Identification and Quantitation of Riboflavin in Vitamin Tablets by Total Luminescence Spectroscopy

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Fluorescence spectroscopy is a powerful tool for the identification and quantitation of fluorescent compounds at low concentrations (*1*). The advent of inexpensive electronic components has made available inexpensive dual scanning monochromator spectrofluorometers and the acquisition of spectra that were previously available only from expensive systems.

Riboflavin displays a green fluorescence, maximum at 530 nm, when illuminated with 470 nm light. The concentration of riboflavin has been determined by fluorescence (*2*), as well as by HPLC with various methods of detection (*3–7*). If one considers the makeup of a typical vitamin tablet, many of the components, including the dyes, will obscure the riboflavin absorbance spectrum. This makes the determination of the riboflavin concentration by spectrophotometric methods impossible. However, the total amount of light absorbed at wavelengths longer than 400 nm is not great, and the fluorescence of riboflavin is largely unobscured, making riboflavin in vitamin tablets an excellent candidate for determination by fluorescence.

Figure 1 shows the total luminescence spectrum of a riboflavin solution, while Figure 2 shows the total luminescence spectrum of the dissolved vitamin tablet. A student can easily see the fluorescence due to riboflavin, a quick identification of one component within a complex mixture of both known (vitamin and minerals), and unknown (fillers and dyes) components.

The following procedure works well for the determination of riboflavin. A vitamin tablet is crushed and dissolved in 500 mL of 250 mM acetate buffer pH 6.5. After being shaken for 30 min, the sample is centrifuged to remove insoluble material that would contribute to light scattering. A stock solution of riboflavin (5.00 mg/L) is prepared in 250 mM acetate buffer pH 6.5. The preparation of the vitamin extract should be carried out in subdued light. Riboflavin solutions are light sensitive and prone to oxidation, so solutions should be prepared the day of the experiment. An estimate of the concentration of the riboflavin in the vitamin tablet may be made from a standard curve using dilutions of the 5 mg/L riboflavin standard. The major interferents in these determinations are the light scattering due to undissolved particles, and the absorption of the light by the dyes used in the vitamin manufacture. This latter problem can be turned to the instructor's advantage by supplying some dilute food coloring to the students to add to their samples. By considering the absorbance spectra of the food coloring, students can see the effect of both excitation and emission absorbance.

The acquisition of the total luminescence spectrum, the identification of a single compound in a complex mixture, and the appreciation of inner filter effects make this laboratory exercise a vast improvement on fluorescence experiments that rely on a filter fluorometer instrument.

Literature Cited

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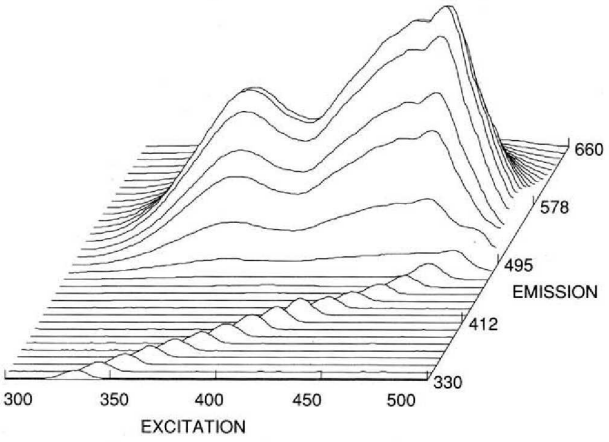

Figure 1. Totalluminescence spectrum of a riboflavin solution.

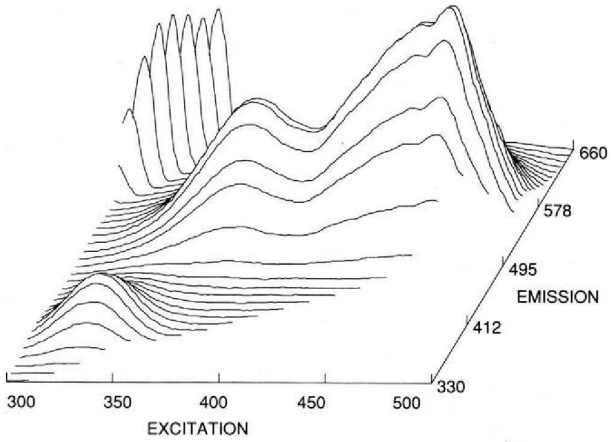

Figure 2. Total luminescence spectrum of a dissolved vitamin tablet.