

Experiment 2: Spectrophotometric Determination of Iron in Vitamin Tablets**C. Instrumental Procedure (revised instructions—25 February 2005)**

1. Sign log book.
2. Turn on the spectrophotometer (the power switch is at the back right corner), monitor, and printer.
3. After the instrument has (successfully) completed its power-up diagnostics, use the mouse to click on **Quit** and then on **WAVELENGTH SCAN** (at the upper left of the screen).
4. Click on **VIS OFF** (at the bottom left of the new screen) to turn on the visible light source (a tungsten filament light bulb!).
5. In the upper panel, click next to Start λ to set it to 400 (nm) and click next to End λ to set it to 700.
6. Set the maximum [Abs] value on the y-axis of the spectrum panel to be 1.0.
7. Fill a plastic cuvet (stored in the Styrofoam box) with your blank solution, wipe the smooth sides of the cuvet with a Kimwipe, and place the cuvet in the back of the instrument's sample tray (**that is, in Slot 1**). Be sure to hold the cuvet by the ribbed sides, and orient the cuvet with the smooth sides exposed to the slits in the side of the tray.
8. Click on **BLANK** in the lower left of your screen. This will store the absorbance in the instrument's memory. Now all subsequent readings will be automatically corrected!
9. Take the blank cuvet out of the sample tray, and replace it with a cuvet filled with your most concentrated Fe^{2+} standard solution.
10. Click on **ReadSamples** (at the upper left of the screen). You should get an absorbance spectrum peaked at around 510 nm.
11. Click on **Print** (upper right) to print out a copy of the spectrum. This should be taped into one of your notebooks.
12. Click on **Tabulate** (upper left). Scroll down to find the wavelength of maximum absorbance, and write down this λ_{max} and the corresponding absorbance. You will use this λ_{max} for the next part of your measurements. **Leave your cuvet filled with your most concentrated Fe^{2+} standard solution in Slot 1.**
13. Click on **Exit**, then **Quit** in the upper right of the screen, then **OK**. (There is no need to save a file.)
14. Click on **FIXED WAVELENGTH** (at the upper left of the screen).
15. Click on all three of the wavelength values (to the right of **Sample ID**) and set all of them to the λ_{max} value you determined earlier.
16. Click on the **None** next to **Sampling Device** (in the upper right). Then click on the box next to **Auto smplr** (short for auto-sampler), and set **Number of cells** to 5.
17. Insert into the sample tray cuvetts containing the other three standard solutions and your unknown **into Slots 2-5**. The front-most cuvet slot (**that is, Slot 6**) should be empty.
18. Click on **ReadSamples** in the upper left of the screen. The instrument should automatically take three readings on each of the five cuvetts: **that is, your four Fe-containing standards and your unknown. Each reading appears in a different column, and all three readings on a given cuvet appear (virtually) simultaneously.**
19. Click on **Print** in the upper right of the screen to get a printout.
20. Click on **Quit** in the upper right of the screen, then **OK**. (Again, there is no need to save a file.)
21. In the log book, note if there were any instrumental problems. (Hopefully there weren't!)
22. Turn off the spectrophotometer, monitor, and printer.
23. Rinse out all of your cuvetts several times with deionized water, and leave them to dry next to the sink.