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Using tattoo inks to develop undergraduate general chemistry and high school level chemistry students' understanding of atomic emission

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Using Tattoo Inks to Develop Undergraduate General Chemistry and High School Level Chemistry Students' Understanding of Atomic Emission

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Submitted in partial fulfillment of the requirements for Distinguished Independent Work in Chemistry at Binghamton University State of New York

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ABSTRACT

An effective way to promote student engagement in the laboratory is to substitute traditional reagents for recognizable, real-world products. In this work, two laboratory experiments are outlined – one geared toward undergraduate general chemistry students and one for high school introductory level chemistry students – which replace solutions of metal salts with tattoo inks in the classic flame test experiment. The main objective of both experiments is to familiarize students with the subatomic mechanism occurring during atomic emission. Additionally, the undergraduate level experiment features the use of spectroscopy to identify metals present within tattoo inks through analysis of atomic emission spectra.

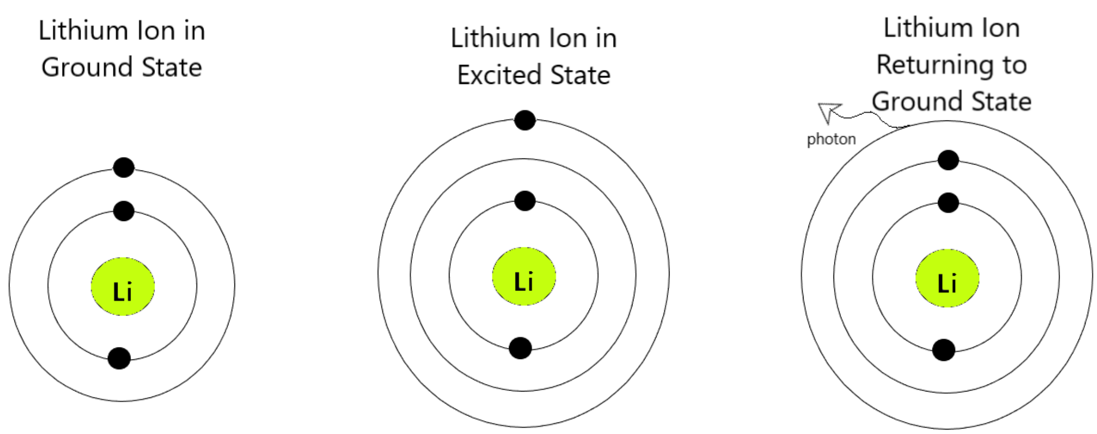
TABLE OF CONTENTS

INTRODUCTION

Educational laboratory sessions are an essential component of chemistry courses. Experiments are designed to expose students to safe lab practices, to teach analytical instrumentation and techniques, and provide dimension and practicality to course topics.[1] At the high school introductory level, instruction typically includes a broad overview of several chemistry topics such as Atomic Structure, Chemical Bonding, States of Matter and Behavior of Forces, Kinetics, Equilibrium, Nuclear Chemistry, and more.[2] Following Next Generation Science Standards, chemistry at the high school level is meant to enhance critical thinking and investigative planning skills.[3] At the collegiate level, many students opt to take General Chemistry, which provides a significantly more in-depth coverage of topics including, but not limited to, Units of Measurement & Significant Figures, Matter and Energy, Stoichiometry and Chemical Equations, Thermochemistry, Electronic Structure of Atoms, Periodic Trends, Molecular Bonding and Structure, Gases, Intermolecular Forces, Solutions, Equilibrium, and Redox Reactions.[4] General chemistry is a required course for many degrees and tracks within STEM.[5] The methods by which material is taught varies with instructor, however, one common goal of the course is to demonstrate the practicality of chemistry in relation to real-world topics and issues, and how material can be useful in other areas of STEM.[6]

A common experiment across curricula at all levels is the Flame Test.[7] Students are provided with solutions of metal salts such as calcium chloride, potassium nitrate, and strontium chloride, which they submit into a flame and analyze for a change in the flame's color.[8] Metals are coupled with chlorides and nitrates for flame tests because these ions' emitted energy is not in the visible region and therefore will not alter the color of the flame.[9] Conceptually, observing a change in flame color enables visualization of atomic emission. Each metal produces a uniquely colored flame due to the promotion of a ground state electron to its excited state when excited by the flame's thermal energy. As the electron returns from the unstable excited state configuration, a photon is emitted, and through emission, the flame color is changed. Figure 1 shows excitation and emission of a lithium ion during a flame test.

Figure 1: Movement of lithium ion's valence electron during flame test



An appropriate extension of a flame test experiment in a more advanced course setting is an introduction to spectroscopy and the utility of emission spectra to identify individual elements. By incorporating spectroscopy, students can also contextualize terms like "wavelength," and "frequency" through calculations based on their findings.

To promote engagement and provide relevance in this sort of educational experiment, instructors can opt to use tattoo inks place of traditional metal salt solutions.[10] A previous report described acid digestion tattoo inks and flame tests to detect specific copper compounds in the inks.[11] The aforementioned experiment was designed for undergraduate analytical chemistry students, however, so the experimental process is not suitable for introductory level courses. Being that a common goal of high school and undergraduate chemistry is for students to better understand how chemistry can be practically valuable, the use of tattoo inks as analytes introduces real-world relevance to laboratory experiments. It encourages students to think about a product that they have seen and heard about outside of the lab, and use their skills to learn more about it using their knowledge of chemistry.

Tattoo inks are minimally regulated in the United States and the quantitative breakdown of their composition is widely unknown.[12] Generally, inks are created by suspending particles of pigments in an aqueous solution.[13] Since the purpose of a tattoo is to permanently mark the skin, inks need to be made up of stable components that can last in the dermis against heat and sun exposure. To create long-lasting pigmented inks, components including binders, molecular dyes, and solid pigments (e.g., TiO_2) are often included in production. Sulfides and oxides of heavy metals (e.g., mercury, lead, copper) are useful in inks to create bright, lasting colors.[14] Thus far, the United States Food and Drug Administration (FDA) has not approved any tattoo inks for injection into the skin, or placed any regulations on the chemical composition of inks. In the past few years, however, the FDA has received numerous reports of adverse effects felt by tattoo consumers including rashes and allergic reactions.[15] As demand for tattoos increases, the lack of ink regulations set forth by the FDA could lead to the production of cheap and dangerous products containing allergens, carcinogens, and otherwise harmful additives including – but not limited to – the usage of pigment from printer toner, car paint, and hair dye.[15]

In this work, 60 tattoo inks from varying United States and international ink brands are analyzed through flame tests and spectroscopy. The techniques and findings were then applied to the creation of educational laboratory content that is suitable for undergraduate general chemistry level students. In addition, the lab can be modified for high school introductory chemistry courses such as New York State Regents chemistry. All supplemental educational materials for this laboratory are included in the appendix of this work, with educators welcome to use them for classroom and experimental purposes. The pedagogical goals of this experiment at the high school introductory level are to:

- Develop students' understanding of the subatomic excitation and emission mechanism occurring during atomic emission
- Demonstrate proper safety when working with open flames

  Use evidence-based reasoning to hypothesize possible metals in sets of tattoo inks

The pedagogical goals of this experiment at the undergraduate general chemistry level are to:

- Develop students' understanding of the subatomic excitation and emission mechanism occurring during atomic emission
- Demonstrate proper safety when working with open flames
- Draw conclusions as to what elements could be present in an ink based on flame and spectroscopic analysis
- Use calculations to describe the relationship between waves and frequency

Recommended assessment for this experiment is based on pre- and post-lab assignments which have been included in Appendixes B and C of this work.

MATERIALS AND METHODS

To establish the procedures for each educational experiment, 60 inks from Intenze, Mom's, Solong, One World Tattoo, and StarBrite were characterized by flame tests. When in their bottles, the inks tended to separate into layers, where the top layer would appear watery while the bottom layer was more viscous, so inks were shaken and then ~1mL of each ink was transferred into centrifuge tubes. Within the tubes, these layers continued to form, so they were shaken prior to testing to ensure proper mixing of particles.

The flame tests were complete using a Bernzomatic flame torch, which produces a flame similar to that of a Bunsen burner. To carry the inks into the flame nichrome wire was initially investigated. The wire was cleaned with hydrochloric acid by dipping the wire in the acid and then submitting it into a flame. Subsequently, the cleaned wire was coated with ink and submitted into a flame. Coffee stirrers were also investigated as carriers for the inks. The stirrers did not need to be cleaned and were immersed in the inks before being dried for 20 minutes.

To analyze inks spectroscopically, the KISS Instruments Smartphone Spectroscope was initially investigated. This spectroscope in particular was chosen because it connects to smartphones through an app and was advertised to display wavelengths on a spectrum that could easily be screen-recorded and shared. The spectroscope itself came in the form of an attachment that is adjustable to fit over a standard smartphone camera. In order to display the spectrum on the phone screen, a mobile app, "CamAtom" was installed. This app was specifically designed by the instrument manufacturer to work with this product. Once the spectroscope was lined up with the phone camera, a visible light spectrum was observed through the camera and displayed on the screen. For the data presented in Appendix A, a FLINN Scientific Handheld Spectroscope was used.

In the high school and undergraduate level experiments outlined, students analyze solutions of metal salts in addition to tattoo inks. These solutions are meant to be standards with a known concentration of metal salt, which allow students to compare the intensity and spectral resolution of a single metal species in solution. This is useful for the comparison of flame colors between standards and inks, which act as unknowns. Sodium nitrate, potassium nitrate, and copper (II) chloride were chosen because they each produce distinctive flame colors that are similar to those produced by inks. To prepare each solution, solid samples of each salt were dissolved in deionized water. Approximately 10.0 g of sodium nitrate and potassium nitrate were dissolved in 50.0mL of deionized water, whereas approximately 15.0g of copper (II) chloride were dissolved to create solutions.

In the high school level experiment, students exclusively analyze inks through flame tests. To prepare for the experiment, instructors should transfer inks from their bottles into a secondary container such as a small beaker or vial. Standard solutions are prepared by dissolving each salt into distilled or deionized – but preferably deionized – water in a beaker (ideally 250mL). Deionized water is preferred because water is treated to remove any ions, as opposed to distilled water which still may contain ions. While the concentration of ions in distilled water is likely too low to be detectable through flame test and spectroscopic analysis, it is still possible that results could be skewed. This experiment works best when inks are dried on the coffee stirrers for approximately 20 minutes prior to flame testing. To allow for multiple trials of flame analysis, multiple coffee stirrers of each ink should be prepared. As the inks are drying, it is also beneficial to place several coffee stirrers into beakers containing standard solutions to allow the stirrers to soak in the solution for a period of time prior to flame testing. As with the inks, several stirrers should be placed into each standard solution to allow for replication of tests. It is also important to set out a waste beaker containing water for stirrers subsequent to flame testing. During the 20 minutes that inks are drying, instructors can take the opportunity to introduce the experiment, provide an overview of the procedure, and talk about safety protocols when working with flames. After drying, inks and standards are analyzed for their flame color by placing the stirrers into the hottest part of the flame. The color will persist from anywhere between one and five seconds and then the stirrer should be placed in the waste beaker to extinguish any flame. Students can record the flame color and any other observations in a table. At the end of the laboratory, students should also complete the post-lab questions provided with the laboratory experiment outlined in Appendix B.

At the undergraduate level, the start of the experiment is comparable to the high school procedure, however, more preparatory steps are involved on the students' part. Prior to coming to lab, students should complete a pre-lab assignment and be familiar with the procedure they will be performing. To begin, inks should be transferred from their containers to small beakers or vials. Then several coffee stirrers can be coated with inks and allowed to dry for 20 minutes. Coffee stirrers should also be placed in beakers of each standard solution. It is important to note that spectroscopic analysis will also be taking place, so each student should prepare at least four stirrers of each ink and standard. Each lab group should also have a waste beaker with water to extinguish any flames.

When completing spectroscopic analysis, especially with tattoo inks, viewing emission lines is easiest when the surrounding environment is as dark as possible. When developing this experiment, analyses were completed in a dark fume hood, however, it is also effective to turn off the lights and block out as much natural light from windows as possible with blinds or shades. Prior to darkening the room, students should become accustomed to looking through the spectroscope and visualizing spectra by pointing the scope at a fluorescent light. Subsequently, the room can be darkened, and students can point the spectroscope at the flame to observe for emitted wavelengths. Observations of atomic emission lines are recorded, and the test should be completed multiple times for each ink and standard solution. Subsequent to lab, students should write a lab report complete with an introduction, procedure, results and discussion, and conclusion section. In the results and discussion section of their report they should answer the post-lab questions from the provided laboratory experiment outlined in Appendix C.

RESULTS

Initially, nichrome wire was investigated as carrier for inks. While inks loaded on the nichrome wire did produce different colored flames, the wiring was thin and produced quick and dull flames when coated with tattoo ink. In place of the nichrome wiring, generic wooden coffee stirrers were used in further analyses, and proved to be the preferred method for flame testing. Specifically, the stirrers appeared able to hold more tattoo ink, which was beneficial because the colors of each flame persisted longer. For an educational setting, they are also more cost effective and simpler to work with since there is no cutting of wire or cleaning involved.

Originally, the KISS scientific spectroscope was intended for use in data collection so that images of the flame tests could be readily captured. However, after extensive testing, the spectroscope did not provide readings when directed toward flames and it was later discovered that the instrument itself only works well with sodium salts that are dissolved in methanol. Due to the fact that tattoo inks contain a wide variety of metals along with the fact that the American Chemical Society's Committee on Chemical Safety recommended the discontinuation of methanol use in flame tests, use of this spectroscope was abandoned.[8]

Instead of the of KISS spectroscope, a FLINN scientific spectroscope was used instead. Initially, the clarity of emission lines was poor, and several steps were taken to improve them. The lights in the lab space were turned off, though significant natural light was still present in the laboratory. It was determined that the best conditions for analysis took place in a dark fume hood. This helped with the visibility of less intense lines. Secondly, the inks themselves were dried in order to allow for a hotter and brighter flame. To start, drying took place over five minutes, but no visible difference in emission lines occurred. After allowing inks to dry for 20 minutes, an improvement in the intensity and resolution of lines was observed. To see if the resolution could improve even more, the inks were dried for 30 minutes, however the quality of emission lines did not visibly improve from after 20 minutes of drying.

A total of 60 inks from various brands were analyzed through flame tests and spectroscopy, with Appendix A detailing the observed flame color and emission lines for each individual ink. Overall, flame test analysis of the inks produced a variety of colors including orange, blue, green, and one ink even had hints of pink in its otherwise green flame. Across each brand of inks analyzed, every blue ink produced a green flame, indicating the presence of copper. Spectroscopic analysis confirmed the presence of copper in blue inks by showing wavelengths at approximately 520-550 nm. The most common flame color seen across all brands of ink was orange which indicates the possible presence of elements like sodium, calcium and iron.[16] Iron is likely to be present in inks because iron oxide is a naturally brown pigment, which is useful when creating red, yellow, and brown inks.[17] Intense emission lines were observed between 500 and 600 nm across multiple red, yellow, and brown inks indicating the presence of iron.

Some inks did not produce a stable flame color, or began to smoke or bubble immediately after being placed in the flame. White inks most commonly exhibited this behavior including Intenze Snow White Opaque, StarBrite Brite White, and Solong Snow White Opaque. The pigmentation of white inks is known to be derived from lead, barium, zinc, and titanium. Across the brands surveyed, most white inks were labeled to contain titanium dioxide. In spite of the smoking, spectroscopic analysis for multiple white inks did briefly show emission lines between 580 and 650nm, which confirms the presence of titanium.

Sodium nitrate, potassium nitrate, and copper (II) chloride were chosen because they are known to produce bright, easily identifiable flame colors when undergoing a flame test. Additionally, being that many tattoo inks contain copper, it is beneficial for students to compare the standard flame with the ink flame. At the undergraduate level, students can also compare the emission lines of copper (II) chloride, which fall between 520 and 550 nm – to the emission lines of blue inks which fall within the same range $\pm$10nm. Sodium and potassium nitrate are beneficial for students to see a wider range of flame colors and atomic emission lines. A sodium nitrate flame is bright orange and produces the most intense atomic emission lines around 580 nm, while potassium nitrate produces a pale purple flame and features intense atomic emission lines between 480 and 520 nm.[19]

DISCUSSION

Experimental Design and Development

The use of tattoo inks in the lab demonstrates the practicality behind course topics. Most tattoo inks are appropriate reagents for a flame test because they contain a detectable amount of metallic particulates.[15] At the high school level, experimental design and assessment follows the Next Generation Science Standards (NGSS) outlined by New York State for physical science/chemistry.[8] Specifically, this experiment follows the NGSS Science and Engineering Practice of formulating explanations of phenomena based on students' own knowledge and experimental results.[8] Whereas at the undergraduate level the experiment is designed to expose students to more instrumentation and critical thinking based on mathematical reasoning.

When conducting this experiment, it is recommended that between three and four different tattoo inks are analyzed. This way, students can be exposed to multiple different flame colors in a time efficient manner. Additionally, it is recommended that one of the inks analyzed is blue because blue inks typically contain copper and therefore produce green flames. The standard metal salt solutions can realistically be any chloride or nitrate-based salt; however, copper (II) chloride is optimal so students can compare the flame color to that of blue inks, and at the undergraduate level they can also compare the emission lines for each. Sodium nitrate produces an orange flame, much like iron, so it is useful to demonstrate to students that multiple different elements can have similar flame colors. Finally, potassium nitrate is recommended in the sample experiment because it produces an extremely pale purple flame, so students can view another unique flame color and also compare its emission behavior to other, similar metal salt solutions including sodium nitrate. Furthermore, undergraduate students can compare potassium and sodium based on their spectroscopic observations. Potassium will have lower energy than sodium because the gap between energy levels n=3 and n=4 is smaller than sodium's gap between n=4 and n=5. Due to this lower energy, potassium will show its most intense emission lines in the red region, whereas sodium shows its most intense emission lines in the yellow region.

One aspect that cannot be defined by flame test and spectroscopic analysis is the amount of metals in each ink. When using a spectroscope intended for classroom use, the limit of detection is expected to be lower than if a high-quality spectroscope were used. Therefore, it is possible that there is a metallic component to some inks that cannot be seen when analyzed by spectroscope. Intenze Brand Ink has a published chemical technology lab certificate confirming that the amount of metals in each ink are below the recommended limit. Metals like copper, zinc, and barium have higher limits of 25, 50, and 50 ppm respectively, whereas cadmium, chromium VI, and mercury have limits of 0.2 ppm.[18] As copper in Intenze blue inks could be resolved with the spectroscope, this suggests a limit of detection around 25 ppm for copper with the spectroscope used in this experiment.

Assessment Style

Although high school and undergraduate students learn much of the same foundational information in their respective courses, undergraduate students are generally expected to have a higher level of understanding than high school students. As such, the proposed assessment questions vary in style and requirements between the undergraduate and high school level laboratory assessments. Specifically, the pre- and post-lab assignments feature questions that exemplify the pedagogical goals for each experiment. Table 1 below indicates the reasoning behind each question asked in the pre- and post-lab assignments at the high school level.

Table 1: Outline of pedagogical purposes of each pre- and post-lab question for high school introductory chemistry level experiment "Detection of Metals in Tattoo Inks Through Flame Tests"

Assignment	Question	Purpose
Pre-Lab	Tattoo inks are typically composed of a mixture of solid particles, molecular dyes, binders, and water. Often times, heavy metals like copper, mercury, or lead are found in tattoo inks. What do you think is the purpose of including metals in tattoo inks? Explain your reasoning.	Students can hypothesize the usefulness of metals in tattoos
Pre-Lab	On the image of the Bunsen burner flame below, indicate the hottest part of the flame.	Identifying the hottest part of the flame lets students know where they should be placing the coffee stirrer containing ink during flame tests.
Post-Lab	Based on the flame colors you observed for the standard solutions ($NaNO_3$, KNO_3, and $CuCl_2$) which metals do you think could be present in the different tattoo inks you analyzed? Explain your reasoning.	Students should be able to make connections between the colors of the standard flames and the colors of certain tattoo ink flames.
Post-Lab	Using your knowledge and the figure below, describe the mechanism of atomic emission.	Students should be able to provide a guided explanation of the subatomic mechanism that occurred during the experiment they completed.

At the undergraduate level, students are expected to complete a pre-lab notebook entry, which provides an introduction complete with objectives, a procedure that is based on the procedure outlined in the assignment in Appendix C – but in their own words, and tables for data. The post-lab for this experiment involves students completing a detailed lab report that contains an introduction, procedure, results and discussion, and conclusion section. In the discussion section of their report, students are asked to provide answers to several specific questions. Table 2 below outlines each post-lab question and its pedagogical purpose.

Table 2: Outline of pedagogical purposes of each post-lab question for undergraduate general chemistry level experiment "Detection of Metals in Tattoo Inks Through Flame Test and Spectroscopic Analysis"

Question	**Purpose**
In your own words, explain the subatomic mechanism occurring during atomic emission.	Students can use critical thinking skills and their knowledge of the experiment to detail the subatomic mechanism occurring during atomic emission.
Define wavelength, frequency, and intensity.	By defining each of these terms, students can better understand the technicalities of spectroscopic analysis.
Using one of your recorded wavelengths from this experiment, calculate the frequency at that wavelength. Be sure to define the formula needed to calculate this value and show each step of the calculation.	Being able to calculate a frequency from an experimentally obtained wavelength demonstrates students ability to identify data and apply it to a mathematical equation.
During today's experiment, it is likely that you observed some atomic emission lines that were more intense than others. Provide a hypothesis as to why this occurs.	Students can use critical thinking skills to demonstrate their understanding of the relationship between atomic emission line intensity and number of atoms of an element.
During today's experiment, it is likely that you observed sharper atomic emission lines for the standard solutions than for the tattoo inks, making them "easier" to analyze. Provide a hypothesis as to why this occurs.	Students can use critical thinking skills to differentiate between a known lab standard and a real-world reagent that contains an unknown concentration of metals and may contain multiple metals.

Both the undergraduate and high school level experiments are complete with grading rubrics that specifically state the expectations for each section of the experiments. At the undergraduate level, effectiveness of the experiment can be based on the quality of lab reports submitted. Students who can write clearly about the experiment while defining concepts, listing and explaining their observations, and drawing conclusions about the inks demonstrate proficiency in this topic. At the high school level, since students are not expected to write a lab report, analysis of the effectiveness of the experiment should be based on the post lab assignment and future examination questions pertaining to the concept of atomic emission. To demonstrate proficiency, students should be able to craft thoughtful and thorough answers to post-lab questions. During examinations, questions relating to the topic of atomic emission or the experiment specifically can be used to show retention of lab concepts. Assessment for the actual experimental portion is based heavily on student conduct. Instructors should take note of students who do not actively participate and deduct points from their lab grade.

CONCLUSION

Identifying the metallic component of inks is important because it is thought that metals can cause adverse effects when injected into the dermis. Being that many tattoo inks contain a relatively high concentration of metals (20 to 50 ppm), inks can be used as reagents in educational flame test and spectroscopy experiments. The use of inks as reagents exemplifies the practicality of chemistry in a real-world context.

In order to develop the proposed high school and undergraduate lab experiments, 60 commercial tattoo inks were analyzed by flame spectroscopy. The process of flame test and spectroscopic analysis uncovered the identities of metals including copper, titanium, and iron in tattoo inks. Of the inks surveyed, every blue ink produced a green flame, and showed atomic emission lines corresponding to copper between 520 and 550 nm. Most red, yellow, and brown inks are hypothesized to include iron because they produced orange flames, which produced emission lines between 500 and 600 nm. Analyzing each of the white inks spectroscopically, gave emission lines between 600 and 650 nm, confirming the presence of titanium. Additionally, development demonstrated the limitations of instrumentation such as handheld spectroscopes, which are not capable of detecting lower concentrations (<2 ppm) of metals in inks. Optimal conditions for the high school and undergraduate experiments were also determined. When conducting experiments, the preferred carrier for inks are generic wooden coffee stirrers because they effectively transport ink into the flames and do not require any cleaning or cutting during preparation. The room in which experiments are conducted should be as dark as possible to allow for best possible visibility of flames and intensity and resolution of atomic emission lines. Drying the inks on coffee stirrers for approximately 20 minutes prior to flame testing was also shown to improve the intensity and resolution of atomic emission lines.

In order to deliver the concept of atomic emission, tattoo inks can be used as unknowns in an educational laboratory setting. The benefit to involving tattoo inks is to convey the utility and practicality of chemical concepts. The above results were then developed into a laboratory appropriate for either the high school or general chemistry level, which are presented in Appendix B and C.

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APPENDIX A

Table 3: Experimental data obtained through flame test and spectroscopic analysis on commercial tattoo inks

Ink Brand	***Ink Name***	***Flame Color***	***Most Intense Wavelengths Observed (nm)***	***Additional Observations***
Intenze	Mario Light Blue	Green	520-550	
Intenze	Mario Blue	Dark Green	520-550	
Intenze	Light Green	Orange	580-620	
Intenze	Dark Green	Orange	580-620	
Intenze	Snow White Opaque	Orange	650-700	Smoked and bubbled in flame
Intenze	Snow White Mixing	Orange	650-700	Smoked and bubbled in flame
Intenze	True Black	Blue	~590	
Intenze	Dark Brown	Orange	450-500	
Intenze	Light Brown	Orange	~620	
Intenze	Dark Red	Orange	500-590	
Intenze	Bright Red	Orange	400-450	
Intenze	Dark Purple	Orange	~590	
Intenze	Light Purple	Orange	~650	
Intenze	True Magenta	Orange	580-620	
Intenze	Light Magenta	Orange	500-600	
Intenze	Lemon Yellow	Orange	~580	
Intenze	Golden Yellow	Orange	580-600	
Intenze	Soft Orange	Orange	500-590	
Intenze	Hard Orange	Orange	590-620	
One Tattoo World	Tangerine	Orange	500-600	
One Tattoo World	Rose Pink	Orange	500-600	
One Tattoo World	Tomato is Red	Orange	~580	
One Tattoo World	Dark Red	Orange	500-600	
One Tattoo World	Lavender	Orange	~580	
One Tattoo World	Violet	Orange	540-580	
One Tattoo World	Baby Blue	Light Green	520-550	
One Tattoo World	Tsunami Blue	Green	520-550	
One Tattoo World	Slight Green	Orange	560-600	
One Tattoo World	Forest Green	Orange	540-580	
One Tattoo World	Black	Orange	~580	
One Tattoo World	Dark Chocolate	Orange	550-580	
One Tattoo World	Deep Yellow	Orange	500-600	
One Tattoo World	Bamboo	Orange	540-600	
Mom's	Ectoplasmic Green	Green	520-600	
Mom's	Hello Yellow	Orange	580-620	

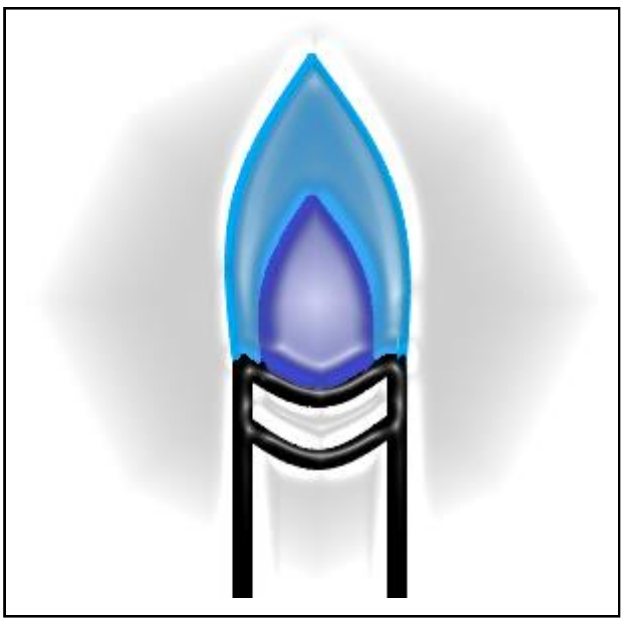
Mom's	Greyhound	Orange	~580	
Mom's	Monthly Red	Orange	500-600	
Mom's	Blue Balls	Dark Green	520-560	Most of the flame was dark green, but also contained sparks of pink on the outside of the flame
Mom's	Black Onyx	Orange	~580	
StarBrite	Brite White	Orange	650-700	Smoked and bubbled in flame
StarBrite	Scarlett Red	Orange	500-600	
StarBrite	Country Blue	Light Green	520-550	
StarBrite	Canary Yellow	Orange	~580	
StarBrite	Tribal Black	Orange	~580	
StarBrite	Jet Black Outliner	Orange	~580	
StarBrite	Lime Green	Orange	600-620	
Solong	Rose Pink	Orange	~580	
Solong	Bright Red	Orange	~580	
Solong	Medium Brown	Orange	500-600	
Solong	Snow White Opaque	Orange	650700	Smoked and bubbled in flame
Solong	Silver	Orange	500-580	
Solong	Lemon Yellow	Orange	~580	
Solong	Banana Cream	Orange	~580	
Solong	Fuchsia	Orange	520-600	
Solong	Baby Blue	Light Green	520-600	
Solong	Mario's Blue	Green	520-560	
Solong	Dark Purple	Orange	550-600	
Solong	Light Green	Orange	~580	
Solong	True Black	Orange	~580	
Solong	Bright Orange	Orange	550-600-	

APPENDIX B: High School Laboratory Experiment Resources

DO NOW

A. Tattoo inks are typically composed of a mixture of solid particles, molecular dyes, binders, and water. Often times, heavy metals like copper, mercury, or lead are found in tattoo inks. What do you think is the purpose of including metals in tattoo inks? Explain your reasoning.

B. On the image of the Bunsen burner flame below, indicate the hottest part of the flame.



Detection of Metals in Tattoo Inks Through Flame Tests

Introduction:

In the United States, approximately 30% of adults have tattoos.[14] Although tattoo artists and shops are heavily regulated to ensure the environment and equipment is sanitary and medicinally "safe" (i.e., proper licensing, appropriate disposal of needles; usage of PPE), the inks themselves have not been regulated by the United States Food and Drug Administration. Between 2004 and 2016, the US FDA received 363 reports of negative reactions to tattoos including rashes, fevers, redness and bumps, and more.[15] With increasing demands for tattoos, the lack of regulations for inks could allow for the production of cheap and dangerous products containing allergens, carcinogens, and otherwise harmful additives including – but not limited to – the usage of pigment from printer toner, car paint, and hair dye.[12] Additionally, certain heavy metals are added to tattoo inks to produce bright and stable colors, however the presence of metals can be harmful to consumers' health causing unwanted side effects.[15]

One way to analyze metals in tattoo inks is by conducting a flame test. Typically, electrons exist in a ground state configuration where each electron occupies its sequential energy level. When heated, however, an electron from the outer most shell is promoted to the next highest energy level. This unstable configuration is called the excited state. As the electron returns to the ground state, a photon – or particle that carries electromagnetic radiation – is emitted in the form of light, which is seen in the color of the flame. Due to each ion having a unique configuration, several different flame colors can be observed.

Safety and Environmental Concerns:

Safety goggles and appropriate clothing and shoes are to be worn to complete this experiment. This experiment deals with fire, and therefore long hair should be tied back, and students should take extra precautions when working with open flames.

Materials:

Four tattoo inks
Standard metal salts of $NaNO_3$, KNO_3, and $CuCl_2$
Three 100mL beakers
Coffee Stirrers
Butane flame torch or Bunsen burner
250mL beaker (for waste)
Water (for waste - does not need to be DI, tap water is fine as this is for waste purposes)

Procedure:

1. Tattoo inks will already be drying on stirrers when you come into the lab, but check to be sure you have enough stirrers for everyone in your group to test each color once.

2. Dissolve ~15.0 mL of each metal salt in 50.0 mL of deionized water. Place at least two wooden coffee stirrers (per person) in each beaker containing the standards.

3. Fill a large beaker halfway with water. This will serve as your waste beaker

4. Check to be sure you know the location of exits, fire blanket, and fire extinguisher in case of fire. For those with long hair, tie it back away from your face in a ponytail, bun, or other secure hairstyle.

5. When directed to by the teacher, light your Bunsen burner

6. Submit a coffee stirrer containing standard solution into the hottest part of a flame, place the stirrer into your waste beaker and record the flame color and any other observations

7. Repeat step 6 for each other standard solution and each tattoo ink

8. Turn off your Bunsen burner and clean up your workspace following the cleanup instructions provided by your teacher

9. Complete the exit ticket assignment

Data Collection:

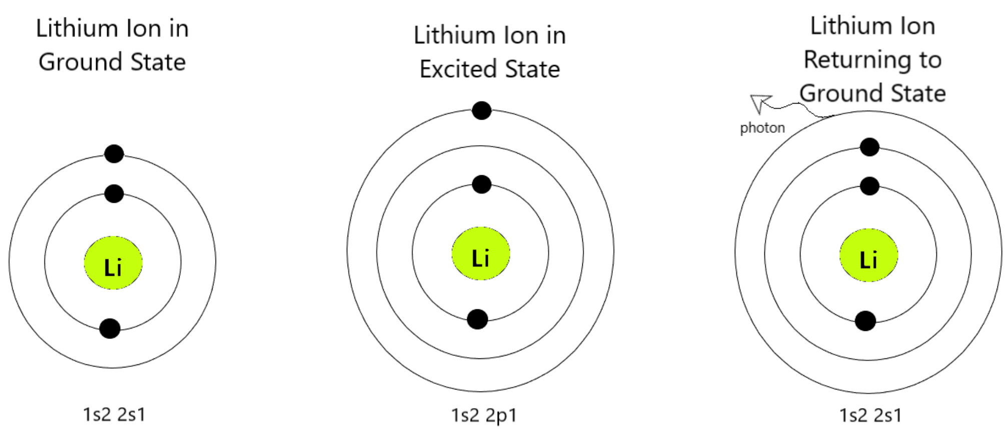
Table 1: Flame colors of various tattoo inks and standard solutions observed

Tattoo Ink or Standard	Flame Color	Additional Observations
Example Ink #1	*Orange*	*Color lasted for 3 seconds*

EXIT TICKET

A. Based on the flame colors you observed for the standard solutions ($NaNO_3$, KNO_3, and $CuCl_2$) which metals do you think could be present in the different tattoo inks you analyzed? Explain your reasoning.

B. Using your knowledge and the figure below, describe the mechanism of atomic emission.



GRADING RUBRIC			
	Full Credit	Partial Credit	Zero Credit
Do Now Assignment	Answer to Part A indicates a clear hypothesis with examples and reasoning. Answer for Part B correctly labels hottest part of flame	Answer to Part A indicates a hypothesis but lacks either examples or reasoning Answer to Part B correctly labels hottest part of flame	Answer to Part A lacks a hypothesis or is irrelevant or incomplete Answer to Part B is blank or illegible
Lab Session	Student appropriately follows procedural and safety instructions Student works cohesively with their group to analyze flames	Student follows procedural and safety instructions Student puts adequate effort into group work	Student does not follow procedural and safety instructions Student copies data from peers or is uninvolved in the process of analyzing flames
Exit Ticket	Answer to Part A thoughtfully identifies metals present in tattoo inks with clear reasoning. Answer to Part B correctly describes the subatomic mechanism of atomic emission	Answer to Part A identifies metals present in tattoo inks providing evidence for most inks. Answer to Part B indicates an adequate level of understanding of atomic emission	Answer to Part A is incomplete or blank Answer to Part B is irrelevant or blank

APPENDIX C: Undergraduate General Chemistry Laboratory Resources

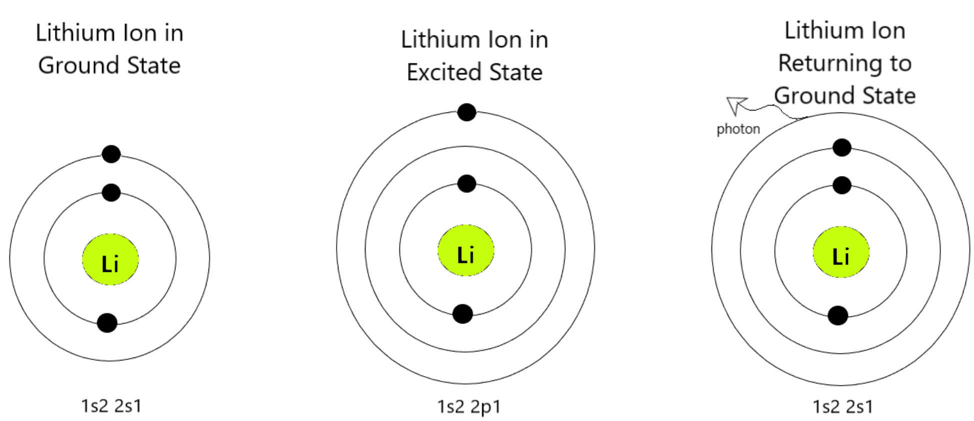
Detection of Metals in Tattoo Inks Through Flame Test and Spectroscopic Analysis

Introduction:

In the United States, approximately 30% of adults have tattoos.[14] Although tattoo artists and shops are heavily regulated to ensure the environment and equipment is sanitary and medicinally "safe" (i.e., proper licensing, appropriate disposal of needles; usage of PPE), the inks themselves have not been regulated by the United States Food and Drug Administration. Between 2004 and 2016, the US FDA received 363 reports of negative reactions to tattoos including rashes, fevers, redness and bumps, and more.[15] With increasing demands for tattoos, the lack of regulations for inks could allow for the production of cheap and dangerous products containing allergens, carcinogens, and otherwise harmful additives including – but not limited to – the usage of pigment from printer toner, car paint, and hair dye.[12] Additionally, certain heavy metals are added to tattoo inks to produce bright and stable colors, however the presence of metals can be harmful to consumers' health causing unwanted side effects.[15]

One way to analyze metals in tattoo inks is by conducting a flame test. Typically, electrons exist in a ground state configuration where each electron occupies its sequential energy level. When heated, however, an electron from the outer most shell is promoted to the next highest energy level. This unstable configuration is called the excited state. As the electron returns to the ground state, a photon – or particle that carries electromagnetic radiation – is emitted in the form of light, which is seen in the color of the flame. Due to each ion having a unique configuration, several different flame colors can be observed. Figure 1 below shows this process at the subatomic level.

Figure 1: Demonstration of the movement of electrons during a flame test.



To further confirm the identity of metals in tattoo inks and other samples, spectroscopic analysis can be extremely helpful. Each element has its own unique line emission spectrum, which serves as a fingerprint for easy identification. Spectroscopes function by measuring electromagnetic radiation in the form of visible light in wavelengths. Wavelengths measure the distance between waves in nanometers. The color spectrum in Figure 2 below shows the differences in wavelengths amongst visible light.

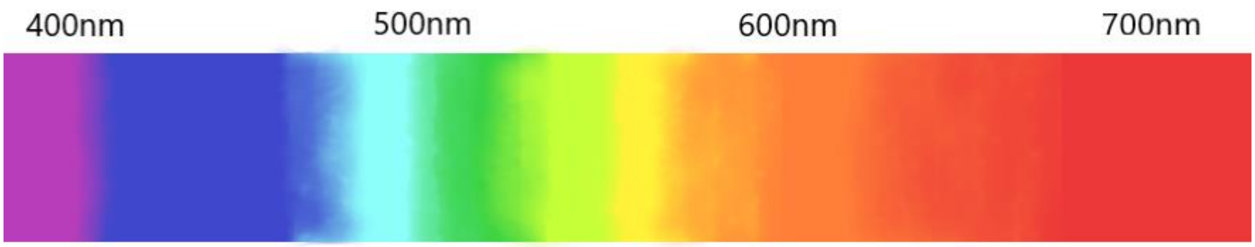


Figure 2: Wavelengths (nm) of electromagnetic radiation in the form of visible light

The purpose of this experiment is to determine what metals are present in a set of tattoo inks through flame tests and spectroscopic analysis.

Safety and Environmental Concerns:

Chemical splash goggles, a lab apron/coat, and appropriate clothing and shoes are to be worn to complete this experiment. This experiment deals with fire, and therefore long hair should be tied back, and students should take extra precautions when working with open flames. Additionally, while tattoo inks will not harm or permanently stain skin, it is recommended you wear gloves when preparing inks to prevent them from getting on skin, as they will take some time to come off.

Materials:

Four tattoo inks
Standard metal salts KNO_3, $NaNO_3$, and $CuCl_2$
Deionized water
Three 100mL beakers
Four 1.5mL vials
Coffee Stirrers
Butane flame torch or Bunsen burner
250mL beaker (for waste)
Water (does not need to be DI, tap water is fine as this is for waste purposes)
Spectroscope

Procedure:

Dissolve ~15.0 mL of each metal salt in 50.0 mL of deionized water. Place at least four wooden coffee stirrers (per person) in each beaker containing the standards. Obtain ~1 mL samples of each tattoo ink and the standard solution into the small vials. Once ready to conduct flame tests, submerge wooden coffee stirrer into ink and be sure to thoroughly coat the stirrer. Carefully light your torch or Bunsen burner and submit the stirrer with ink into the hottest part of the flame. Record the color of the flame and any further observations. Repeat for all tattoo inks and the standard solution. Subsequently, repeat this process, but this time using the spectroscope to observe the spectral lines of each ink. Record the spectral lines you observe. Repeat this process three times for each ink.

Data Collection:

Table 1: Flame colors of various tattoo inks and standards observed

Tattoo Ink or Standard	**Flame Color**	**Additional Observations**

Table 2: Emission spectra wavelengths of various tattoo inks observed

Tattoo Ink	**Trial 1 Wavelengths Observed (nm)**	**Trial 2 Wavelengths Observed (nm)**	**Trial 3 Wavelengths Observed (nm)**

Post Lab Assessment Questions – *To be answered in the Discussion section of your lab report*

1. In your own words, explain the subatomic mechanism occurring during atomic emission.
2. Define wavelength, frequency, and intensity.
3. Using one of your recorded wavelengths from this experiment, calculate the frequency at that wavelength. Be sure to define the formula needed to calculate this value and show each step of the calculation.
4. During today's experiment, it is likely that you observed some atomic emission lines that were more intense than others. Provide a hypothesis as to why this occurs.
5. During today's experiment, it is likely that you observed sharper atomic emission lines for the standard solutions than for the tattoo inks, making them "easier" to analyze. Provide a hypothesis as to why this occurs.

Be sure to label your answer to each question in your lab report

Grading Rubric

	Full Credit	Partial Credit	Zero Credit
Pre-Lab Preparation	Student comes to class with a completed pre-lab entry in their laboratory notebook that demonstrates they have read the lab and thoroughly understand the objectives and expectations for this session. Student is dressed appropriately for lab	Student comes to class with a complete pre-lab entry in their laboratory notebook that indicates some level of preparation and an adequate level of understanding of objectives and expectations for this session. Student is dressed appropriately for lab	Student comes to class with an incomplete pre-lab, no laboratory notebook, or is dressed inappropriately for lab **In this case, the student should <u>not</u> be admitted into the lab**
Lab Session	Student appropriately follows procedural and safety instructions. Student works cohesively with their partner to analyze flames	Student follows procedural and safety instructions. Student puts adequate effort into partner work	Student does not follow procedural and safety instructions. Student copies data from peers or is uninvolved in the process of analyzing flames
Lab Report	Student crafts a well-organized lab report that includes complete Introduction, Procedure, Results & Discussion, and Conclusion sections. Each section indicates an exceptional level of understanding of the experiment performed	Student writes an organized report with Introduction, Procedure, Results & Discussion, and Conclusion sections. One or more sections indicates a lack of comprehension of the experiment	Student submits a disorganized, irrelevant, or blank report. It is clear that the student either lacks an understanding of the material or did not put effort into the assignment
Assessment Questions	Questions are answered and labeled in the appropriate section of the report. Answers are organized, thoughtful, and provide explanations and reasoning.	Questions are answered and labeled in the appropriate section of the report. Answers are thoughtful, but demonstrate a lack of conceptual understanding	Answers are incomplete, irrelevant, unanswered, or unlabeled.