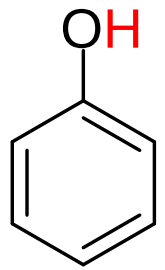
Experiment 10: Antioxidant Assay to Investigate the Radical Scavenging Properties of Anthocyanins

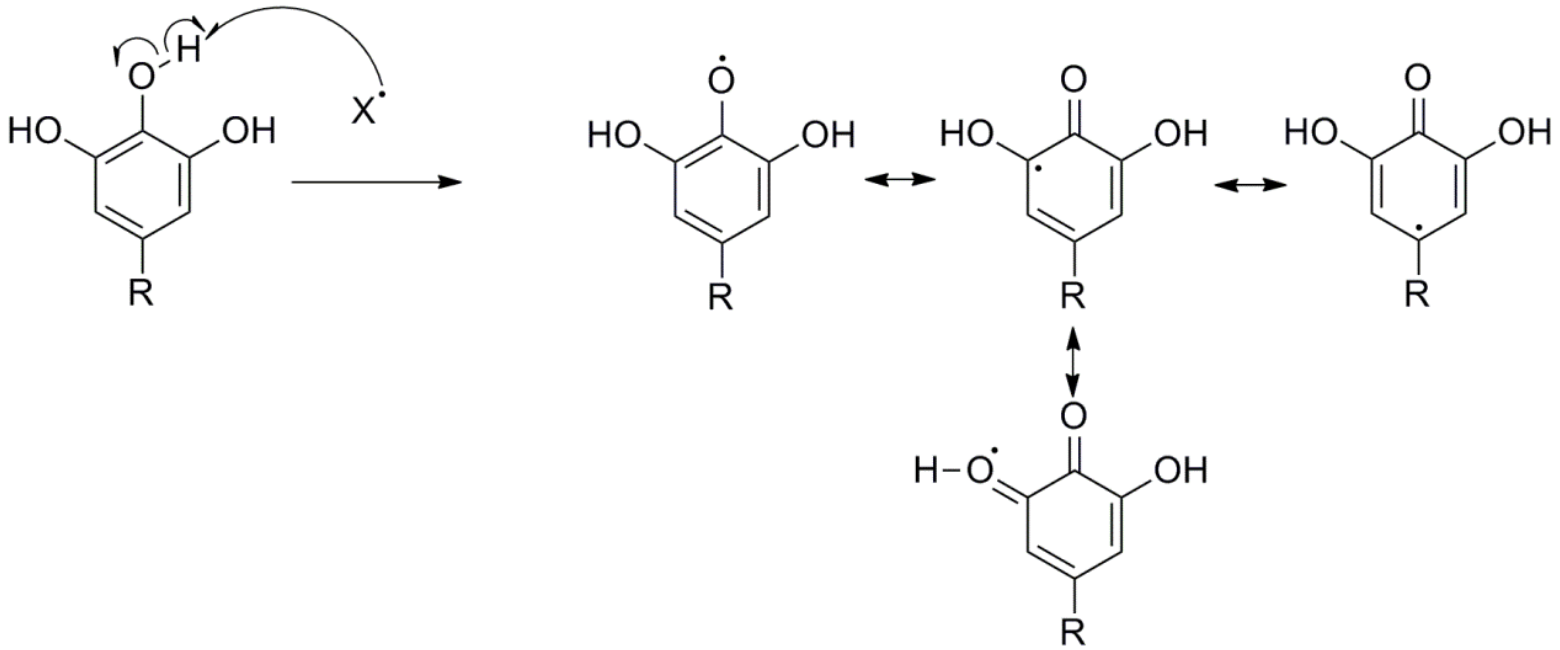
- This experiment is an investigation into specific properties of the anthocyanins you investigated in experiment 6.
- The goal of this experiment is to determine the relative antioxidant potential of the berries.
- As mentioned previously in experiment 6, anthocyanins are part of the flavonoid family which have similar properties to a group called polyphenols. There is substantial research that polyphenols are efficient radical scavengers and that polyphenols have positive health benefits.
- It is known that free radicals in the body can damage the body. Thus, research on naturally occurring radical scavengers has been underway for several decades.
- The radical scavenging ability of anthocyanins have been of particular interest lately because although they have aromatic hydroxyl groups, they do not have the number of aromatic rings or hydroxyl groups that true polyphenols have.

Anthocyanins as Radical Scavengers

Like polyphenols, anthocyanins have the ability to donate phenolic hydrogens. A phenolic hydrogen is the hydrogen attached to the oxygen of a phenol. The scheme below shows how a generic polyphenol donates a phenolic hydrogen and then shares the radical throughout the molecule via resonance.



Phenol

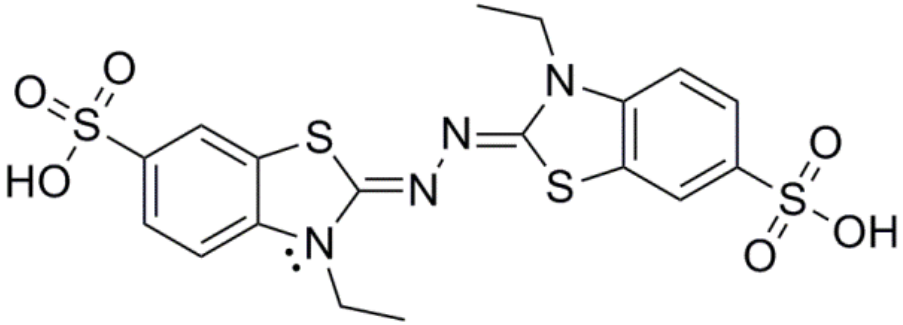


Antioxidant Assay

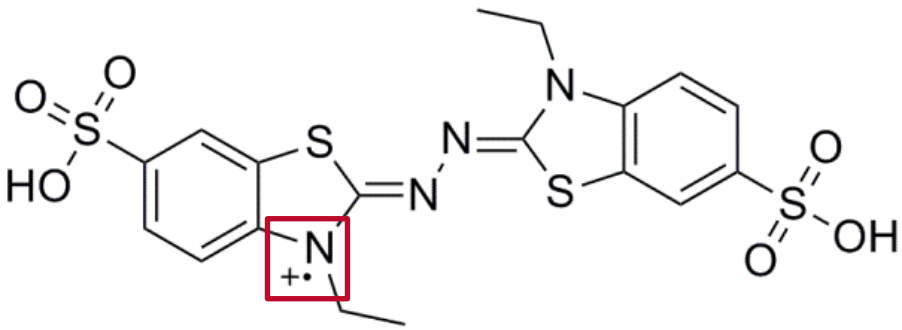
- In this experiment, you will set up an antioxidant assay in which you will be able to monitor how the concentration of a radical changes based on the amount of berry extract added to it.
- An assay is an analytical technique used to measure the amount or functional activity of a target compound or compounds.
- In this case, the target compounds are anthocyanins and other anti-oxidants that may be in the berry extracts.
- Each sample for the assay will have a fixed amount of a radical solution so that the quantity of anti-oxidant is the limiting factor in the reaction.
- UV-Vis spectroscopy will be used to monitor the changes in concentration of the radical.

Radical for the Assay

- The radical to be used for the assay is the stable free radical $ABTS^{\cdot+}$.
- Neutral ABTS, [2,2'-azinobis-(-ethyl-benzothiazoline-6-sulfonic acid)], is colorless in solution.
- When ABTS reacts with potassium persulfate ($K_2S_2O_8$), ABTS is oxidized to the radical cation $ABTS^{\cdot+}$. The radical cation $ABTS^{\cdot+}$ forms a blue solution that has a characteristic UV-Vis spectrum consisting of absorbance maxima at 415 nm, 645 nm, 734 nm and 815 nm.



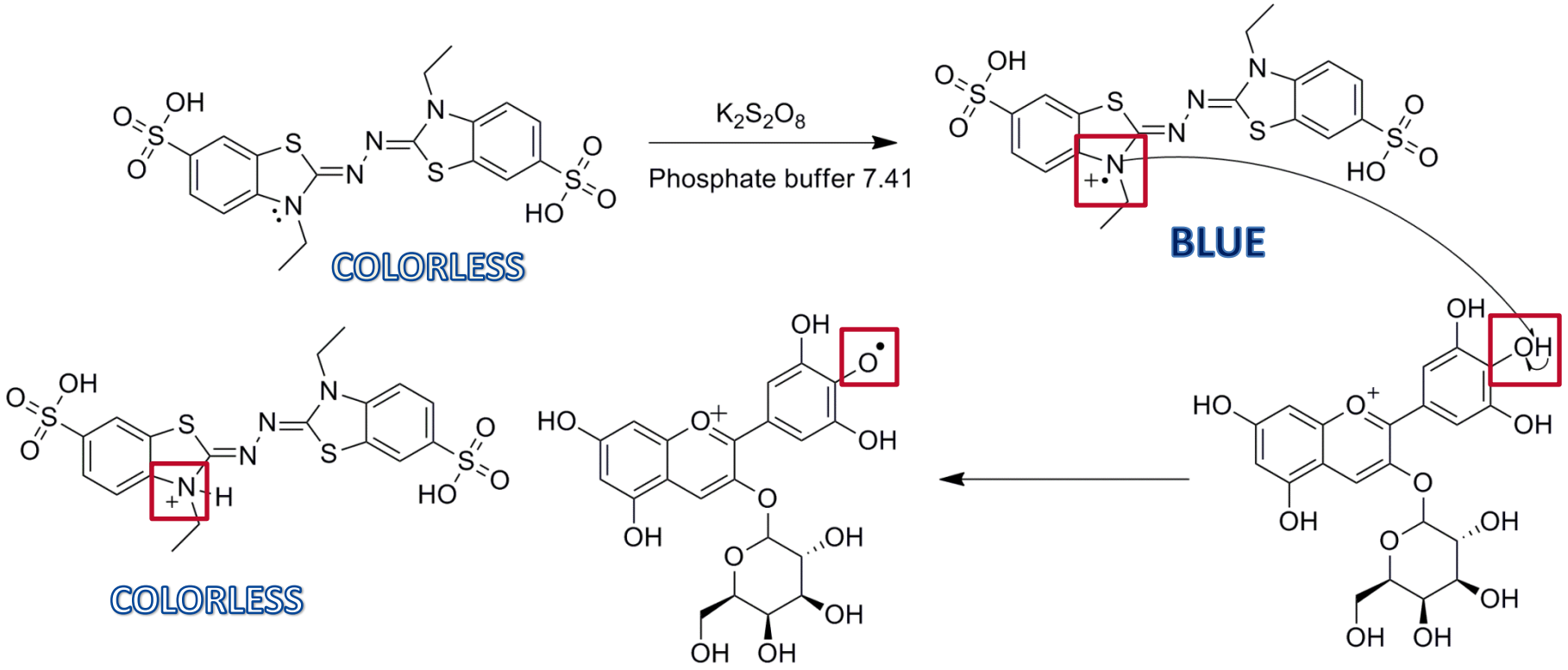
ABTS



$ABTS^{+\cdot}$

$ABTS^{\bullet+}$ Antioxidant Assay

- The following scheme shows the reactions that produce the radical cation and how an anthocyanin interacts with the radical.
- First, neutral ABTS is oxidized by potassium persulfate.
- Upon addition of an anthocyanin (using delphinidin-3-galactoside as an example), a phenolic hydrogen scavenges the radical on the $ABTS^{\bullet+}$.
- The resulting products are an $ABTS^{+}$ (which is colorless) and a highly delocalized (stable) anthocyanin radical.



$ABTS^{\cdot+}$ Antioxidant Assay

Tube	mL extract	mL solvent	mL $ABTS^{\cdot+}$
Control (1)	0.00	3.00	12.00
(2)	0.50	2.50	12.00
(3)	1.00	2.00	12.00
(4)	1.50	1.50	12.00

- After extracting the anthocyanins from your assigned berry using procedures you are already familiar with, you will set up the assay.
- In 4 different tubes, you will keep the amount of $ABTS^{\cdot+}$ constant at 12.00 mL and vary the amount of crude extract added to each tube. The extraction was performed with acidic methanol, so acidic methanol will be added to each tube to keep the volume of acidic methanol constant and the total volume of the solution constant. Note that the total volume for each tube is 15 mL.
- The initial reaction of the $ABTS^{\cdot+}$ with the anti-oxidant is rapid and then levels off. After 10 minutes, you will take the UV-Vis spectrum for each solution.

Experimental Hints

1. You have experience performing the anthocyanin extraction from experiment 6. Don't waste time during the extraction process.
2. Choose a wavelength to monitor for $ABTS^{\cdot+}$ that would not interfere with the spectrum for any anthocyanin.
3. There are only 3 UV-Vis spectrophotometers per room. Be familiar with the procedures for the UV-Vis so that you can take your spectra efficiently.
4. Label your tubes for the antioxidant assay and be precise when measuring the different volumes for the $ABTS^{\cdot+}$, the acidic methanol, and the berry extract.
5. This is a two week experiment. If you receive unexpected results during week 1, you will be able to repeat your experiment during week 2.
6. Week 2 will also be a time to analyze and discuss your data as a group.
7. You will need to bring your laptops with Logger Pro downloaded for both weeks of the experiment.
8. You will share data with the group of six students who you shared data for the 3 berries in experiment 6. Make sure you obtain all the data before you leave lab.