VII. DETECTION LIMIT

Connect the SCM to the ADC channel 0 and use the DVM data acquisition program in the automatic mode with a 3-s spacing. With blank solution in the sample cell and an SCM gain (g) set to 100, record 10 readings of the voltage. Be sure that the blank signal is much less than 0.5 V but greater than 0.010 V and is close to E_{bk} for the 0.1 μg/mL standard. Add comments to the comments box so that you will know what experiment this data represents later when you are working on the report! If the standard deviation is zero, something is wrong - check with one of the instructors. Print and save the file from the DVM program.

The fluctuation in the signal voltage for the blank is used to determine the detection limit (DL). The detection limit is based on an equivalent concentration that yields a signal equal to three times the noise in the blank signal ($3s_{bk}$), or DL = 3 s_{bk} / m, where m is the slope of the calibration curve.

VIII. LAB REPORT

This entire lab report is an individual effort. Your lab report should include: the completed data sheets; the "Summary Checklist for Results Sheet"; answers to the following questions; duplicate pages of your lab notebook showing actual sample preparation; copies of the spectra acquired with the commercial PE spectrofluorometer, and copies of the spectra acquired with your PTR station (spreadsheet version). No abstract is required for 2B.

Section *DATA, CALCULATIONS, QUESTIONS*

III-V

Spectra on the commercial Perkin Elmer *(PE) and PTR (your station) spectrofluorometers.*

1. Include the labeled excitation and emission spectra for the riboflavin standard acquired with the PE.
2. Include the labeled excitation and emission spectra for the sample acquired with the PTR instrument and plotted with Excel.
3. In the report, make a 2 X 3 table comparing the excitation wavelength maxima of all bands in the excitation spectrum and the wavelength maxima of all bands in the emission spectrum as observed on the commercial Perkin Elmer fluorometer with those from the PTR fluorometer you built.

4. Specifically for the copies of the spectra from the PE spectrofluorometer, identify which bands in the excitation spectrum and in the emission spectrum correspond to the scattering band (by labeling the wavelength maxima on the chart) and briefly discuss in a sentence or two how you made this decision for each case. Also identify which bands in the excitation spectrum and in the emission spectrum correspond to the molecular transitions for riboflavin (and label the wavelength maxima on the chart).

5. Using the spectra from the PTR fluorometer, report the half-width of the fluorescence band (in nm) and the difference in wavelength between the excitation and emission maxima (in nm). Mark these on your copy of the Excel plot.

6. Compare the shapes and relative intensities of bands in the excitation spectrum obtained using the PTR to the those obtained from the PE spectrofluorometer (note: absolute intensities are not comparable for different instruments). Define the differences and indicate some reasons for these differences. Hint: review section III of this manual and eqs. 5 & 6 and your 2B lecture notes.

7. Compare shapes and relative intensities of bands in the emission spectrum obtained on the PTR to those obtained from the PE spectrofluorometer (again, absolute intensities taken on different instruments are not comparable) . Discuss differences, similarities and give possible explanations after reviewing this lab manual and lecture notes.

VI *Calibration Data*

1. Construct an analytical curve of the normalized emission signal (E_f') vs. riboflavin concentration in μg/mL for the 0.1 - 1.0 μg/mL range. Fit the data to a linear model and report the slope and intercept along with the standard errors for both..

2. Use the **linear calibration** determined in VI 1 and calculate the riboflavin concentrations (μg/mL) in the synthetic unknown and the three vitamin tablet Z solutions. Report the results in a proper table.

3. Use the concentration of each the Z solutions to back-calculate the corresponding mass (mg) of B2 in the vitamin tablet. **Show an example calculation for one of the samples** (give equation(s) and values from 1 trial with the result). Report the results in a proper table. Also report the mean, the standard deviation, and RSD(%) of the mass

(mg) of B2 per vitamin tablet. Compare in a proper table the mean and RSD(%) to the same quantities you obtained in Experiment 2A (section V, question 7).

4. **Plot a second non-linear analytical curve** (different graph and scale) of E_f' vs. riboflavin concentration in μg/mL for 1, 2.5, 10, 25, 75, and 100 μg/mL. Fit the data with a non-linear equation and report the exact equation and coefficients determined. Carefully consider your choice for the type of fit (e.g., polynomial, log, etc.) and make sure that the fit to the data is reasonable (e.g., E_f' is predicted to be 0 for c = 0 and relative standard errors for the coefficients are not too large). You may find it necessary to exclude the data for the 75 and 100 μg/mL B2 standards to obtain a good fit for the rest of the curve. **Briefly explain in a sentence or two why the plot is not linear**.

5. Is there a background signal above the dark current? If so, what are potential causes of this signal?

VII *Detection Limit*

1. From the normalized standard deviation of the blank signal and the slope of the calibration curve, calculate the detection limit. The detection limit for fluorescence should be well below 0.1 μg/mL but not zero.

2. Calculate the **absorbance (AU)** for a solution with a riboflavin concentration equal to the fluorescence detection limit reported above. Use your calibration curve equation with the slope from experiment 2A to calculate the AU value and assume a zero intercept.

3. Based on the AU value from 2 above, do you think that you would be able to detect the concentration of riboflavin with the absorption technique you used in part 2A and why? Remember that the minimum detectable absorbance is typically between 0.001 to 0.0001 AU.

Name _____ Station # = _____ Date_____

DATA SHEET FOR EXPERIMENT 2B. Molecular Fluorescence. CH 461/461H

Synthetic unk # _____; Which team member has charts from PE? _____

VI: PMT bias voltage (HV) = _____ V (should be about -400 to -600 V)

Frequency cutoff on SCM = _____ Hz (should be 0.3 Hz)

For each standard or sample solution, record E_{total} and measure a new blank and record E_{blank}. Promptly calculate the fluorescence signal E_f (eq. 7) and normalized fluorescence signal E_f' calculated as E_f/gain. All fluorescence signals are normalized back to the value at the output of the PMT module by dividing by the SCM gain. Assume that g is known to three significant figures. Start a chart in Excel as you collect the data. Recall that: $E_{total} = E_f + E_{blank}$

Dark current voltage (E_d) = _____ V (measure once)

Table I. Fluorescence Calibration Data

Conc. (µg/mL)	E_{total} (V)	E_{blank} (V)	E_f (V)	**SCM Gain (g) (note changes)**	E_f' (V)
100				**1.00**	
75				**1.00**	
25				**1.00**	
10				**1.00**	
2.5				**2.00**	
1.0				**5.00**	
0.25				**20.0**	
0.1				**100**	
syn. unknown				**20.0**	
Z1				**20.0**	
Z2				**20.0**	
Z3				**20.0**	

VII: Detection Limit Data

Include the computer printout of repetitive blank measurements and report the following:

mean blank voltage = _____

standard deviation in blank voltage = _____

gain on SCM = _____

gain normalized standard deviation (referenced to PMT module output gain) = _____
(the normalization is the same as used previously)

Show calculation of gain normalized standard deviation:

SUMMARY CHECKLIST FOR RESULTS FOR EXPERIMENT 2B

Section II: Solution Preparation - Mass data same as that given in experiment 2A (+/- 0.1 mg)

mass of whole vitamin tablet	
mass of sample for solution X1	
mass of sample for solution X2	
mass of sample for solution X3	

Any requested proper Tables and short answers to questions on pages 14-15 PLUS the following summary:

Question	Done?	Information Requested
V. 1.		From the PTR fluorometer: emission wavelength maximum and half-width of emission band
V1. 1.		Calibration equation from regression curve for linear region (normalized signal vs conc.)
2.		B2 concentration in the synthetic unknown
3.		Mean and RSD for amount of B2 in pill found with fluorometry (this study); Mean and RSD of amount of B2 in tablet found with absorption spectrophotometry (from exp. 2A)
VII. 1.		normalized standard deviation in the blank voltage; and detection limit
2.		absorbance for a solution of riboflavin of concentration equal to the above detection limit