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Activity

K-5 Thin-Layer Chromatography: Three-Dimensional Analysis of Pigments from Plant Materials Using an Interlocking Building-Block Photography Box

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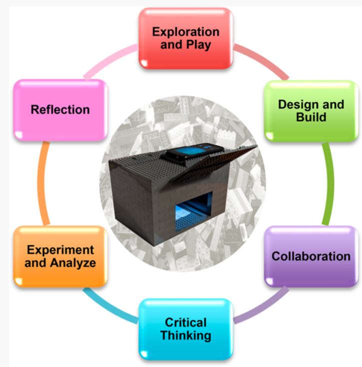
ABSTRACT: It is widely understood that students are able to learn and comprehend topics better as active participants. Topics related to science, technology, engineering, and mathematics are often mistakenly considered too difficult for elementary students to grasp. Conducting simple hands-on science experiments can be a useful instructional approach, allowing young scholars the ability to better grasp key scientific principles. We felt chromatography would make an ideal technique to incorporate into the elementary school curricula due to its ubiquitous use throughout the field of chemistry. We have designed an activity that combines chromatography with pigments extracted from plant materials to create a new, interactive activity that allows students to take on the role of a scientist. Students extract pigments such as chlorophyll, anthocyanins, flavonoids, and carotenes from plant materials, and separate the pigments using thin layer chromatography. The students construct a photography box from interlocking building-blocks and build a circuit that is used as a light source for the box. The developed thin layer chromatography paper is placed inside the box, and using a smartphone, a photograph is taken for analysis. Using a python script developed for this activity, students are able to analyze the photograph of the thin layer chromatography paper in a three-dimensional space that determines the red, green, and blue values, and the hue and saturation values of the pigments. This activity allows students to actively learn important scientific principles such as chromatography, color theory, and circuitry, all while working with common household items and toys, such as interlocking building-blocks, and smartphones, for instrument construction and analysis.



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Students have a greater comprehension of scientific concepts when they are able to participate and perform the experiments themselves.[1] Bransford and colleagues have shown that exposing pupils to scientific concepts at a young age, such as kindergarten through fifth grade, hereafter referred to as K-5, generates an aptitude and motivation for science that is carried forward throughout the pupil's education and vocation.[2] Students in K-5 classrooms, however, generally do not have an opportunity to conduct hands-on scientific research experiments, often due to the lack of equipment and tools for analysis. However, there are tools, instruments, and machines used in research settings that can be incorporated and brought into the classroom to help K-5 students.[3]

It is widely understood that exposing students to science-based concepts at an early age helps the development of the student to become more versed and confident in their abilities to pursue their education in science, technology, engineering, and/or mathematics (STEM) related fields.[4−7] Developing elementary school student's growth-mindset, the belief we are inherently able to learn, grow, and change through experiences and failures, is key for the student's ability to pursue an education in STEM.[8] Thus, bridging the gap between research science and K-5 classrooms curricula will motivate students to pursue an education in STEM fields.[9−12]

Since the early 19th century, thin-layer chromatography (TLC) has been a common technique used in chemistry to separate a mixture of compounds into their individual components. One of the first influential reports of using TLC was by Richard Willstätter, who won the Nobel Prize in Chemistry for his discovery of chlorophyll using TLC.[13] Since then, TLC has become a widely used teaching tool in undergraduate level chemistry courses to illustrate chromatography; one of the fundamental concepts in chemistry.[14−19] Thin-layer chromatography experiments are relatively quick to perform, safe, easy to dispose of waste, and cost-effective.[20]

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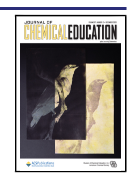

Simple experiments, such as TLC, would help elementary students be exposed to and learn one of the central concepts taught in undergraduate level chemistry courses, chromatography.

While previous experimental reports use TLC to target the undergraduate level of education,[14,21] we are building upon current frameworks to effortlessly bring the concept of chromatography into elementary school curricula.[22−25] We have designed a kid-centric activity that involves the use of interlocking building-blocks, photography, circuitry, TLC, and three-dimensional (3D) visualization. We report transforming a previously used tool from a chemistry laboratory, a nerve gas detector built out of interlocking building-blocks, into a TLC photography device.[26] The elementary school students extract a mixture of chlorophyll, flavonoids, carotenes, and/or anthocyanins from plant materials. As previously reported, thin-layer chromatography has been used to separate plant pigments, and based on their color students can differentiate the pigments present.[27−29]

We have taken this precedent to generate an experiment in which elementary school students learn to carry out collaborative work as they build a photography box and light source from interlocking building-blocks, and circuitry materials, respectively. To further analyze the pigments on the TLC paper, we have developed a kid-friendly computer program to provide a full comprehension of the pigments, red, green, and blue (RGB) values, and the hue saturation values (HSV). The RGB value and HSV of a color describes the prominent color we detect with our eyes, and the amount of gray present within the color, respectively.[30,31] In contrast to a two-dimensional TLC paper, this program allows students to explore and visualize the TLC paper in a 3D color space. The students thereby take on the role of a scientist to learn key scientific concepts such as chromatography, instrumental analysis, mixtures in solutions, 3D visualization, and circuitry, all while using a construction medium with which they are familiar, interlocking building-blocks. We demonstrate that the activity described helps students learn key scientific concepts while having fun with interlocking building-blocks.

■ EXPERIMENTAL OVERVIEW

Thin-Layer Chromatography

Colorful nontoxic plant materials were used as samples for spotting a TLC paper. Nontoxic plant materials, such as deciduous tree leaves, spinach, beets, and carrots contain chromophores that can be readily extracted.[32−35] For this activity vegetal samples such as spinach, beet root, beet leaf, and carrot root (Figure 1A) were the selected plant materials used. Pigments from the plant materials were isolated by grinding the plant foliage using a mortar with a pestle, and extracting the pigments in isopropyl alcohol (Figure 1B). To make a concentrated solution of the pigments, 3 mL of isopropyl alcohol was used for 3 large spinach leaves, 1/6 of a small beat root, 1 beet leaf, and 1/5 of a medium carrot root. If the solution is too concentrated, isopropyl alcohol can be added to dilute the solution. If the solution is too dilute, additional ground plant material can be added to the solution. To follow their work flow, the students can fill out an Assessment Work Sheet (English and Spanish, see Supporting Information) or write their procedure in a designated laboratory notebook.

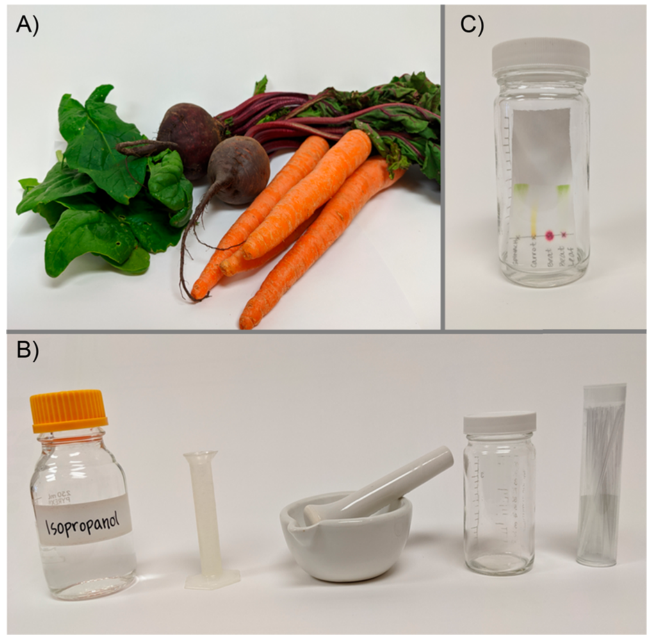

Figure 1. (A) Spinach, beets, and carrots were the plant materials used for sample mixtures; (B) items used for extracting the pigment from the plant materials: isopropyl alcohol, graduated cylinder, mortar and pestle, glass jar with a secure top, and capillary tubes or dropper (left to right); (C) TLC chamber containing the TLC paper using isopropyl alcohol as the eluent.

Using a dropper, the solutions of the plant material were spotted on chromatography paper (2 in. × 4 in.). A pencil was used to mark the chromatography paper in advance, indicating where each spot originated. Once spotted, the TLC paper was put into the TLC chamber and eluted using isopropyl alcohol as the eluent (Figure 1C).

Circuitry

To build the light source that goes inside the photography box, a simple circuit was assembled by the students. The circuit consisted of a single pole throw electrical knife switch, alligator clip leads, light emitting diode (LED), a 3-V button cell battery, and a coin cell battery holder. Once the leads connect the battery source to the LED and switch, as shown in Figure 2, the switch can be in the open (Figure 2A) and closed (Figure 2B) configuration to turn the light off and on, respectively.

Once the circuit was made it was placed inside the box and used as a light source to illuminate the TLC paper for a photograph. The leads were secured inside the box by using removable mounting putty. One or two LEDs can be used to illuminate the interior of the box. If one LED is used as a light source, the LED is placed in the center of the ceiling inside the box. If two LEDs are used, then the LEDs are placed in opposite corners of one another on the ceiling of the box.

Photography Box Construction

The photography box consists of a door with a hinge, a smart phone holder, and a tray inside the box for the TLC paper (Figure 3). The smart phone holder has a customizable hole for the camera of the smart phone to view what is inside the box. There is a holding tray inside the box where the TLC paper can be placed for optimal quality for photography of the TLC paper. The door is constructed to prevent light entering inside the box. Pictures of the TLC paper inside the box are

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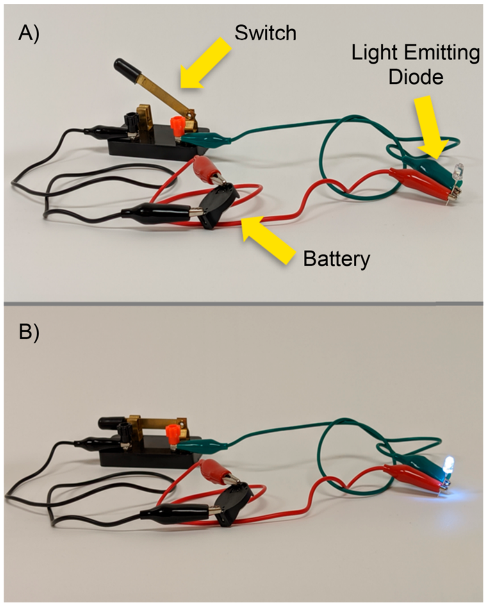

Figure 2. (A) Circuitry with switch open; (B) circuitry with switch closed.

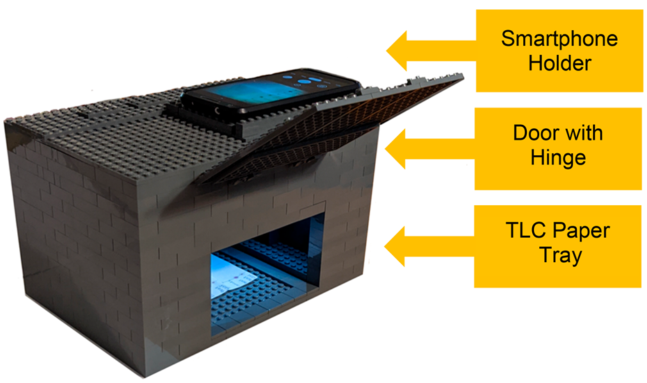

Figure 3. Photography box fully constructed.

done with the flash turned off. For further details with the construction of the photography box please see ref 26.

■ DATA ANALYSIS

Once the TLC paper was spotted with the desired plant materials (Figure 4A), and eluted (Figure 4B), the students processed the finished TLC paper using the photography box to take a picture of their developed TLC papers. To avoid the pigments from fading, photography analysis of the developed TLC paper was done within 48 h. In Figure 4, green is chlorophyll, yellow is the carotene, and red represents the anthocyanins.

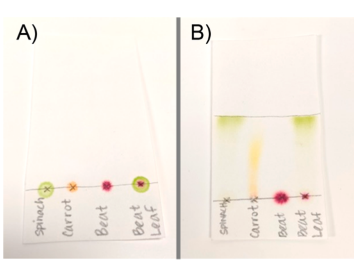

Figure 4. (A) Spotted TLC paper prior to elution in the TLC Chamber (spinach, carrot, beet, beet leaf); (B) postelution.

To analyze the developed TLC paper, students place the TLC paper inside the box equipped with the light source. Then using a smart phone, the students take a photograph of the developed TLC paper inside the photography box. This picture can be used for presentations, as a hard copy for their records, and/or can be used in the following extension segment.

Extension

Using a program our group developed for this activity (see Supporting Information), the students are able to analyze the pigments present on the photographed TLC paper in two different color spaces, RGB and HSV. The RGB color space shows a band of the colors present on the TLC paper because it describes the association between the colors. While in contrast, the HSV color space gives discrete color blocks due to the amount of gray that is present with each designated color. Using either a Macintosh (Mac) and Windows operating system, the photograph can be uploaded to the programs software to generate interactive 3D graphs that determine the RGB and HSV color spaces for the pigments present on the TLC paper (Figure 5A,B). The program implements the use of OpenCV to provide image manipulation tools along with Matplotlib, a python library designed for creating interactive visualizations. To run the program, one simply needs to double click the executable file and select the file of the desired image in the file finder, and then the analysis will begin (see Supporting Information). The informative, interactive 3D

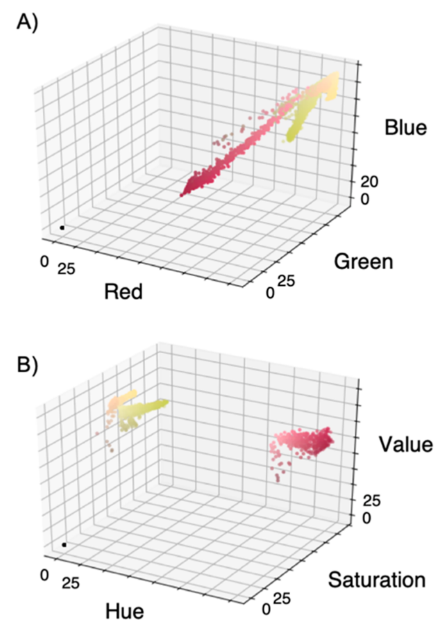

Figure 5. (A) Interactive 3D graph of the RGB values of the developed TLC paper in Figure 4; (B) interactive 3D graph of the HSV of the developed TLC paper in Figure 4. Here, in both plots we have displayed three colors: green, yellow, and red corresponding to the three different colors found in the TLC for chlorophyll, carotene, and anthocyanins, respectively. However, each color is generated by combinations of RGB and/or HSV. Each individual dot in the figures represents a pixel from the original TLC image that was subsequently analyzed by our Color Spotter software.

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visuals of the TLC paper, make this program an invaluable tool to educate young students about colors.

■ HAZARDS

In preparation for the experiment, read the safety data sheets (SDS) of isopropyl alcohol and any other chemicals with which the students may come in contact during the process (see Supporting Information). Personal protective equipment, such as safety glasses, gloves, and laboratory coats are highly recommended while handling isopropyl alcohol. All experiments should take place in a well-ventilated room.

Plant materials can be toxic to humans. It is strongly encouraged to avoid toxic plants by having a general knowledge and awareness of the plant materials being used for the experiment. To determine if a plant is toxic (1) call your local land grant extension agent, (2) conduct a thorough online search of the plant materials, or (3) go to your local library and conduct research regarding known toxic plants in the community.

If isopropyl alcohol and/or a toxic plant material has been ingested consult with American Association of Poison Control Centers.

Waste Disposal

Please review your federal, state, and local environmental control regulations prior to disposing waste materials such as isopropyl alcohol. In general, once the material is dry (all of the isopropyl alcohol has evaporated) the waste material can be thrown away with general waste.

■ RESULTS AND DISCUSSION

The experiment described above was conducted by a fourth grade class with a total of 13 students. The students worked in a total of four research groups, three groups of three and one group of four. The teacher used terms that embodied the students experience as a scientist.

Each student brought plant materials from home for the extraction process. Students were able to use a mortar and pestle to extract the pigments from their plant material using isopropyl alcohol. Each student successfully spotted their solution onto a precut chromatography paper and put their initials in pencil below their spot for identification. The students were able to put their TLC paper into the TLC chamber with the solvent. After the TLC paper eluted the students were able to directly compare each other's spot. From this point they were able to work together to figure out how to develop a procedure to either (1) create a sample that will create the greatest contrast in color, or (2) create a sample that would have little to no visible color contrast on the TLC paper. The majority of the research groups optimized the extraction process to make a concentrated sample to have the greatest contrast in color on the TLC paper.

As a class, the students were able to work together as a team to build one photography box. Then each research group was given circuity materials to work with and learn how to make a light-source for the box. For students that had difficulty grasping the concept of circuitry, the circuit was modified by either not incorporating the switch, or by only using the LED and a coin battery (see Supporting Information).

The students are able to use the photography box to take a picture of the developed TLC paper using their own smartphone, or that provided from the teacher. For further analysis, the students can use the computer program developed to determine the RGB values and HSV of the pigments present on the TLC paper (Figure 4). Using the computer program, students are able to upload the photograph of the developed TLC paper, and a series of interactive 3D graphs allow students to determine the RGB- and HSV-color space.

The computer program can be used to prompt a discussion of color with the students. For example, of the two different color spaces, RGB is more widely known due to the use of primary colors (red, green, and blue, Figure 5A) in elementary schools, mainly used for mixing paints or dyes to make tertiary colors. In contrast, the HSV color space is less intuitive by name (Figure 5B), and it is what students are accustomed to seeing every day in landscapes with natural lighting. The hue, which is one of the components of HSV, is the color ranging from Red at 0 degrees to Magenta at 360 deg. The second component of HSV, saturation, is the amount of gray producing a color's faded appearance. The colors faded effect is described by how much white or black is present within the given color. An example of the difference between RGB and HSV color space is a shadow being cast on an object in a landscape. In RGB color space, the shadow cast on an object will be distinctively different to the eye compared to the part without the shadow. The hue component of an object with a shadow will be relatively the same, while in contrast, the saturation value will be different due to the amount of gray present in the shadow compared to where there is no shadow. Learning distinctions between these two different color spaces and the effects altering the x, y, and z axes of these color spaces will have on colors should provide exciting insight into what makes up colors.

The RGB and HSV issues just discussed can be seen in Figure 5 as a spread of dots, which represent the pixels analyzed and plotted by our computer program, for the individual three colors green, yellow, and red. This figure demonstrates that the colors seen by one's eye are actually made up of many mixtures of RGB and HSV, and helps the teacher to demonstrate the lessons given in elementary schools that colors are actually composites of many different colorimetric features.

Incorporating interlocking building-blocks into the student's curricula helped to break down the barrier of learning chromatography and circuitry and made the topics fun. Students were able to work together as a team to build the photography box while simultaneously "playing" with interlocking building-blocks and learning key concepts. This made the learning process easier for the students and they were able to focus on learning the fundamental concepts, such as circuitry, chromatography, extractions, color processing, and mixtures and solutions. The students understood how to make a circuit that allows the LED to illuminate. Furthermore, the addition of the switch helped to further enhance their knowledge that you are able to have a closed loop that allows the LED to illuminate, and in contrast, you can break the closed loop by opening the switch.

Further, the students understood they were extracting pigments from the plant materials to make a solution that is composed of a mixture of pigments. The students reasoned that they were able to separate and determine the different colored pigments in their mixture by eluting them on a TLC paper. They were able to understand that the pigment can be separated because each colored material interacts differently with the chromatography paper and solvent.

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One of the benchmark achievements of this activity was to expose the students to the concept of chromatography as a separation of a mixture into individual components, and a second was a mastery of constructing instrumental analysis platforms as a scientific skill. While the students were able to master these topics, softs skills such as collaboration, team work, constructive communication, and empathy were also promoted. After the students were able to extract their plant materials' pigment and develop their TLC paper, they were able to collaborate within research groups, and with other research groups, to determine the optimal ratio of plant materials to solvent for making the brightest colored TLC paper. Within their research groups they were able to work together to figure out how to put the circuit together to make the LED work. Students who had prior experience using interlocking building-blocks took on leadership roles, developing empathy for students who did not know how to construct objects with interlocking building-blocks. They began using the same terms used by the teacher, such as research groups, primary colors, solvent, extraction, and scientists.

Overall, the students were able to embrace a growth-mindset which allowed them to respond and adapt appropriately to making mistakes, an essential part of any investigative process. As a result of this growth-mindset, the students took ownership of their own research, allowing them to communicate, adjust, and troubleshoot issues; such as how much plant material, and/or solvent, they should add to get optimal results for a strong pigment on the TLC paper. The elementary school students responded positively to the opportunity to take on the role of a scientist and being in charge of their own independent investigative work.

■ SUMMARY

We have been able to successfully transform a device used in academic research into a teaching tool for elementary school students. The students were able to successfully gather plant material, extract the pigment from the plant materials, use TLC separate the pigments, build a light source from circuity materials, and construct the photography box for the data analysis. Students were able to learn the key concepts of chromatography, experimental analysis, colors, and circuitry, while working with interlocking building-blocks. In addition, they learned how to collaborate, work in a team, and have empathy toward one another. This activity helped to demote any preconceived notion that learning STEM related concepts is difficult, and it built confidence in the student's own ability to learn.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.0c00625.

> Safety data sheet of isopropyl alcohol; instructions for using the color spotter software and script; lesson overview; assessment work sheets and instructor handouts (English and Spanish); circuitry instructions and modifications; results from the activity (PDF)

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Notes

The authors declare no competing financial interest.

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