2. Absorption and Emission: Interplay in the Horse Chestnut

This experiment is about the phenomena of fluorescence using Aesculin as a natural fluores-cent dye.

Materials: 7 snap-capped vials with a seal, ultraviolet torch (λ = 366 nm), scissors, beaker (100 mL), glass rod, shoe box (see photo for the location of holes), a horse chestnut twig

Note: the recommended ultraviolet torch is the Pet Urine Detector 365NM Black Light Flash-light: PeeDar-Precision UV LED. It is available on Amazon.

Chemicals: Distilled water

Time: 15 minutes

Procedure

a. Using the scissors, cut a hole in the box for the ultraviolet torch and cut another small hole to see into the box.
b. Insert an approximately 10-cm-long horse chestnut twig in a beaker (100 mL).
c. On one side of the twig, remove up to half of the bark using the scissors.
d. Add 50 mL of distilled water to the pieces of bark and the twig and stir several times with the glass rod.
e. Place the beaker containing the horse chestnut twig and extract in the shoe box, aligning it with the holes, and put the lid on the box.
f. Insert the UV torch into the suitably-sized hole in the box and switch it on. The second hole in the shoe box is used for making observations during the experiment (see figure 6).
g. Now turn off the UV torch, remove the beaker, and use the extract to evenly fill six snap-capped vials.
h. Fill one snap cap vial with distilled water to serve as the blank.
i. Line up the seven snap-capped vials behind one another inside the box in front of the UV lamp hole. The position of the blank can be varied arbitrarily.
j. Shine the UV light through the lined up vials, which now block each other, so that the first vial gets full light and the last vial gets the least light (see figure 6).

Disposal

All of the solutions can be kept for subsequent experiments.

Explanation

Aesculin is a substance occurring in the leaves, seeds and bark of the horse chestnut (*Asculus hippocastanum*). This substance is soluble in polar solvents such as water and it fluoresces blue. Fluorescence occurs when UV absorption and visible light emission interact. High-energy, short-wave, and invisible UV radiation excites electrons of the aesculin molecule. As a result of this absorption, the aesculin molecule temporarily transforms into an unstable state. However, the excited electrons immediately return to the energetically more favorable ground state. The energy resulting from this process is released in the form of visible light (emission).

The blue light of the extract in the lined-up tubes diminishes from one snap-capped vial to the next. The first aesculin solution absorbs the majority of the UV radiation, producing the most intensive fluorescence in this tube. Only a small proportion of the UV radiation passes through all of the snap-capped vials, which is why the last tube with aesculin solution hardly emits light at all. Regardless of the sequence of the tubes and the position of the blank, the weakening of fluorescence is identical because water does not absorb UV radiation.

Fluorescence occurs when UV absorption and visible light emission interact. The picture on the left shows the blue fluoresces of horse chestnut extract on the left side and ash extract on the right side. The blue fluorescence in the second picture diminishes from one snap capped vial to the next. The first snap-capped vial serves as a blank containing water.

3. Acid-base reaction with horse chestnut extract

This research task is about the pH dependency of the fluorescent dye. Acid-base reactions illustrate reversible fluorescence changes.

Research task: Verify the extent to which the fluorescence of the horse chestnut extract depends on the pH value of the solution. Also show how possible changes can be reversed. To enable comparison, use water as a blank and unchanged horse chestnut extract as a control sample.

Materials: 7 snap-capped vials from experiment 2, ultraviolet torch ($\lambda = 366$ nm), felt-tip pen, small spoon, glass rod, shoe box, pH indicator paper, pipette, waste container

Chemicals: Lemon juice, baking soda, 5% hydrochloric acid and 5% lye in eyedroppers

Time: 15 minutes

Safety instructions: Safety goggles should be worn when handling acidic and alkaline solutions.

Procedure

Unless students are unable to work out a solution by themselves, please keep the following hidden from them. If they cannot, the following steps will help guide them. Note too that in Germany, students over 10 are allowed to work with hydrochloric acid and lye. Please check local regulations.

Part 1

a. Drip lemon juice or hydrochloric acid into three snap-capped vials containing horse chestnut extract.
b. Use a snap-capped vial with distilled water as the control sample.
c. Use one snap-capped vial with horse chestnut extract as a blank.
d. Put these five vials into the shoe box and expose them to ultraviolet radiation using the UV torch. Observe how the pH levels affect the fluorescence.

Part 2

e. Add baking soda to a sixth snap-capped vial with horse chestnut extract.
f. Add lye to a seventh snap-capped vial with horse chestnut extract.
g. Place these two vials also into the box.
h. Shine the UV lamp into the box to observe how the pH levels affect the fluorescence.

Part 3

i. Use one of the three vials with acid from part 1 that contains extract and either lemon juice or hydrochloric acid.
j. Either baking soda is spooned into it or lye is dripped into it to change the pH level.
k. Place all seven vials (horse chestnut extract with lemon juice; horse chestnut extract with hydrochloric acid; horse chestnut extract with baking soda; horse chestnut extract with lye; horse chestnut extract with lemon juice and baking soda or with hydrochloric acid and lye; horse chestnut extract as a blank; distilled water) into the box and shine the UV light to compare the florescence and see how the change in pH has affected the solution.

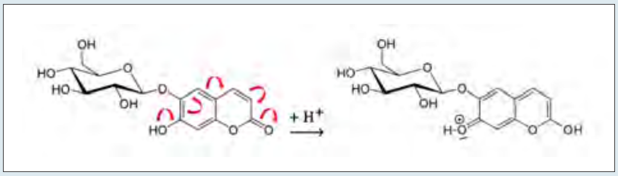
Disposal

Collect all remaining solutions in a waste container.

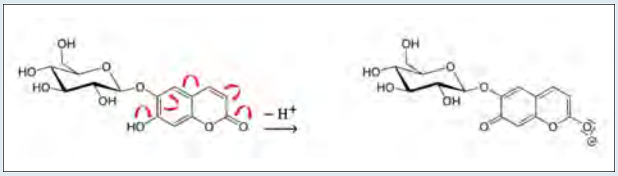
Explanation

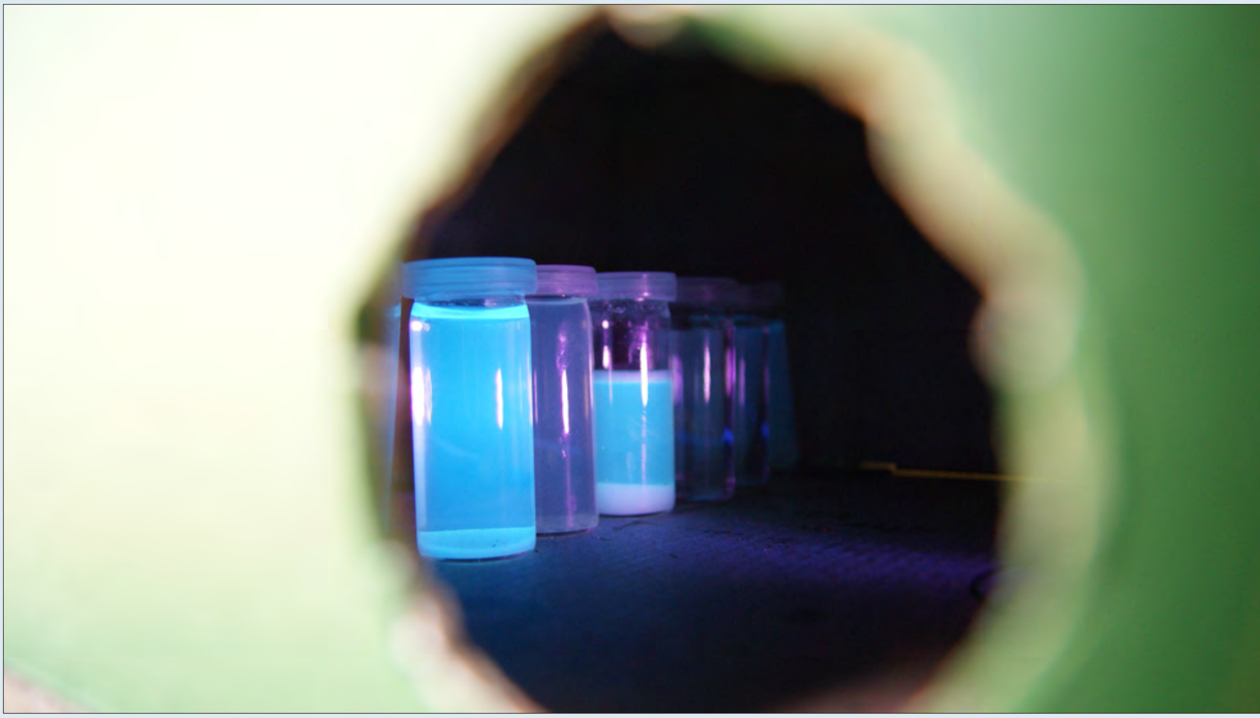
The aesculin molecule is prevented from fluorescing by adding acid. Bases, on the other hand, intensify fluorescence of the aesculin molecule. A neutralization reaction causes the base in part three of the experiment to move the acidic pH value of the horse chestnut extract into a neutral area, making the solution fluoresce blue again. The fact that the fluorescence of aesculin is dependent on the pH value can be explained by the structure of the molecule. In an acidic environment, the molecule is protonated, destroying the conjugated π-electron system and resulting in no more UV radiation being absorbed. In a basic milieu, the deprotonated molecule is stabilized by resonance structures and the free electron pairs of the oxygen atoms extend the conjugated π-electron system, causing fluorescence to acquire more intensity.

9. protonated



10. deprotonated





The fluorescence of the horse chestnut extract depends on the pH value of the solution. Acids like lemon juice or hydrochloric acid prevent it from fluorescing and bases like baking soda or lye intensify fluorescence. In the picture above, the first snap-capped vial serves as a blank containing horse chestnut extract, the second snap-capped vial contains horse chestnut extract and lemon juice, and the third snap-capped vial contains horse chestnut extract and baking soda.