Use the SpectroVis Plus to Explore Fluorescence Spectroscopy

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PROCEDURE

1. Prepare a 1 ppm solution of phenolphthalein and a 1 ppm solution of fluorescein. Neither of these compounds are very soluble in water. You may need to make stock solutions using 70% ethanol as a solvent and then dilute to 1 ppm using distilled water. Follow the instructions below to compare the fluorescence of fluorescein and phenolphthalein.
    a. Use a USB cable to connect the SpectroVis Plus to your computer. Choose New from the File menu.
    b. Fill a cuvette with 2 mL of fluorescein solution and place it in the cuvette slot of the Spectrometer.
    c. Choose Change Units ▶ Spectrometer from the Experiment menu and select Fluorescence 500 nm.
    d. Choose Set up Sensors ▶ Spectrometer from the Experiment menu and change the sample time to 100 ms.
    e. Click ▶ Collect. A full spectrum graph of the fluorescence of the fluorescein will be displayed. Note that one area of the graph contains a peak at approximately 515 nm. This peak is from fluorescein. Click ■ Stop.
    f. Adjust the sample time to increase or decrease the size of the fluorescent peak. If the peak intensity is above 1, decrease the sample time by 10 ms and collect a new fluorescent spectrum. Continue to decrease the sample time until the peak is fully visible. If the fluorescent peak is below 0.3, increase the sample time by 10 ms and collect a new fluorescent spectrum. Continue to increase the sample time until the peak fluorescent amplitude for fluorescein is above 0.8.
    g. Once you have a nice peak, store your data by choosing Store Latest Run from the Experiment menu.
    h. Collect a full spectrum graph from a cuvette containing 1 ppm phenolphthalein. Do not adjust the sample time.
    i. Compare the fluorescent spectra of the two compounds. Phenolphthalein is not fluorescent. A small peak may be visible for the phenolphthalein sample at 500 nm. This is peak is from the excitation LED used in this experiment.
    j. Print or save your experiment file as directed.