Week 3 – % Riboflavin in a Multivitamin

**Figure 1.** Molecular structure of riboflavin, C_{17}H_{20}N_{4}O_{6}.

- Found in many foods (eggs, nuts, grains, dairy products)
- Biochemical: proper utilization of carbohydrates, fats, proteins as energy sources
- Component of two coenzymes (FAD and FMN):
  - Aid certain enzymes in oxidation and reduction rxns in e⁻ transport chain and a wide range of metabolic pathways
Objective: Determine Riboflavin Concentration

- Riboflavin: yellow $\rightarrow$ absorbs blue and green strongly
- Can absorbance spectroscopy be used to measure riboflavin concentration?
  - **NO!** Many other components of vitamin absorb visible light

- Fluorescence spectroscopy
  - Little interference
Fluorescence Spectroscopy

• Much more sensitive than absorbance spectroscopy → detection of smaller concentrations possible (small increase in signal on top of near-zero background is easily detected)
• Only a small fraction of molecules will fluoresce (most lose energy thermally or break apart)
• Capable of detecting single molecules
  – Human genome sequencing, in vivo cell imaging
Fluorescence and Standard Addition Measurements

- Fluorescence calibration curve
- Standard addition curve (fluorescence vs. standard concentration)
  - Eliminates matrix effect
  - Make unknown solution that falls within range of fluorescence calibration curve (dilute if needed)
  - Keep unknown concentration constant; vary standard concentration
  - Extrapolate data to determine concentration of unknown (distance between origin and x-intercept)