Anthocyanin Watercolors – Part 2

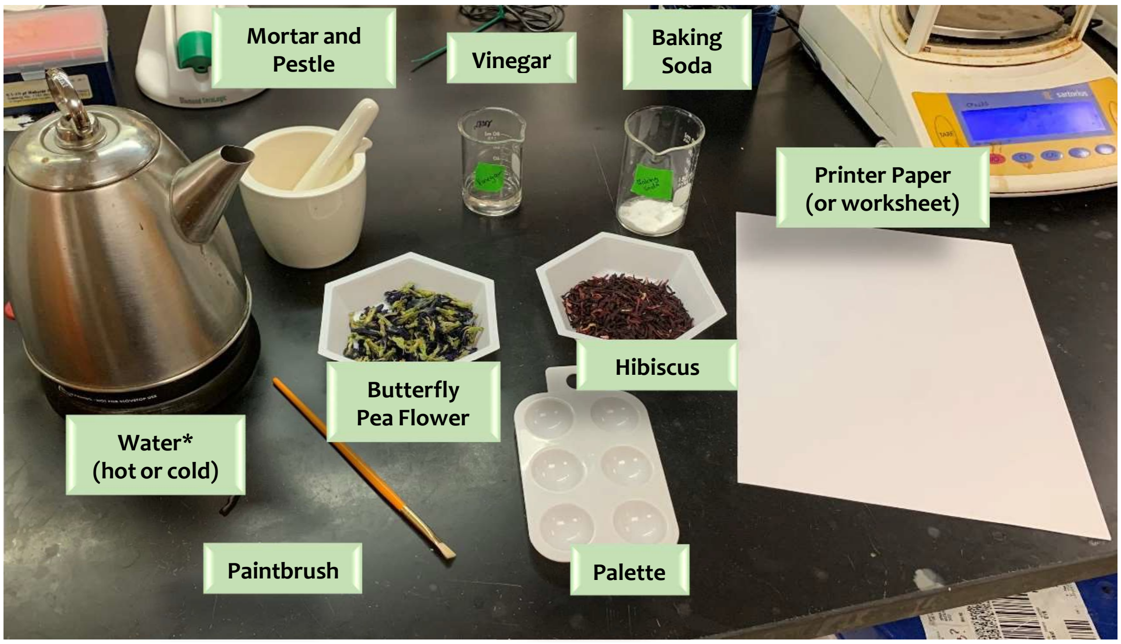
In Part 1 of this activity, you made paints using anthocyanins from two types of flowers. If, however, you tried to paint with hibiscus on printer paper, you likely noticed an unusual feature – color change! In this activity, we'll explore the chemistry behind these changes, and use it to make our paints a little more dynamic. *This activity uses weak acids (white vinegar) and bases (baking soda), as well as common allergens (flowers) – take extra precautions if you have allergies or are skin-sensitive to pH changes.*

Martin Lab at UC Irvine YouTube Video: https://youtu.be/x8g1hQHrVFc

In Part 2 of this activity, you will learn:

- The relationship between pH and anthocyanin color.

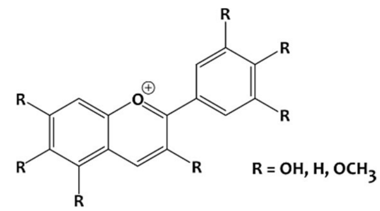
You will need:



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

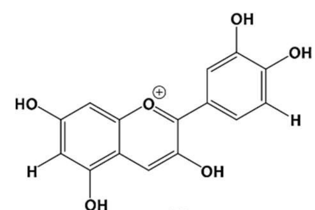
Background – Anthocyanin Structure and pH:

If you've ever tried drinking unsweetened hibiscus tea, you'll know just how acidic it is – almost as sour as a lemon! This acidity, or low **pH,** is what gives hibiscus anthocyanins their bright red color. When those same anthocyanins come into contact with more basic (high pH) printer paper, they almost instantly change color. What's going on?

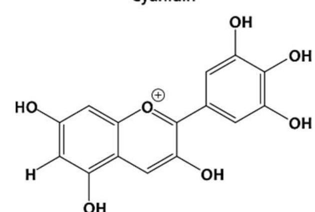


General Anthocyanidin Backbone

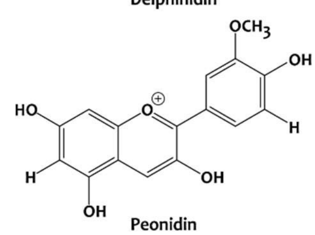
Anthocyanins all have the same basic structure: an **anthocyanidin** backbone with various sugars in place of the hydrogen (H) on one or more hydroxyl (OH) groups. A molecule with this kind of sugar substitution is called a **glycoside**, and as a result, anthocyanins can be categorized as glycosides of anthocyanidins. Chrysanthemin, for example, is a glycoside of cyanidin, and contains a *glu*coside (Glu) group (derived from glucose).



Cyanidin



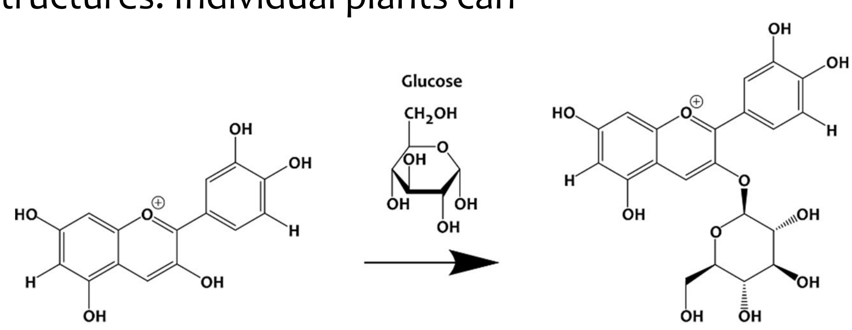
Delphinidin



Peonidin

Image credits: Red Cabbage – I, KENPEI/Wikimedia Commons; Cabernet Sauvignon Grapes – Agne27/Wikimedia Commons; Peony – Fanghong/Wikimedia Commons

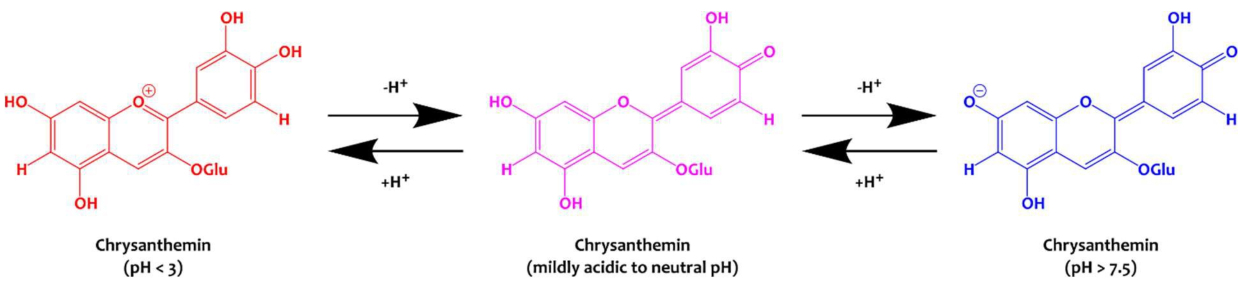
Many different anthocyanins can be made from the same anthocyanidin backbone, by varying the type and location of sugar substitutions. In addition, anthocyanins may produce slightly varied ranges of colors depending on the details of their molecular structures. Individual plants can



Cyanidin Chrysanthemin

have a complex mixture of different anthocyanins, each generated by a set of **enzymes** adapted to catalyze reactions like the sugar substitutions that turn anthocyanidins into anthocyanins.

When these anthocyanins are exposed to different pH, they either gain (with decreased pH) or lose (with increased pH) hydrogen ions (H^+) in a reversible reaction. This in turn changes the wavelengths of light they can absorb. As a result, they tend to range from red at low pH to blue at higher pH (with some variation). In even more basic environments, some anthocyanins turn green or yellow.



Adapted from (Rose et al., 2018).

In the last experiment, you witnessed this exchange of hydrogen ions in real time. The highly acidic hibiscus flower paint was red to start – its anthocyanins had gained hydrogen ions – but when it touched the basic printer paper, it quickly turned blue. This indicated a loss of hydrogen ions and an increase in the wavelength of visible light those anthocyanins could absorb.

Anthocyanins can therefore be considered pH-sensitive – they easily take part in acid-base reactions. Because of this immediate and reversible property, anthocyanins are often used as universal pH indicators.

Optional Question: Do you get the same results on paper towels? Paper plates? Why do you think that is? Be careful though – these things stain!

Answer: ____

Instructions:

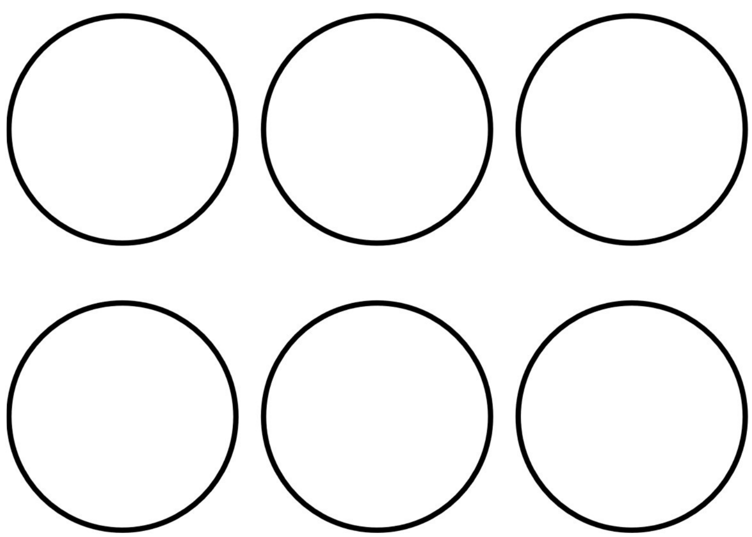
Now that you understand this property of anthocyanins, let's put it to use in our paints.

1. First set up your palette. For each column of three wells, fill one well with nothing, one with vinegar, and one with baking soda. This will set up your control (rough flower pH), acidic, and basic environments respectively.
2. Follow steps 1-3 of Part 1 for butterfly pea flowers; as before, you may optionally filter your paint with a coffee filter to remove bits of material.
3. Pour the finished product into one control, one acidic, and one basic well. Mix each well until the contents are homogeneous.
4. Repeat steps 2-3 with hibiscus.

Question: What color is each well? Why? What wavelength of visible light does each well absorb?

Answer: ____

Now that you have some extra colors, let's see how they turn out on paper. Use the circles below to see your true color palette.



(They're likely to be mostly blue, green, or purple after drying.)

Contact us at: **martinlabuci@gmail.com** Find out more about our research at: **www.probemonkey.com**

Using your new palette, you can paint a blue-green-purple masterpiece!

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Image Credits:

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.