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Anthocyanin Watercolors – Part 1

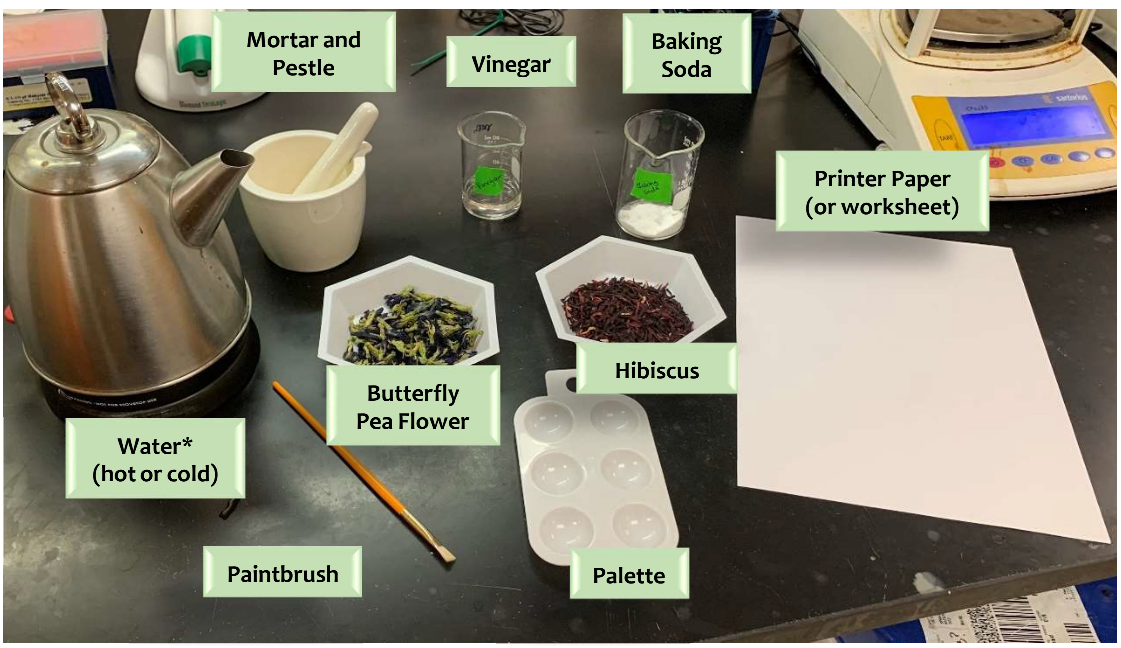
Biochemistry is art! In this activity, you will be making watercolors using the **anthocyanins** in hibiscus and butterfly pea flowers. These are the same flowers used in popular teas (though we wouldn't recommend eating the ones in this kit). *This activity uses weak acids (white vinegar) and bases (baking soda), as well as possible allergens (flowers) – take extra precautions, like wearing gloves, if you have allergies or are skin-sensitive to pH changes.*

Martin Lab at UC Irvine YouTube Video: https://youtu.be/7K37wQvJrpU

In Part 1 of this activity, you will learn:

- The relationship between visible light absorbance and the color of an object.
- The relationship between wavelength and energy.

You will need:



* Hot or cold water will work, but be safe if you choose to use hot water.

We recommend first going through this worksheet sequentially, with the flowers we have provided, but we will never discourage experimentation!

Background – Anthocyanins:

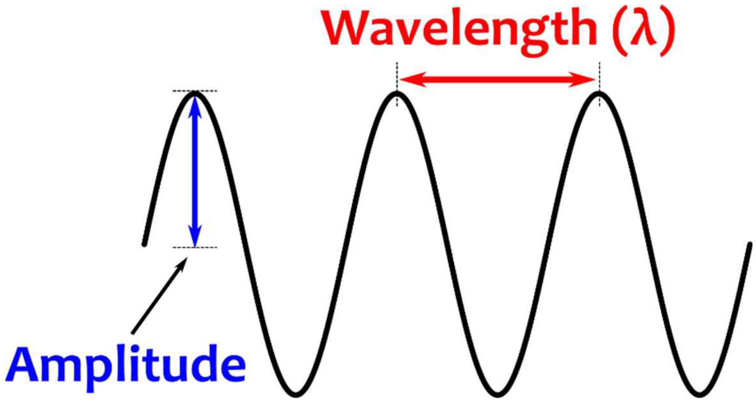
Anthocyanins are plant pigments that give flowers, fruits, and vegetables their red, blue, and purple colors. They belong to a class of molecules called **flavonoids**, which are known for their antioxidant properties. In both plants and humans, antioxidants play a role in protecting cells against unstable molecules, called **reactive oxygen species (ROS)**, which can damage genetic material and proteins. In plants, anthocyanins also serve in reproduction, producing the vibrant colors that attract pollinators and seed dispersers.

Their colorful properties and nontoxic nature make them good candidates for home-made food dyes, and have recently been explored as possible hair dye alternatives (Rose *et al.*, 2018). Since a number of anthocyanins can be found in the waste products of important crops, transitioning some of our dyeing needs to these biomolecules may also help eliminate agricultural waste. Our goal in the Martin lab is to contribute to the utility of these multifunctional pigments by characterizing as many as we can in a variety of different plants.

In this activity, you'll be using anthocyanins to make watercolors – but first, let's dive into a bit of the physics behind their vibrant color palettes.

Background – Visible Light and Color:

Have you ever wondered why black cars heat up more quickly in the sun than white cars? It has little to do with tricks of design or types of metal, and everything to do with the wavelengths of visible light they absorb. In fact, all colorful objects, including the flowers used in this activity, appear the way they do because of the way they absorb light. To understand this, we first must understand what visible light is.



Light acts like a wave. It has an **amplitude** and a **wavelength**. The amplitude determines the intensity of the light, while the wavelength, denoted λ (lambda), determines the type of light and its **energy**.

The inverse relationship between energy and wavelength can be seen in this equation:

$$E = \frac{hc}{\lambda}$$

$E = energy, h = Planck's\ constant, c = the\ speed\ of\ light, \lambda = wavelength$

In other words, the longer the wavelength, the lower the energy and vice versa. Ultraviolet (UV) light, which includes the kind that gives you sunburns, is relatively high energy, ranging from around 10-400 nm in wavelength. **Visible light**, on the other hand, is lower energy, with wavelengths around 400-700 nm.

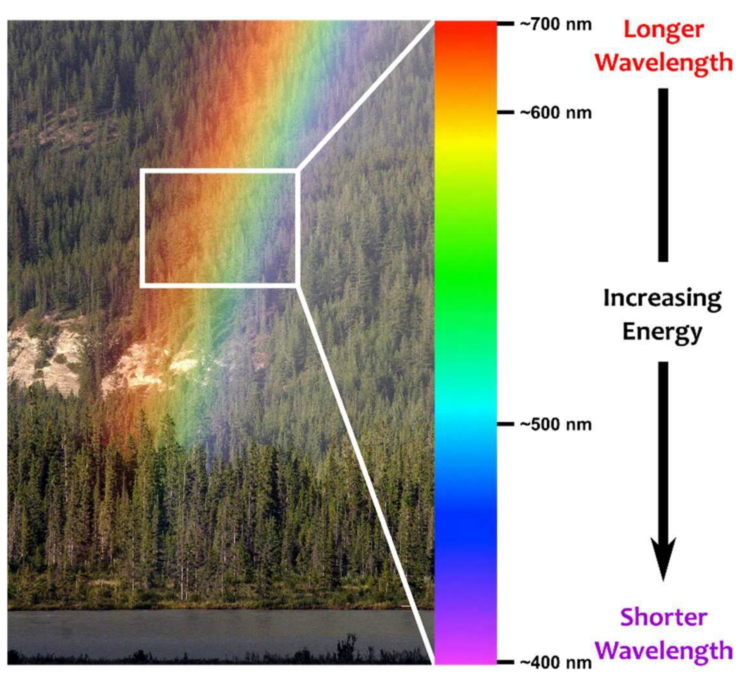
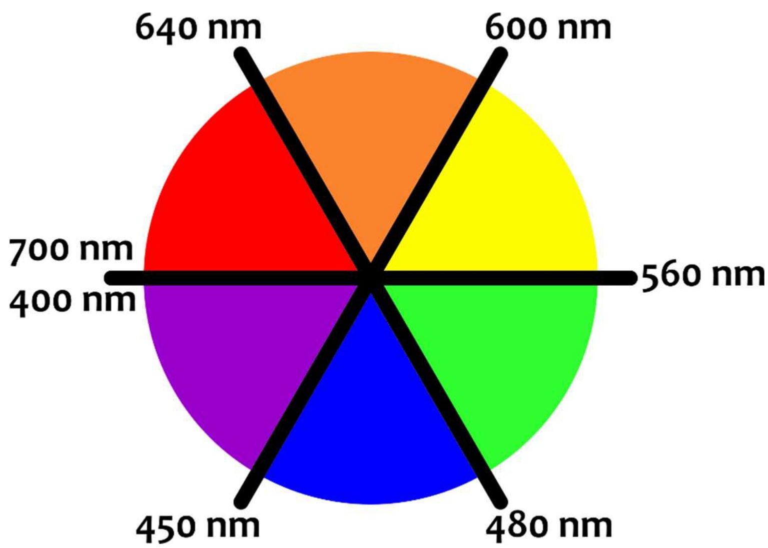


Image credits: Rainbow – Wing-Chi Poon/Wikimedia Commons; Spectrum adapted from (Gonsher, 2019).

If you've ever seen a rainbow, you've witnessed the full **spectrum** of visible light. Each wavelength corresponds to a different color, ordered longest to shortest. In a rainbow, these wavelengths are visible as separate colors – this is due to the way in which light is refracted and reflected in water droplets – but normally, these wavelengths combine to make white light. Likewise, an absence of all visible light appears black, which is why you can't see anything in the dark – so what does this have to do with flowers and hot cars?

It turns out that the perceived color of an object depends on the wavelength(s) of visible light it absorbs. An object that absorbs *no* visible light will appear white, while one that absorbs *all* visible light will appear black. What we see is a combination of wavelengths that were reflected (not absorbed). Black cars heat up faster because they absorb all visible light and its energy, while white cars reflect it.

For humans, when an object primarily absorbs one color of visible light, it will appear as the **complementary** color on the color wheel. For example, a shirt that primarily absorbs wavelengths around 670 nm (red) will appear green. The anthocyanin pigments that give flowers their vibrant reds and blues absorb light in the same way.



This is a result of how our eyes work; we have three types of receptors, called **opsins**, which absorb different wavelengths and allow us to see in combinations of red, green, and blue-violet. Other animals have different opsins, and see color differently. Dogs, for instance, see in combinations of blue and yellow because they only have two opsins. Birds have four, and can see into the UV part of the spectrum!

In this activity, we'll be exploring the range of colors anthocyanins can produce. As you paint, see if you can tell which wavelengths are being absorbed!

Instructions – Butterfly Pea Flower:

Let's start with butterfly pea flowers. They are a favorite in tea colorants.

1. Take 3 or 4 butterfly pea flowers and remove the tough green base from the blue petals (this doesn't have to be perfect). This green part of the plant does not contain many anthocyanins and is tough to break down. Place the petals in the mortar.
2. Begin grinding the petals with the pestle. They may be difficult to grind down, and that is ok! Mash them up as much as you can, then proceed to the next step.
3. Add just enough water to cover the petals and continue grinding. Hot water may speed up the process, but cold water works well too. You likely will end up with squishy clumps of petals – press as much pigment out of them as you can. Adding more or less water will change the concentration – play around with different dilutions and see what you like.
4. (Optional) Use a coffee filter (not provided) to filter the finished product into your palette. If you do not have a filter on hand, painting with the unfiltered product will still work, but you may have solid grains in your painting.

Question: Roughly what wavelength of visible light is being absorbed by this paint? What color light is that wavelength? Is it low or high energy on the visible light spectrum?

Answer: ____

You are now ready to paint! Grab your paintbrush and give us your best in the box below:

Instructions – Hibiscus:

Now we will try hibiscus. Be sure to rinse out the mortar and pestle before using it again. The process is much the same as with butterfly pea flower.

1. Grab 2 or 3 pinches of hibiscus and place them in the mortar.
2. Grind these pieces into a rough powder with the pestle before proceeding to the next step. These will be easier to break down than the butterfly pea flower petals were.
3. Add just enough water to cover the powder and continue grinding. Hot water may speed up the process, but cold water works well too. Adding more or less water will change the concentration – play around with different dilutions and see what you like.

4. (Optional) Use a coffee filter (not provided) to filter the finished product into your palette. If you do not have a filter on hand, painting with the unfiltered product will still work, but you may have solid grains in your painting.

Question: Roughly what wavelength of visible light is being absorbed by this paint? What color light is that wavelength? Is it low or high energy on the visible light spectrum?

Answer: ____

You are again ready to paint! Show us your art in the box below:

Did anything unexpected happen? Check out Part 2 for more on the biochemical properties of anthocyanins!

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Image Credits:

Full red cabbage image credit: By Wing-Chi Poon - self-made; at Jasper National Park, Alberta, Canada (along Yellowhead Highway 16 between intersection to Malign Valley Road and intersection to Snaring River Campground, overlooking Colin Range in the south)., CC BY-SA 2.5, https://commons.wikimedia.org/w/index.php?curid=405080

Gonsher, Ian. "Why Isn't There a Seam on the Color Wheel: Brown Faculty Responses." Medium, (May 2, 2019). https://iangonsher.medium.com/why-isnt-there-a-seam-on-the-color-wheel-brown-faculty-responses-3657abfd8249.

References:

Rose, Paul M., Victoria Cantrill, Meryem Benohoud, Alenka Tidder, Christopher M. Rayner, and Richard S. Blackburn. "Application of Anthocyanins from Blackcurrant (Ribes Nigrum L.) Fruit Waste as Renewable Hair Dyes." *Journal of Agricultural and Food Chemistry* 66, no. 26 (July 5, 2018): 6790–98. https://doi.org/10.1021/acs.jafc.8b01044.