1. Manual – spectrophotometer UV/VIS VIS-723G

1. Turn **ON**: spectrophotometer, computer and printer.

2. Prepare the different concentrations of riboflavin solutions (consult with the teacher)

3. Initialization of the system

a) Click „**Initialization**" (button on the lower-left corner of the screen). You can see the following information: "**Initializing…please wait**".

b) If initialization is OK, it will inform you that "**Initialization success!**". Click "**OK**".

c) Attention: One initialization is succeeded, system will store the settings as default, then for later operation, user do not need to input parameters every time.

4. Spectrum scan – enter spectrum scan mode

6.1. Click "**Spectrum**" button to enter spectrum scan mode. Then click "**Param**" to set parameters.

    a. Data Mode: Abs
    b. Wavelength Range: 330-530nm
    c. Display Range: -0.010 – 1.000
    d. Scan mode: Once
    e. Save mode: Manual
    f. ☑ Overlay
    g. Sampling Interval (nm): 1
    h. Scan Speed: Middle
    i. Decimals: 3
    j. Reference Test: Once

6.2. Then click „OK" button.

5. Measurements

a) Click „**Start**" to start measurement.

b) System will inform you about consecutive operations:

c) System will inform you to pull reference into light path shows: "**Please set the reference position.....**"

d) Open sample chamber, place the reference cuvette with distilled water, close the lid and click "**OK**".

e) After test of reference is finished, system will inform you to pull sample into light path shows: "**Please set the SAMPLE position…..**".

f) Then pull sample into light path and click "**OK**" to finish test.

g) After test finished, system will inform you that scan is complete, click "**OK**" on window "**Scan complete!**"

6. Registration of Absorption spectra - several samples

a) Click „**Start**" button again. System will inform you to pull sample into light path shows: "**Please set the SAMPLE position…..**".

b) Remove cuvette with starting solution. Empty cuvette. "Wash" cuvette twice with next solution. Fill cuvette with this solution again for absorption spectrum mesurement. Then pull this sample into light path, close the lid and click "**OK**".

c) After measurement finished, system will inform you that scan is complete, click "**OK**" on window "**Scan complete!**"

d) Consecutive spectra must be registered for solutions with increased concentration of riboflavin.

e) Absorption spectra of the sample with unknown concentration of RF: Clean cuvette with distilled water. Fill cuvette with solution of unknown concentration of RF (consult with an assistant). Then start measurement.

7. Save data

   Click "Save" to save all spectra from measurements. Follow the path displayed in the program.

8. Overlay spectra

   Chose "**Overlay**" mode – Spectrum overlay can show all the test spectrum on one data page. Select spectrum you want to overlay. Click "**OK**". The overlay spectrum that you selected will be shown in Data page

9. Peak picking

   Select the spectrum that you want to pick peak. Click "Peak Picking" button on tool bar. Peak picking window will come out (this function is used for certain spectrum, not for overlay spectra).

10. Print registered spectra on the printer

   Follow proper instruction.

11. Calculation of unknown concentration of riboflavin.

12. Chose certain wavelength ($\lambda_{observation}$) from the range of absorption spectrum – the same (constant) for all registered spectra. (Consult the assistant)

13. Read absorbance value for chosen $\lambda_{observation}$: from absorbance spectra of known concentrations of riboflavin: $\lambda_{observation} \rightarrow A_{known}$

14. Read absorbance value for chosen $\lambda_{observation}$ from absorbance spectrum of unknown concentration: $\lambda_{observation} \rightarrow A_{\textbf{un}known}$

15. Put these values into "**Table of results**".

16. Start (if not ON) the Grafpad Prism Program. Follow instruction for making plot y=f(x), plot absorbance as a function of concentration: Absorbance = f(c), and calculate linear correlation.

17. Knowning $A_{unknown}$ value calculate unknown concentration ($c_{unknown}$).