SYNTHESIS OF FLUORESCEIN, a fluorescent dye

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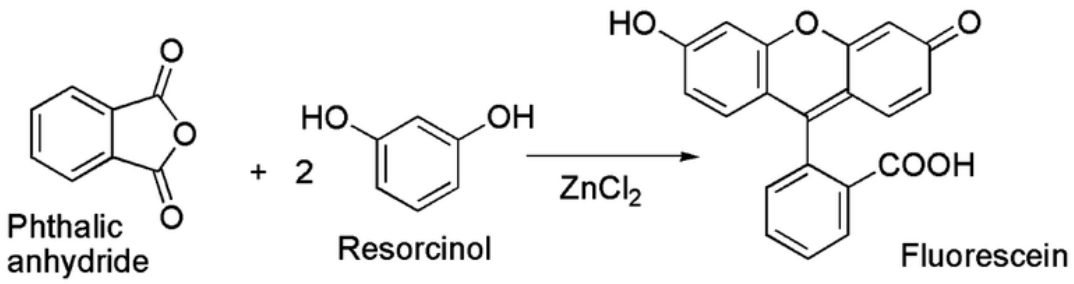
Fluorescent detection technique has played a significant role on the advancement of modern medicine and molecular biology and has achieved rapid development. Fluorescein is one of the widely used fluorescent probes in such applications. Fluorescein was first synthesized by Bayer from the reaction of resorcinol and phthalic anhydride in the presence of zinc chloride.



Scheme 1. Synthesis of fluorescein from resorcinol and phthalic anhydride.

The fluorescence of this molecule is very high, and excitation occurs at 494 nm and emission at 521 nm. Fluorescein has a pKa of 6.4, and its ionization equilibrium leads to pH-dependent absorption and emmission over the range of 5 to 9.

Figure 1. Fluorescein under UV illumination.

Synthesis of Fluorescein

(a) Reaction Setup

Set an oil bath to a temperature between 180° and 200°C. To a large test tube (15 X 150 mm) or small Erlenmeyer flask add 0.3 g of resorcinol and 0.2 g of ground powdered phthalic anhydride. To this mixture of powders add 6 drops of 2M H_2SO_4 (DO NOT ADD MORE THAN 6 DROPS). Stir the mixture briefly with a spatula. Place the test tube in the preheated oil bath. The reaction should be run at a temperature between 180° and 200°C. *(Note: It is extremely important to monitor the temperature and keep it within this range. Overheating will cause the product to decompose.)* The reaction should run for 30 minutes within this temperature range. Once the reaction time is up, remove the test tube from the oil bath and allow it to cool for about 5 min.

(b) Reaction Workup

To the test tube add 10 mL of acetone and a stir bar. Using a ring stand and clamp, place the test tube over a magnetic stir plate and stir the solution for 5 to 10 minutes. The solution should turn yellow as the crude fluorescein dissolves. If the entire product did not dissolve, repeat the process with an additional 5 mL of acetone until the entire product dissolves (do not use more than 25 mL total). Combine the acetone layers in a 50 mL beaker.

Boil off the acetone leaving a crude orange residue. Take this crude residue and dissolve it in 30 mL of diethyl ether and 1.5 mL of water. (*Note: Even though most of the dye will end up in the organic layer it will not dissolve unless a small amount of water is present*.*)* Place a stir bar in the solution and put the beaker over a magnetic stir plate for several minutes until all the solids dissolve. Transfer this organic solution in a separatory funnel and add 15 mL water for washing. Discard the aqueous wash. Following this, extract the ether layer once with 10 mL of a saturated NaCl solution.

Dry the organic layer over anhydrous sodium sulfate. Pre-weigh a small beaker. Place the dried organic solution in the beaker and evaporate it to dryness in a water bath to yield the product as an orange solid.

(c) Observation of fluorescene

Prepare a solution of fluorescein by dissolving 5 milligrams (or spatula tip size) of the sample in 50 mL 0.1 M NaOH solution. Place the solution in a vial and place it on a black non-reflective surface, such as a lab bench. Place a bright light source (sunny window works well) on the opposite side of the bench from the observer and note the appearance of the solution. In this case, the color observed is primarily due to absorbance of some wavelengths of visible light passing through the sample from the light source.

Next observe the same solution at a position 90 degrees from the light source. The color observed from this perspective is primarily due to fluorescence.

Finally, in a darkened room shine a long wave UV lamp at the sample vial. The vials should visibly glow from the fluoresced light.

References

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