Fluorescence of scopoletin and related coumarins

Joy Yoo
Professor Mark Muyskens
Department of Chemistry of Biochemistry, Calvin College, Summer 2016

Muyskens group's research on sycamore trees started about four years ago when Professor Muyskens became interested in naturally occurring fluorescence in local tree materials. His students found that sycamore trees give off fluorescence when put in water, so he decided to further study the compound that makes sycamore extracts fluoresce. Last summer, the Muyskens group identified the coumarin compound called scopoletin as the fluorescent compound present in sycamore, and our goal for this summer was to further characterize the fluorescence of scopoletin and two other related coumarins, esculetin and umbelliferone. The goal was to measure its maximum absorption and emission wavelengths, the fluorescence lifetime (the rate at which fluorescence occurs), and observe how these fluorescent properties depend on pH. The lifetime of these compounds have not yet been reported in the scientific literature, so we are striving to be able to contribute new information about their fluorescent properties.

To study how fluorescent properties of the three coumarins depend on pH, we made 20 pH buffers, ranging from pH 1 to pH 13. To avoid the complication of the inner filter effect, we made sure our samples have UV absorbances at around 0.1. From the fluorescence excitation data, we are able to determine the pKa of our coumarins, which denotes where the molecular shift from low to high pH occurs. To measure the lifetime, we developed a nanosecond-resolution fluorescence lifetime set up. We measured the full width half max of few standards with known lifetimes and constructed a calibration curve with the lifetime values of our standards from the literature. In collaboration with Professor Gary Blanchard at Michigan State University, we were able to analyze selected samples of ours using their picosecond-resolution fluorescence lifetime apparatus. Surprisingly, the data we got from MSU matched very well with our data and we constructed another calibration curve with the experimental data we got from MSU. Analyzing the data from MSU, we also found that there is a more complicated fluorescent process going on for low pH scopoletin because the exponential decay curve was clearly not a simple single exponential function.

Using our method of measuring full width half max of fluorescence signal, we measured the lifetime of scopoletin to be 4.55 ns for low pH and 4.15 for high pH. We also noted few observations for esculetin: fluorescence for low pH was not strong enough to get a solid signal to measure a reliable full width half max and borate buffer for pH 8-9 quenched fluorescence of esculetin. Overall, we were glad to see that our simple approach to measuring lifetime works and also observed that fluorescent properties of our coumarins depend on pH to some extent.

This research experience allowed me to gain skills on using different instruments used in labs and solving problems. I was able to explore several questions and learned how to think critically, and I also got to have more confidence in working in the lab. I learned to take different paths when things do not work as we expected and learned that research takes both hard work and patience. I am really thankful for this opportunity to work as an undergraduate researcher and for the valuable experience of collaborating with faculty and another student.