lab handout is extremely detailed to a fault, with the stepwise calculations for analyzing results practically “spoon-fed” to the experimenter. This tends to be the case when the mathematical skills that are required to complete a lab may not have been covered in the lecture, adding to the frustration level among students who perceive the meaning behind the lab as too theoretical.

Although cookbook labs are beneficial because they can be done by a large group of students in a cost-effective manner and with little time investment by the instructor (Domin, 1999), a few studies show that they do not contribute to the development of student’s critical



Since CHEM 141 is the first of a two-semester introductory chemistry sequence for many STEM majors, this honors thesis was set up to help the department restructure this course. For learning to be more meaningful to students, CHEM141L must move away from cookbook labs to more engaging instruction styles such as inquiry-based labs (IBLs) that teach students how scientific discovery actually happens. In other words, IBLs teach students how to do chemistry. In Fall 2019, the Chemistry-CUR curriculum team proposed a restructured CHEM 141 laboratory curriculum that focuses on teaching students “how to do chemistry” (CHEM 141L/142L CUR Committee, 2019). The specific learning goals and course learning outcomes of the restructured CHEM 141 laboratory are detailed in Figure 2.

CHEM 141 Lab Learning Goals	Course Learning Outcomes
The primary objectives of CHEM 141 laboratory will be to provide the context of how to do chemistry. Specific goals include: 1) developing laboratory skills; 2) developing fundamental mathematical skills; 3) learning basic laboratory techniques, safe laboratory practices, and record-keeping; 4) responsible use and treatment of data	1) Students will learn the following laboratory skills:
	a) measure physical properties using appropriate equipment
	b) follow written procedures
	c) analyze and treat data using spreadsheets and graphing software
	d) prepare solutions
	2) Students will learn the following laboratory techniques:
	a) chemical separations
	b) aqueous solution reactions
	c) volumetric analysis
	d) spectroscopy
	3) Students will perform accurate and complete record keeping:
	a) collect and accurately record data in a lab notebook
	b) treat data in a responsible manner
	4) Students will be able to comply with laboratory safety regulations:
	a) carry out responsible disposal techniques
	b) properly use personal protective equipment to minimize exposure to hazards
c) understand the categories of chemical hazards (physical, health, environmental)	

Figure 2: Learning goals and learning outcomes of the proposed CHEM 141 laboratory curriculum. Highlighted in blue are the goals and outcomes related to this thesis project. (CHEM 141L/142L CUR Committee, 2019)

The text highlighted in blue (Figure 2) are the focus of this honors thesis, which is addressed in more details in the next paragraph. As described earlier, laboratory curriculum reforms for introductory chemistry focus on engaging students in the practice of science. Of the various laboratory instruction styles that help students do science, an inquiry-based laboratory (IBL) instruction style is one of the most commonly mentioned approaches for introductory chemistry labs. IBLs have been reported to increase students' confidence (Tomasik et. al, 2013), critical thinking (Domin, 1999) and problem-solving skills (Mandler et. al, 2014; Goeden et. al, 2015; Flynn et. al, 2012), and improve attitudes towards chemistry (Tomasik et. al, 2013; Kerr et. al, 2012). There are different levels of IBLs that range from structured to authentic inquiry (Bruck et. al, 2008), and the main difference they have is on what type of information is given to the students. Essentially, the higher the level of inquiry (e.g. authentic), the less information is given to the students (Bruck et. al, 2008). Higher levels of inquiry require that students experience more scientific practices such as asking questions, planning and executing an experiment, and forming a conclusion (NRC, 2011).

For CHEM141L, structured inquiry-based lab, the level above “cookbook” lab, is best suited for the type of students. To many CHEM 141L students, this course provides their first lab experience. Many did not take high school chemistry, and if they did, there was very little lab instruction due to limited resources and lack of room in the curriculum. In structured IBLs, the research question, experimental procedure, and methods of analyzing results are provided (Bruck et. al, 2008). However, students are encouraged to decide on the best approach to report their results, and they must form their own conclusions, thereby experiencing some of the practices of science (Bruck et. al, 2008). Finally, structured IBL experiments can be carefully selected so that



CHEM141L students will be able to use real-world samples, allowing them to relate to the activity from their own daily experiences. Figure 3 shows the experimental procedure for a structured IBL titled “How Much Cranberry is in Cranberry-Apple Juice?”. This IBL focuses on determining the percentage of cranberry juice present in cranberry-apple juice blend. Note that the procedure shown in Figure 3 does not explicitly state what the expected results are, providing an opportunity for students to engage with the material they are presented, which could aid in the development of critical thinking and problem-solving skills.

**Calibration Curve of Dilutions of 100% Cranberry Juice**

The calibration curve of 100% cranberry juice will be used to help determine the concentration of cranberry in cranberry-apple juice (Cranberry Delight). The data collected from the dilutions of 100% cranberry juice (All Cranberry) will be used to generate the calibration curve. 100% cranberry juice will be diluted to the following five percentages: 4%, 8%, 12%, 16%, and 20%. The absorbance of these five concentrations will be measured at the  $\lambda_{\max}$  section A.7.

- 1. Dilutions of 100% Cranberry Standard Solution.** Obtain a pipet, five 100-mL volumetric flasks, and five clean cuvetts. Use the pipet to measure 4mL of 100% cranberry juice into a 100mL volumetric flask. Fill up the volumetric flask with deionized water to the 100ml mark. Cap and shake the flask to ensure that the cranberry and deionized water have mixed thoroughly. Label the volumetric flask with its corresponding concentration. Repeat this procedure to make up the 8%, 12%, 16%, and 20% dilutions of 100% cranberry juice. Fill each cuvet with each of the diluted concentrations of 100% cranberry juice.
- 2. Measuring the Absorbance of the Diluted Concentrations of 100% Cranberry Juice.** Measure each cuvet at the  $\lambda_{\max}$  determined in section A.7. Recalibrate the spectrophotometer each time a new diluted concentration of cranberry juice is to be measured. Read and record the absorbance values.
- 3. Graph the Data.** Plot an absorbance vs. concentration graph in Excel to turn in with your laboratory report. Use the data collected in section B.2. Also use the concentration and absorbance data from section A.7 (the 10% dilution of 100% cranberry juice), at your identified  $\lambda_{\max}$ . This means that your graph will have six points: five points from section B.2, and one point from section A.7. In Excel, obtain the equation of the line. The line may not go through zero, which is acceptable; this may happen due to random error effects being noticeable since there are just a few samples being used for the calibration curve, but with a greater number of samples, the calibration curve should go through zero. The graph generated is the calibration curve.

Figure 3. Experimental section of a structured IBL “How Much Cranberry Juice Is In Cranberry-Apple Juice? (Edionwe et. al, 2011).

Implementing structured IBLs in CHEM141L will hopefully help BSU students learn how to do chemistry, which is the primary objective of the restructured CHEM141 lab. Conversations among faculty and upper-level chemistry majors who experienced CHEM 141/142 labs identified three skills that will be crucial in teaching students how to do chemistry: (1) preparation of solutions; (2) use of standard curves in quantitative analysis; and (3) graphical analysis of data in Excel. These three skills all require good foundation in math. Thus, my thesis focused on development of interrelated, multi-week IBLs geared towards teaching those essential skills. These IBLs would be scheduled in the later half of the semester to ensure that students have learned the appropriate math skills in the lecture during the first half of the semester. The specific aims of this honors thesis were to: (1) select inquiry-based lab experiments from the Journal of Chemical Education that teach at least two of three aforementioned skills; (2) test, modify and refine the experiments to ensure that their level is appropriate for introductory chemistry lab and based on resources available in the department; and (3) write a CHEM141 laboratory handout for each inquiry-based lab that includes background information, a set of prelab questions to guide students in their inquiry, experimental procedure, and post-lab questions so students can reflect on what they learned.

## **MATERIALS AND METHODS**

### *A. Literature search and selection of viable IBLs*

The first step into this research involved literature search for IBLs that address three essential skills described in the Introduction: preparation of solutions; use of standard curves in quantitative analysis; and graphical analysis of data using Excel. The searches were limited to

the Journal of Chemical Education (JCE) to ensure that results will be specific to the undergraduate chemistry curricula. Seventeen JCE scientific articles were chosen for the initial pool of viable IBLs as depicted in Figure 4, which were narrowed down to three articles based on the following criteria: (1) level appropriateness for introductory chemistry lab; (2) safety based on type of chemicals used, and hazard and volume of waste generated; (3) the cost needed to perform the experiments, and (4) availability of materials/instrumentation at BSU's Chemistry department.

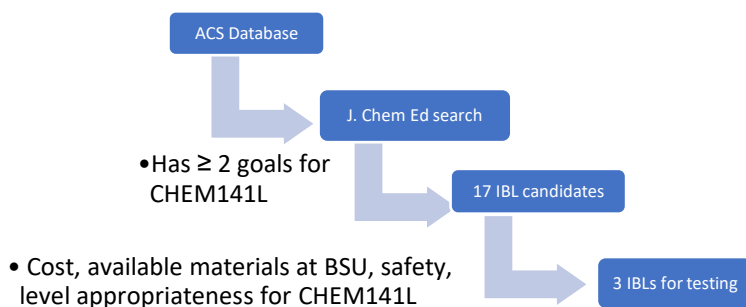


Figure 4. Summary of methodology for selection of CHEM141L IBLs.

## B. Testing of selected IBLs

### 1. IBL 1: Determination of Percent Cranberry in Cranberry-Apple Juice Blends

#### i. Reagents

There were no reagents for this experiment.

#### ii. Selection of cranberry juice for use in the preparation of calibration standards

Ocean Spray Cranberry Juice, Langers Cranberry Juice, Langers Cranberry-Apple Juice, Light Ocean Spray Cran-Grape Juice, and Northland Cranberry blend were all purchased from

local supermarkets for this experiment. Unfortunately, during the time of purchase, Ocean Spray Cranberry-Apple juice blend was out of stock, which limits the sample used for the analysis of cranberry juice content to Langers Cranberry-Apple Juice.

The selection of calibration standards was between the Ocean Spray Cranberry Juice Cocktail and Langers Cranberry Juice samples; the other samples' absorbances of 520 nm light, the wavelength of light absorbed by natural and artificial red dyes, were used for comparison with the two cranberry juice samples in order to determine the sample dilution needed for subsequent analysis. Sufficient volume of each undiluted cranberry juice and juice blend samples were transferred with a disposable plastic pipet onto separate plastic cuvettes. The absorbance of each sample at 520 nm was measured on the UV/vis spectrometer. The brand with the highest absorption intensity, Ocean Spray Cranberry Juice Cocktail, was selected for use in the preparation of standard solutions.

### iii. Preparation of Ocean Spray cranberry juice standard solutions

Undiluted Ocean Spray Cranberry Juice Cocktail was selected as reference solution and was designated as "100 % cranberry juice" from which the standard solutions for calibrating the UV/vis spectrometer were prepared. Dilutions of 100 % cranberry juice in deionized (DI) water with the following concentrations in % by volume were prepared in 100-mL volumetric flasks: 4.0, 8.0, 10, 12, 16, and 20.

### iv. Preparation of Cran-Apple sample

A 10 % by volume Langers Cranberry-Apple juice, or simply Cran-Apple, sample solution was prepared by measuring 10.00 mL of the juice with a 10-mL volumetric pipet, transferring it into a 100-mL volumetric flask and diluting to the line with DI water.

v. Analysis of cranberry juice content of Cran-Apple by UV/Vis spectroscopy

a. Spectrometer calibration: A portion of each cranberry juice standard solution (4.0, 8.0, 10, 12, 16, and 20 % by volume) was transferred into a plastic cuvet and the absorption spectrum was recorded from 350 nm to 750 nm wavelength using a HP 8453 diode array UV/Vis spectrophotometer utilizing UV/Vis Win system software. A calibration blank made of DI water was used to set the absorption of light to zero in the absence of red dye, after which the absorption of 520 nm wavelength of light of each cranberry juice standard solution was recorded. Finally, a calibration curve was generated by plotting the absorption at 520 nm wavelength against the concentration of standard solutions.

b. Analysis of Cran-Apple sample: A portion of 10.0 % by volume Cran-Apple solution was transferred into cuvette and the absorbance was measured at 520 nm. The recorded absorbance was compared with the calibration standards' absorbance at 520 nm to determine the % cranberry juice content in Cran-Apple. Details of calculation can be found under section vii, Treatment of Data.

vi. Hazards

There are no chemical hazards involved in this experiment.

vii. Treatment of Data

To teach CHEM 141L students how to generate a calibration curve in Excel, a calibration curve was regenerated by plotting the absorbance at 520 nm on the y-axis against concentration of cranberry juice standard solutions on the x-axis. A linear regression analysis was performed in

order to fit the data points into a straight line. The resulting line equation was then used to calculate the % cranberry juice content of Langers Cranberry-Apple juice.

## 2. IBL 2: Separation of Red and Blue Dyes in Kool Aid by Solid Phase Extraction (SPE)

### i. Reagents

HPLC-grade 2-propanol (Fisher Scientific) was used to prepare two different dilutions of mobile phases to be used in the separation of artificial dyes Red 40 and Blue 1 in Kool-Aid Grape sample by solid phase extraction (SPE).

### ii. Solid-phase extraction (SPE) cartridge

Systems LLC BOND ELUT JR-C18 500 MG-1/UN-SPE cartridges were purchased from Fisher Scientific for about \$316 per 100-pack. The C18 SPE cartridges' solid stationary phase packing material consists of silica particles covalently bonded to non-polar 18-carbon hydrocarbon chains.

### iii. Preparation of mobile phases

Two separate 100-mL dilutions of HPLC-grade 2-propanol were prepared in 100-mL volumetric flasks as mobile phases for the separation of food dyes using the SPE cartridge: A = 30 vol % 2-propanol and B = 70 vol % 2-propanol, both in deionized water.

### iv. Sample preparation

A packet of grape flavored Kool-Aid powder was dissolved in 500 mL of DI water in a large beaker. The sample was stirred thoroughly with a spatula until all the powder dissolved.

This sample solution is more than sufficient for the whole class consisting of 8-10 groups of students.

v. Separation of Red 40 and Blue 1 dyes in Grape Kool Aid using Solid Phase Extraction (SPE) chromatography

a. Equilibration of SPE cartridge:

Exactly 5.0 mL of mobile phase B (70 vol % 2-propanol in DI water) was measured on a 5-mL plastic syringe. The tip of the syringe was inserted into a Systems LLC BOND ELUT JR-C18 500 MG-1/UN-SPE cartridge similar to the image shown in Figure 5. The cartridge was equilibrated with 5.0 mL of solvent B by slowly pushing the plunger for about one minute. The empty syringe was replaced with another 5-mL syringe containing 5.0 mL of DI water and the SPE cartridge was then “washed” with DI water by slowly pushing the plunger down for about one minute.

b. Loading Kool-Aid sample onto SPE:

After equilibration and washing, 2.0 mL of dissolved Kool-Aid Grape sample, measured with a 3.0 mL plastic syringe (Figure 5), was loaded onto the SPE cartridge by slowly pushing the plunger down for approximately one minute.



Figure 5. SPE cartridge set up showing how a plastic syringe on the left with grape Kool Aid sample solution, should be attached to the cartridge (left). The image on the right shows the process of elution of red dye by passing mobile phase through the cartridge.

c. Chromatographic separation of dyes:

After loading the Kool-Aid Grape sample, the SPE cartridge was washed with 5.0 mL of DI water from a separate 5.0mL plastic syringe in order to flush out sugars and other highly water-soluble components. Thereafter, 5.0 mL of mobile phase A (30 vol % 2-propanol) from a 5.0 mL plastic syringe was used to elute, or wash out, Red 40 dye onto a clean, dry 15-mL plastic centrifuge tube. Finally, 5.0 mL of mobile phase B (70 vol % 2-propanol) from a separate syringe was used to elute the Blue 1 dye, which was collected onto a separate 15-mL plastic centrifuge tube. The SPE cartridge was washed with another 5.0 mL of mobile phase B for reuse and/or storage.

vi. Hazard

2-propanol is a flammable organic solvent and should be disposed of in a properly labeled waste container.

vii. Treatment of Data

Other than qualitatively analyzing the color separation between both Red 40 and Blue 1 dyes from the Kool-Aid Grape sample, there is no treatment of data in this experiment.



### 3. IBL 3: Total Phenolic Content of Green Tea using FAS Reagent

#### i. Reagents

Gallic acid crystals (ACROS Organics; 98 %), a 100 % pure or 200-proof ethanol (Fisher Scientific), reagent grade  $\text{Na}_2\text{CO}_3$  or sodium carbonate (Fisher Scientific), and ACS grade ferrous ammonium sulfate (FAS) crystals (ACROS Organics; 99% pure) were used in this lab.

A saturated  $\text{Na}_2\text{CO}_3$  solution was prepared by setting up a 500-mL beaker full of deionized water on top of a magnetic stirrer plate with a stir bar inside of it.  $\text{Na}_2\text{CO}_3$  powder was added repeatedly with constant stirring until some powder added no longer dissolve in water.

The 1.00 m/v % FAS reagent was prepared by dissolving 0.100 g FAS crystals in DI water to make 10-mL of solution.

#### ii. Preparation of gallic acid standard solutions

A 0.150 mass-to-volume (m/v) % of gallic acid stock solution was prepared by weighing 0.150 gram of gallic acid crystals, transferring to a 100-mL volumetric flask and filling it up to the line with 70 vol % ethanol. The 0.150 (m/v) % gallic acid stock solution was used to prepare the following concentrations of gallic acid calibration standards in (m/v) % using deionized water as diluent in separate 15.00 mL plastic centrifuge tubes: 0.0375, 0.0750, and 0.115. Using a P5000 micropipet, exactly 1.00, 2.00, and 3.00 mL of the 0.1500 % gallic acid stock solution were transferred to separate centrifuge tubes, then diluted with deionized water up to the 4.00-mL line. Afterwards, two drops of 1.00 (m/v) % FAS in deionized water were then added to each tube. The tubes were heated in 40-50 °C water bath for 15-20 minutes, and then cooled in a beaker with cold water.

### iii. Sample preparation

Exactly 1.00 mL of Pure Leaf honey green tea was measured with a micropipet and transferred onto a 15-mL centrifuge tube. Five drops of saturated Na<sub>2</sub>CO<sub>3</sub> solution was added to neutralize the acids in tea. To this, 2.75 mL of DI water was added, followed by two drops of 1 (m/v) % FAS reagent. The resulting solution was heated in the 40-50 °C water bath for 15-20 minutes, and then subsequently cooled in a beaker with water.

### iv. Analysis of total phenols in Honey Green Tea using UV/Vis Spectroscopy

#### a. Spectrometer calibration

Approximately one milliliter each of the gallic acid standard solutions was transferred to separate cuvettes for UV/Vis analysis using the HP 8453 UV/Vis diode array spectrophotometer. Their absorption spectra were recorded at a range of 400-700 nm, together with their absorbance at 575 nm. A calibration curve was generated by plotting the absorbance at 575 nm against concentration of gallic acid standard solutions.

#### b. Analysis of Pure Leaf honey green tea sample

Approximately one milliliter of Pure Leaf honey green tea was transferred onto a cuvette using a plastic pipette for UV/Vis analysis. The sample's absorption spectrum was recorded at a range of 400-700 nm and its absorbance was recorded at 575 nm.

### v. Hazards

Sodium carbonate is an irritant, while ethanol, an organic solvent, is flammable. Gallic acid and ferrous ammonium sulfate reagent can cause irritation to the skin, eyes and respiratory tract if not handled carefully. Thus, gloves, goggles, and protective clothing that cover arms,

legs, and feet should be worn during this experiment. All waste should be properly disposed of in properly labeled waste containers.

#### vi. Treatment of Data

A calibration curve was generated in Excel by plotting the absorbance of 575 nm light on the y-axis and concentration of gallic acid standard solutions on the x-axis. A linear line fit was obtained by regression analysis and the resulting equation was used to calculate the total phenolic content in honey green tea sample measured as gallic acid equivalent (GAE) based on its absorbance of 575 nm light.

## **RESULTS AND DISCUSSION**

### *A. Literature search and selection of viable IBLs*

Table 1 shows in detail fourteen IBLs from the initial pool of 17 for CHEM 141L curriculum development, together with the reasons for non-selection, ranging from lack of required student skills to complexity of experimental procedure as illustrated in Figure 4.

The titles and attractive features of three IBLs selected for this project are summarized in Table 2. Two of three IBLs focus on the teaching three essential skills for CHEM 141L students, whereas one has the potential to extend from teaching preparation of solutions to calibration and use of Excel in data analysis.

Table 1. Titles of viable IBLs from the *J. Chem Ed.* literature search, skills taught (preparation of solution, PS, graphical analysis GA, and use of Excel, UE) and the reasons for non-selection.

<b>TITLE and SKILLS TAUGHT</b>	<b>REASON FOR NON-SELECTION</b>	<b>References</b>
Determination of Stability of Vitamin C (PS, GA, UE)	<ul style="list-style-type: none"> <li>Complex reagent (<math>\text{Fe}^{3+}</math> + 1,10-phenanthroline in acidic medium)</li> </ul>	(Adem et. al, 2016)
Hydration of Decorative Beads (GA, UE)	<ul style="list-style-type: none"> <li>No preparation of solutions</li> </ul>	(Hill et. al, 2017)
Activity Analysis of Iron in Water using a LED Spectrophotometer (PS, GA, UE)	<ul style="list-style-type: none"> <li>Iron indicator reagent is expensive</li> <li>Not much preparation of solutions</li> </ul>	(Place, 2019)
Escaping Boredom in First Semester General Chemistry	<ul style="list-style-type: none"> <li>Not a laboratory activity</li> </ul>	(Watermeier et. al, 2019)
Graphing Activity – Introducing Data Processing (GA, UE)	<ul style="list-style-type: none"> <li>No solution preparation</li> </ul>	(Magers et. al, 2019)
Determination of the Percent $\text{Na}_4\text{EDTA}$ in Bathroom Cleaners (PS)	<ul style="list-style-type: none"> <li>No graphical analysis; no Excel</li> <li>Reagents used are very hazardous</li> <li>Large volume and cost of chemicals</li> </ul>	(Kump et. al, 1978)
Spectroscopic Analysis of Aspirin (PS, GA)	<ul style="list-style-type: none"> <li>No Excel (no standard curve)</li> <li>Advanced instrumentation needed</li> </ul>	(Byrd et. al, 2003)
Identifying Unknown Chloride Salts by Titration with Silver Nitrate (PS)	<ul style="list-style-type: none"> <li>Costly and hazardous solid reagents</li> <li>No graphical analysis or Excel use</li> </ul>	(Maines et. al, 2012)
A Caffeinated Boost on UV Spectrophotometry (PA, GA, UE)	<ul style="list-style-type: none"> <li>Complex and not easily available bean samples</li> </ul>	(Dooling et. al, 2013)
Chemical Composition of Sodium Percarbonate (PS, GA)	<ul style="list-style-type: none"> <li>Requires advanced instrumentation</li> <li>No graphical analysis</li> </ul>	(Wada et. al, 2013)
Mass and Time Release of Acetaminophen from Gel Capsules (PS, GA, UE)	<ul style="list-style-type: none"> <li>Highly corrosive reagents</li> <li>Complexity of solution preparation</li> </ul>	(Smith et. al, 2014)
Determining Ethanol in Gasoline by FT-IR Spectroscopy (PS, GA, UE)	<ul style="list-style-type: none"> <li>Use of hazardous chemicals</li> <li>Requires advanced instrumentation</li> </ul>	(Conklin et. al, 2014)
Measuring Vitamin C Content of Orange Juice Using Pencil Lead Electrode (PS, GA, UE)	<ul style="list-style-type: none"> <li>Highly toxic, corrosive, and/or cancer causing reagents</li> </ul>	(King et. al, 2010)
Spectroscopic Determination of Triclosan in Soaps (PS, GA, UE)	<ul style="list-style-type: none"> <li>Requires advanced knowledge of chemical structures</li> </ul>	(Wyllie, 2014)

Table 2: Titles of selected IBLs from the *J. Chem Ed.* literature search, skills taught (preparation of solution, PS, graphical analysis GA, and use of Excel, UE) and attractive features

<b>TITLE and SKILLS TAUGHT</b>	<b>ATTRACTIVE FEATURES</b>	<b>References</b>
Determination of Percent Cranberry in Cranberry-Apple Juice Blends (PS, GA, UE)	<ul style="list-style-type: none"> <li>Relevant to daily life</li> <li>Inexpensive</li> <li>Use of benign chemicals</li> </ul>	(Edionwe et. al, 2011)
Separation of Red and Blue Dyes in Kool Aid by Solid Phase Extraction (SPE) (PS, potential for GA and UE)	<ul style="list-style-type: none"> <li>Visually appealing due to colors of dyes</li> <li>Relevant to daily life</li> <li>Only one organic solvent used</li> </ul>	(Bidlingmeyer et. al, 1984)
Total Phenolic Content of Green Tea using FAS Reagent (PS, GA, UE)	<ul style="list-style-type: none"> <li>Visually appealing due to colored reaction</li> <li>Also relevant to daily life</li> </ul>	(Shaver et. al, 2011)

## B. Testing of selected IBLs

### 1. IBL 1: Determination of Percent Cranberry in Cranberry-Apple Juice Blends

The quantification wavelength of 520 nm was selected based off literature (Edionwe et. al, 2011) and is due to absorption of visible light by naturally occurring anthocyanins responsible to the characteristic red color of cranberry juice and cranberry juice blends. Among the juice samples tested, all prepared at 50 % dilution with DI water, Ocean Spray Cranberry Juice Cocktail showed the most intense absorption at 520 nm (Figure 6 and Table 3), followed by Ocean Spray Light Cran-Grape. Langers Cranberry juice only showed weak absorption at the same wavelength (Figure 6 and Table 3). Thus, Ocean Spray Cranberry Juice Cocktail was selected as reference solution and designated “100 % cranberry juice”, which is used to prepare various dilutions of cranberry juice standard solutions used to calibration the UV/vis spectrometer. Langers Cran-Apple (50 %) showed equally intense absorption at ~ 0.4 as 50 % Langers cranberry juice, indicating similar cranberry juice content. More importantly, the 50 % dilution should yield reasonable absorption signal for quantification.

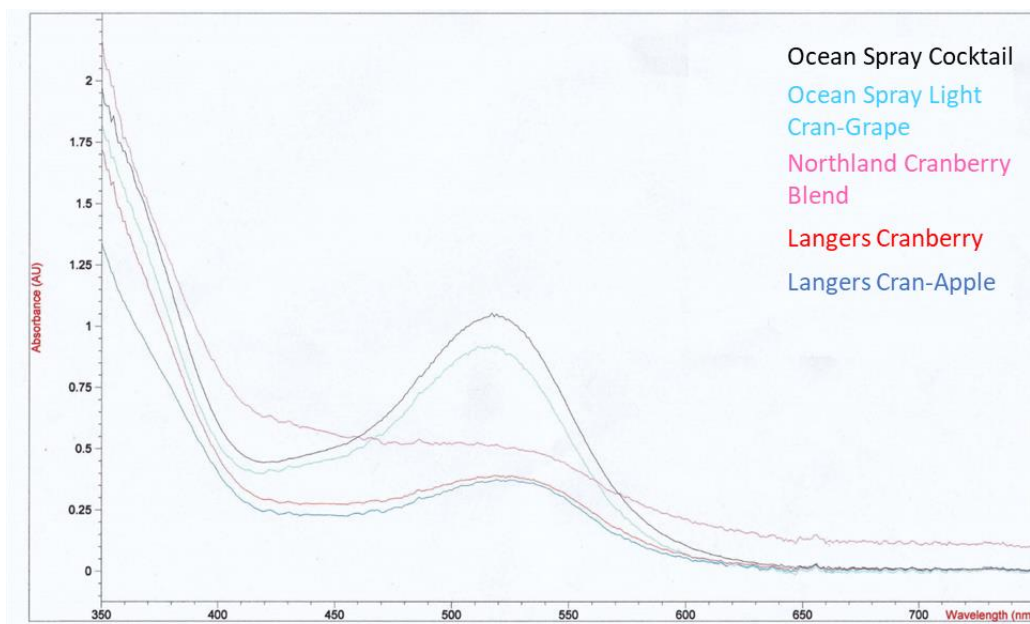


Figure 6. Overlaid absorbance spectra in the visible range of 350-750 nm of 50 % dilution of each of Ocean Spray Cranberry Juice Cocktail, Langers Cranberry, Langers Cran-Apple, Ocean Spray Light Cran-Grape, and Northland Cranberry Juice Blend samples. Each brand of cranberry juice is color-coded and in order of highest to lowest absorbance value shown.

Table 3. Experimentally measured absorbance of 520 nm light of 50 % dilutions of cranberry juice samples

<b>Cranberry Juice Samples</b>	<b>Absorbance at 520 nm</b>
50 % Ocean Spray Cocktail	1.03890
50 % Langers Cranberry	0.38752
50 % Langers Cran-Apple	0.37138
50 % Ocean Spray Light Cran-Grape	0.91157
50 % Northland Cranberry blend	0.51275

The resulting calibration curve from various dilutions of 100 % cranberry juice whose absorbance of light was measured at 520 nm (Figure 7) showed a linear line fit with a correlation coefficient ( $R_2$ ) of 0.9904. An  $R_2$  value of 1.0 means the relationship between concentration of solution and absorbance of light at a given wavelength is perfectly linear. The near 1.0  $R_2$  value obtained also indicates that the preparation of cranberry juice standard solutions was accurate and will yield reliable quantification results of cranberry juice in the Cran-Apple sample.

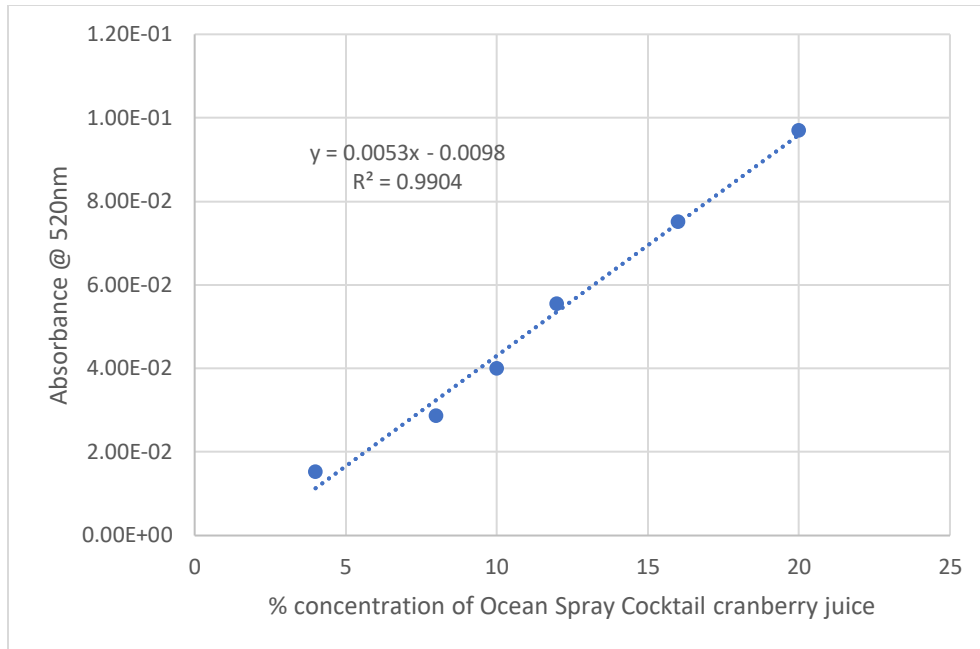


Figure 7. Calibration curve for standard solutions of Ocean Spray cranberry juice cocktail and the resulting line equation. Absorbance was measured at 520 nm.

The measured absorbance of 10 % Langers Cran-Apple in comparison with 10 % Ocean Spray cranberry juice and 10 % Northland cranberry juice blend is shown in Table 4. Using the line equation from the calibration curve (Figure 7), and the dilution factor of 10 (equal to the inverse of 10 % or 0.10), the cranberry juice content of undiluted Langers Cran-Apple was determined to be 16.4 %. Details of the calculation are shown below:

$$y = 0.5289x - 0.0098 ; 10 \% \text{ Cran} - \text{Apple Juice absorbance: } 0.077192$$

$$0.077192 = 0.05289x - 0.0098$$

$$0.086992 = 0.05289x$$

$$x = 1.64 \% \text{ cranberry juice}$$

$$1.64 * 10 (\text{dilution factor}) = 16.4 \% \text{ cranberry juice in Langers Cran} - \text{Apple juice}$$

Table 4. Experimentally measured absorbances of 520 nm light by 10 % dilutions of cranberry juice and cranberry juice blends

<b>Juice Samples</b>	<b>Absorbance of 520 nm light</b>
10 % Ocean Spray cranberry juice cocktail	0.044700
10 % Langers cran-apple juice	0.077192
10 % Northland cranberry juice blend	0.109610

*Preliminary testing and modifications to the procedure for IBL 1: Determination of Percent Cranberry in Cranberry-Apple Juice Blends*

Preliminary measurements of absorbance of light by a semester-old Ocean State cranberry juice cocktail used in a separate study revealed a significant reduction in absorption of 520 nm light compared to the prior semester. It is speculated that the old sample's naturally occurring red anthocyanin content has degraded over time. Thus, it is recommended that juice samples be bought just prior to use. Refrigeration of leftover samples is only recommended for up to one week to prevent further degradation of anthocyanins. To ensure freshness, the absorbance of 520 nm light of newly-purchased samples were measured with the results shown in Table 5. Ocean Spray cranberry juice cocktail showed the highest absorbance, thus confirming its use as reference solution when quantifying the cranberry juice content of cranberry-apple juice blends.

Table 5. Experimentally measured absorbance of 520 nm light by undiluted newly-bought cranberry juice and cranberry juice blend samples

<b>Undiluted Juice Samples</b>	<b>Absorbance at 520nm</b>
Ocean Spray Cranberry Juice Cocktail	2.0952
Langers Cranberry	0.76162
Langers Cran-Apple	0.73458
Light Ocean Spray Cran-Grape	1.8706



Northland Cranberry blend	0.99422
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The protocol in the reference article (Edionwe et. al, 2011) suggested to analyze cranberry juice sample at 41 wavelengths at 5-nm intervals to create a full absorption spectrum using a handheld spectrophotometer, such as the Spectronic GENESYS 20. To cut down on analysis time, the department's scanning HP 8453 UV/Vis diode array spectrophotometer was used. It was able to record the full spectrum in seconds rather than minutes expected of a benchtop, non-scanning spectrometer.

*Recommendation for CHEM 141L adoption:*

Because the department has only one rapid-scanning spectrometer, instead, it is best to rewrite this IBL so that several units of a Perkin Elmer LAMBDA Bio+ benchtop spectrometer, of which are easily accessible to CHEM 141L students, be used when performing this IBL. In addition, the protocol did not suggest performing replicate dilutions of Cran-Apple sample.. To measure the precision (reproducibility) of CHEM 141L students' preparation of solutions, it is recommended that each group prepare their cran-apple sample dilutions in triplicate. This will allow them to perform statistical analyses in Excel, such as calculation of mean concentrations and the corresponding standard deviation. Another suggested change is that it is possible that the newly adapted BSU CHEM141 lab protocol could offer to not dilute the specific Cran-Apple sample or, at the most, only do a 50 % dilution in order to get a more accurate quantification of cranberry juice content. Another suggestion is that, when making 50 % dilutions, students can easily use a P5000 micropipet to measure both the cranberry samples (5.00 mL) and DI water diluent (5.00 mL), then mix them together in one 15-mL centrifuge tube instead of using a narrow-neck 10-mL volumetric flask, which is too narrow to accommodate the thick pipet tip

and is therefore prone to spillage. A final suggestion to note is that if 50 % sample dilution are to be used instead of 10 %, a 50 % cranberry juice standard solution should be included in calibration.

#### IBL 2: Separation of Red and Blue Dyes in Kool Aid by Solid Phase Extraction (SPE)

The separation of artificial dyes Red 40 and Blue 1 in grape-flavored Kool Aid is shown in Figure 8. During the first attempt, the mobile phases or eluent compositions of 30 vol. % 2-propanol in water followed by 70 vol % 2-propanol in water did not achieve clean separation of dyes, resulting to a purple color (mixture of red and blue) in the first fraction collected, and pale blue for the second fraction (Figure 8 a). Changing the initial eluent compositions by decreasing the proportion of 2-propanol to water to 25 vol %, followed by 65 vol. %, resulted in a clean separation of red and blue dyes (Figure 8 b).

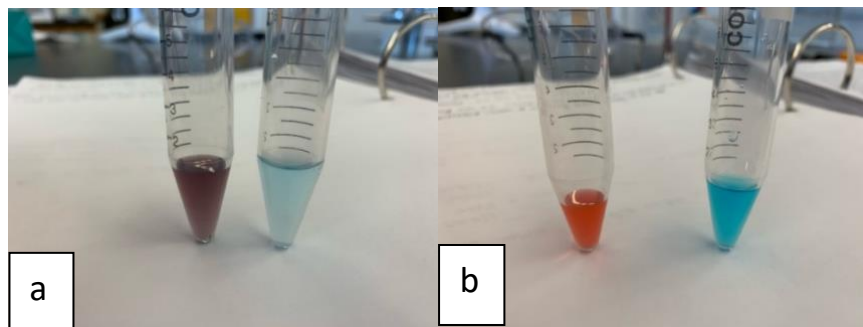


Figure 8. Collected fractions following the use of two different mobile phase (eluent) compositions to separate blue and red dyes: a = 30 vol. % 2-propanol in water followed by 70 vol % 2-propanol in water, and b = 25 vol. % 2-propanol in water followed by 65 vol % 2-propanol in water.

The polarity of eluent, components being separated, and SPE cartridge stationary phase play a key role in separation of components. Water is a polar eluent; 2-propanol, an organic solvent, is less polar than water. Because the C18 stationary is nonpolar, when the sample containing red

and blue dyes is applied on the cartridge, the less polar component will be more strongly attracted to the nonpolar cartridge and therefore will be last to elute. Figure 9 shows the structures of Red 40 and Blue 1 dyes. Although both dyes have polar ends due to the sulfonate ( $-\text{SO}_3^-$ ) group, Blue 1 has more cyclic nonpolar aromatic groups, making it less polar than Red 40. Red 40 eluted first because it is less retained by the nonpolar C18 cartridge.

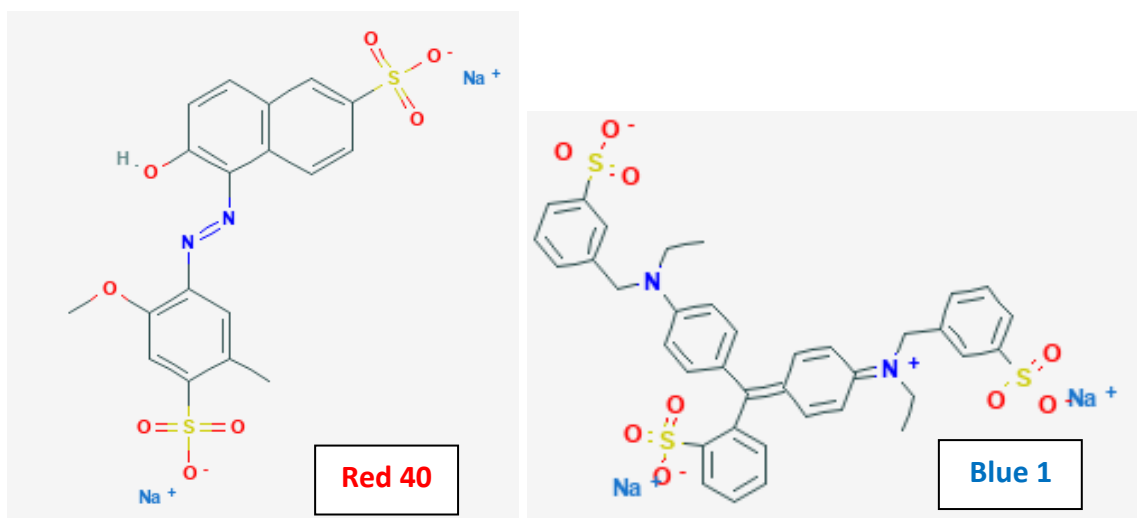


Figure 9. Structures of Red 40 (left) and Blue 1 dyes (right) from PubChem Database (National Center for Biotechnology Information).

During the first trial, Red 40 and Blue 1 eluted out together because the eluent compositions of 30 vol. % 2-propanol in water followed by 70 vol % 2-propanol in water is a little too low in polarity that it also “pushed” the less polar Blue 1 as Red 40 is eluted (Figure 8a). Increasing the polarity of the eluents by increasing the proportion of DI water, which is synonymous to decreasing the proportion of less polar 2-propanol, allowed for the Red 40 to completely separate from Blue 1 (Figure 8 b).

This IBL provides more of a qualitative experience compared to the other two selected IBLs, since I did not have the chance to introduce the other two essential skills to this experiment: calibration standards and use of Excel. The original plan was to continue this work after spring break, which unfortunately did not happen due to the switch to remote learning as a result of the pandemic. However, given more time, this IBL could potentially be expanded to include the two remaining essential skills. Out of all three IBLs that were tested, this one required the least amount of modifications and the least amount of repetitions. The eluent concentrations suggested by the protocol of the original JCE article was 30 vol % 2-propanol in water followed by 70 vol % 2-propanol in water. For the first test run of the experiment, it was found that the recommended eluent concentrations did not completely separate Red 40 from Blue 1 (Figure 8 a) as detailed in the previous discussion. This is why the reason why the eluent compositions were altered in Figure 8 b.

This particular IBL is relatively simple, but CHEM141L students should take care as to being patient while pushing the plunger through the SPE cartridge. The protocol makes it clear that the plunger needs to be pushed slowly so that the cartridge packing material (the stationary phase) is either fully equilibrated and ready to use, or that the dyes have sufficient time to elute out of the cartridge. That being said, this IBL will allow CHEM141L students to once again practice careful preparation of solutions and patience with execution of the procedure in order to achieve the intended results. More often than not, mistakes are made because students are tempted to go through the procedure quickly. This experiment could be repeated in triplicate to gauge student's precision in following experimental protocol.

One main point worthy of discussion is on the reusability of the SPE cartridges. The ones used for this IBL are considered reusable, but the main question is: how many times can they be

reused? A 100-pack SPE cartridge costs ~ \$ 316; compared to the cost of cranberry juice and juice blend samples in IBL 1 (\$20), IBL 2 is indeed more costly if the cartridges cannot be reused. If this IBL were to be experimented on further, it would be interesting to compare how well the two dyes separate with a newly purchased SPE cartridge versus an old SPE cartridge that has been reused various times.

IBL 3: Total Phenolic Content of Green Tea (FAS Reagent):

The calibration curve obtained for gallic acid standard solutions is shown in Figure 10, together with the line equation from linear regression.

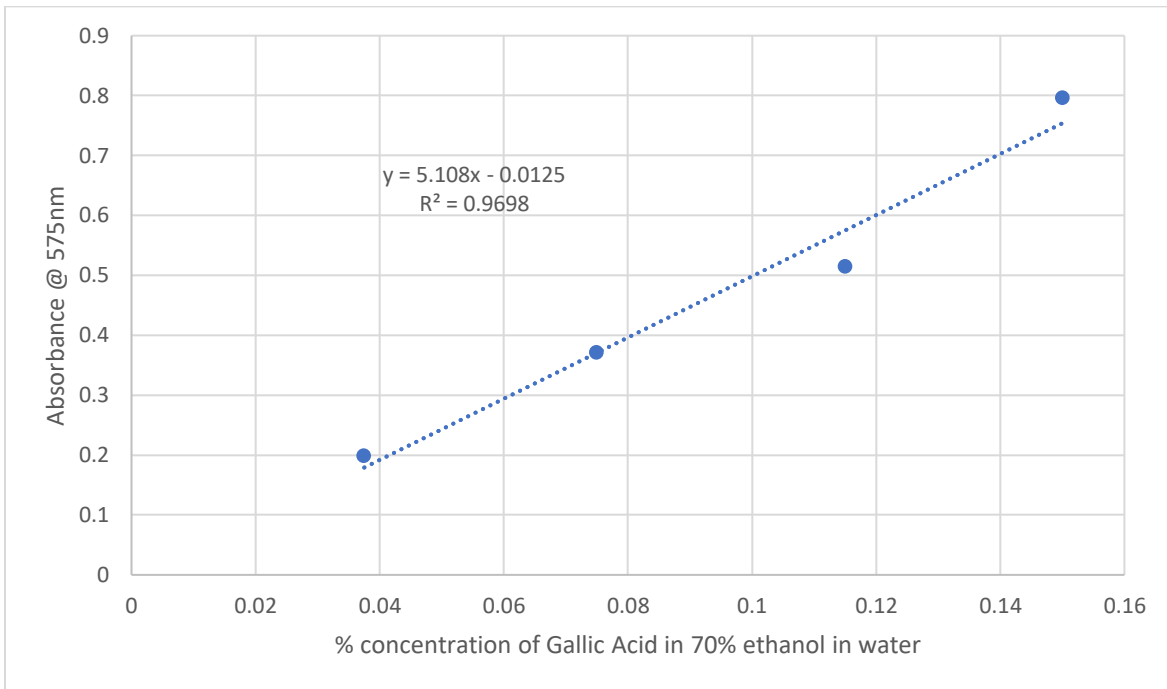


Figure 10. Calibration curve of for gallic acid standard solutions and the resulting line equation. Absorbance was measured at 575 nm.

The  $R^2$  value achieved in this calibration curve is 0.9698, which deviates from the value of 1.0 for perfectly linear relationship between absorbance and concentration by a difference of 0.0302. Compared to the  $R^2$  value achieved in IBL 1 ( $R^2 = 0.9904$ , Figure 7), the  $R^2$  value for this calibration is inferior and could have been the result of incomplete reaction during the assay. It is often difficult to achieve a high  $R^2$  value in assays that utilize chemical reactions because it is always assumed they always go to completion. This is beyond the control of the experimenter performing this particular IBL. For example, there could be compounds that are similar to gallic acid that are present within the sample that would also chemically react with the FAS reagent. There are also a multitude of factors that could prevent the chemical reaction from going to completion, such as temperature of the water bath, which also affects the reaction going to completion. Thus, the calculated total phenolic content of Pure Leaf Honey Green Tea sample, reported as gallic acid equivalent (GAE), may not be as reliable as quantification of cranberry in cran-apple because the latter did not involve a chemical reaction.

The absorption spectra of gallic acid calibration standards and 1:4 (or 25 %) dilution of Pure Leaf Honey Green Tea sample is shown in Figure 11, showing that the absorption of 575 nm wavelength of the sample is very close to that of 0.115 (m/v) % gallic acid.

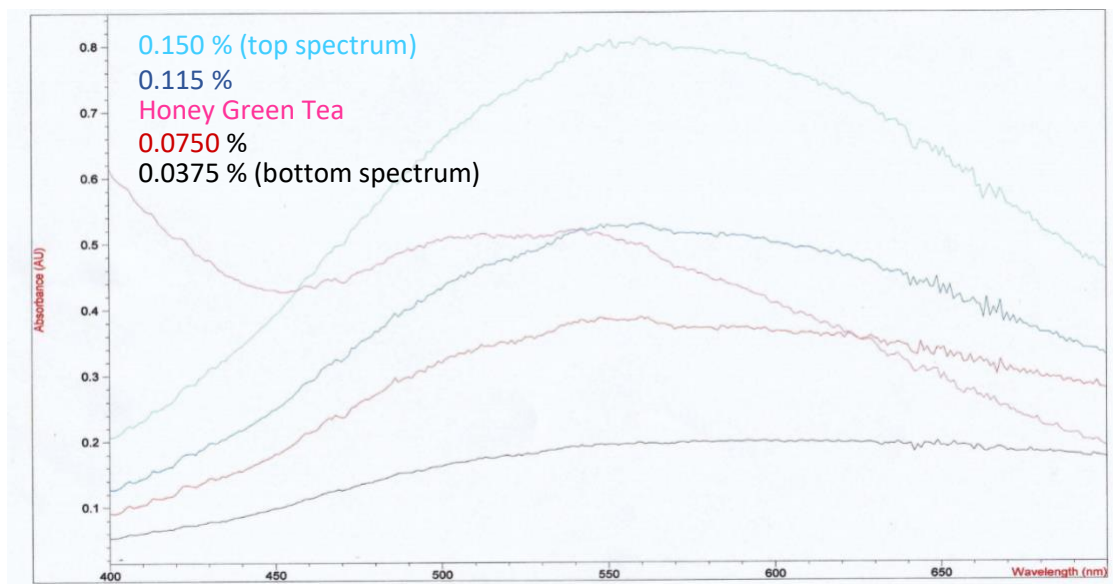


Figure 11. Absorbance spectrum from 400-700 nm of gallic acid calibration standards (insert showing color code by % gallic acid) and 25 % dilution of Pure Leaf Honey Green Tea sample (pink).

Using the line equation from the calibration curve (Figure 10) and the absorbance of tea sample, 0.45912 at 575 nm, the total phenolic content reported as % GAE of the undiluted Pure Leaf Green Tea sample was 0.0923. A detailed calculation follows:

$$y = 5.108x - 0.0125 ; \text{Pure Leaf sample absorbance: } 0.45912 @ 575\text{nm}$$

$$0.45912 = 5.108x - 0.0125$$

$$0.47162 = 5.108x$$

$$x = 0.09233 \left( \frac{m}{v} \right) \% \text{ GAE}$$

$$0.09233 * 4 (\text{dilution factor}) = 0.369318 = 0.369 \% \text{ total phenols as GAE}$$

Interestingly, in the first trial run of this IBL, the protocol was unclear on how to properly neutralize the sample being used so its absorbance could lie in between the absorbances of the gallic acid calibration standards. The protocol from the original JCE article suggested using a

supersaturated sodium bicarbonate solution. Sodium bicarbonate was not easily available in the lab during the time of the experiment, so sodium carbonate was used instead. At first, sodium carbonate powder was added manually to the 15.00 mL plastic tube with the Pure Leaf sample until it could no longer dissolve. This method proved to be unsuccessful because during the heating step of both the Pure Leaf sample and gallic acid calibration standards in a 40-50°C water bath, the Pure Leaf sample developed a much more intense purple color, the color of the reaction product between gallic acid and phenols in the sample, than the calibration standards. This implied that quantification of total phenols in the sample will not be determined accurately since its absorbance would be much higher than the absorbance of the highest concentration of calibration standards used. Due to over-neutralization of the Pure Leaf sample in the first run, it was decided that an accurately prepared saturated solution of sodium carbonate is needed for a successful assay and quantification.

Although this experiment was visually pleasing due to the formation of purple-colored product during the assay, there are a few concerns for adoption of this IBL for CHEM 141L. First, the chemical reaction involved may be too complex or intimidating for first year students who have not been exposed to structures of large molecules like gallic acid and phenols. Additionally, the complexity of performing the assay where pH and temperature controls affect the outcome of the experiment requires advanced laboratory skills that CHEM 141L students have not yet developed at the time of the experiment.

## **Conclusion**

Overall, these three experiments are good IBL candidates for adaptation to the BSU CHEM141L curriculum. However, weighing the pros and cons of each IBL, IBL 1 (Determination of Percent Cranberry in Cranberry-Apple Juice Blends) proved to be the most



cost-effective, safest to perform and level-appropriate for BSU's CHEM 141 students. In addition, there are no reagents required to complete the experiment and only benign waste is generated (i.e. unused cranberry juice can be poured down the drain). Finally, it is highly recommended that this IBL be scheduled for the later half of the semester so students can first improve on their math skills and preparation of solutions from earlier experiments.

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