University Of Anbar

College Of Pharmacy

Advanced Pharmaceutical Analysis

"UV SPCTROPHOTOMETER ANALYSIS"

Photometric Analysis

Laboratory / Class "5"

By:

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UV Spectrophotometer.

Chapter 1/ Photometric Measurement

Key points:

In this lecture, we'll tell of the following contents: What's photometric measurement? How to setup parameters for photometric measurement? How to perform photometric measurement? How to save and printout measured results? It includes:

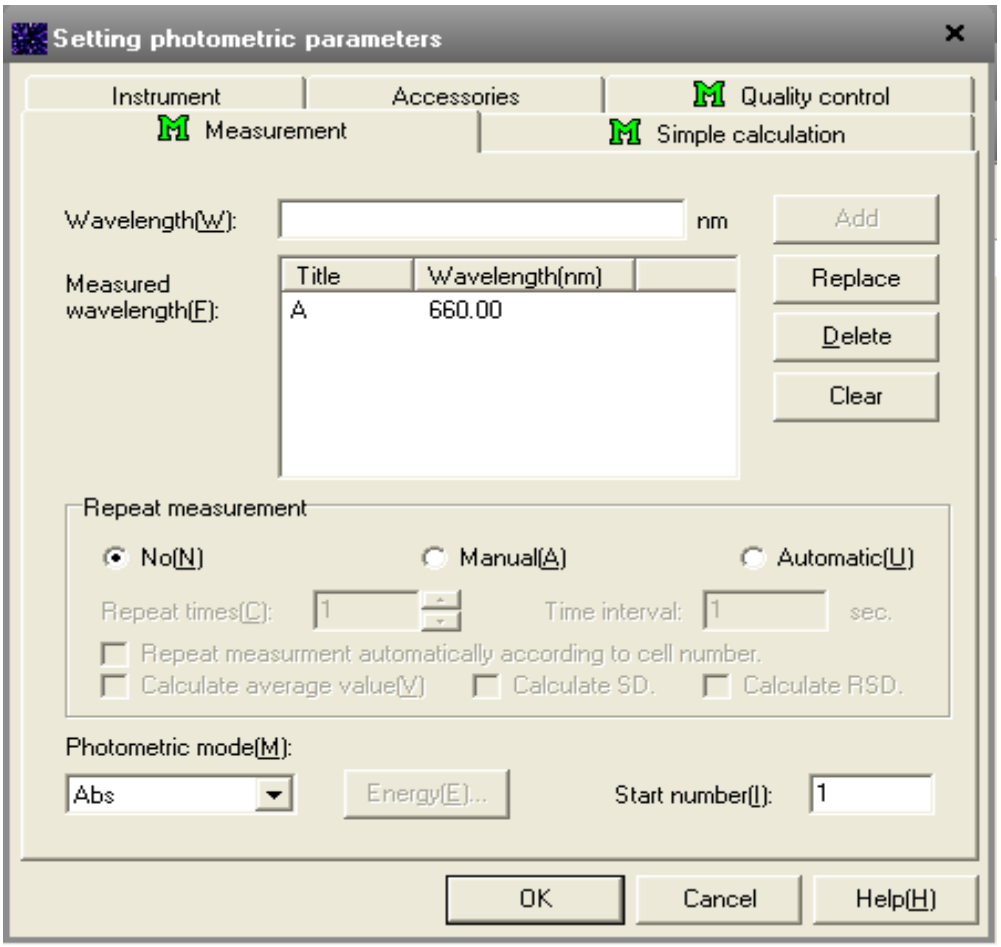
- Brief introduction of Photometric measurement
- Setting of photometric measurement parameters
- Photometric measurement
- Saving and printout of measured results
- Chapter Summary

1.1 Brief introduction of Photometric measurement

"Photometric measurement" is readout of measuring data at the wavelength points can set multiple wavelength points for photometric measurement. Furthermore, it can do some simple calculation on measured data.

1.2 Setting of Photometric measurement parameters

To enable Photometric Measurement, should click on the Photometry tab in Work Space. Select Parameters settings submenu under Measure Menu to activate the Photometric measurement setting dialog box, as shown in Figure 4-1. In this dialog box, there are 5 tabs, and can setup them according to various needs.



Figure; 1,1 Photometric parameters sittings "Measurement Tap"

• *Measurement Tab*

• *Wavelengths:*

In measurement tab, input in Wavelength Edit Box the wavelengths at which want to measure. Then click Add button to add it into its below wavelength list. The number of measuring wavelengths can be up to 26, while one is least. If need to delete wavelength points, or clear the wavelength list, can press Delete Button, or Clear Button. After choose a wavelength in wavelength list, there shows the same wavelength in Wavelength Edit Box. Here, So can amend the wavelength and press Replace Button to update it correspondingly in Wavelength list.

- ***Repeat Measurement***:

Photometric measurement enables to select number of repeat measurements. If not require repeat measurement, select No in Repeat Measurement Option. If manual repeat measurement is required, select Manua! in the same. And then input number of repeat measurements in Repeat Times Edit Box. Same as that of Manual, Automatic is also performing repeat measurement. And for Automatic, it accomplishes multiple repeat measurements automatically, without each press of Measure Button. What Automatic requires specifically is to set a time interval for measurement, that is, waiting time between every two measurements. This interval could be zero, which means to perform continuous measurement without pause. If have chosen Automatic Repeat, it also able to check the option box of "Repeat measurement automatically according to cell number", which will do once measurement over all samples in cells. In this way, there is no need to set repeat times, which will be forbidden. If set cell holder type as fixed cell holder, it cannot check the option box. Anyway, the main purpose of repeat measurement is in fact for averaging of repeat measurement data. So it can check "Calculate average value" to enable average calculation. Then System will calculate and display the average automatically in results table.

- ***Photometric Mode***

Photometric mode means instrument current operation mode, which has options of Abs. (Absorption), I-% (Transmittance), Es (Sample Energy), Er (Reference Energy), and R% (Reflectance).

- ***Start Number***

Setting a start point of sample serial number. What is input here can be any number.

- ***Simple Calculation Tab***

The option facilitates greatly calculation on measured results. By this function, we can work out some professional data and analytic results. Its setting window is

shown as Figure 1-2. can check the option box of "Enable Simple Calculation" to open the simple calculation function.

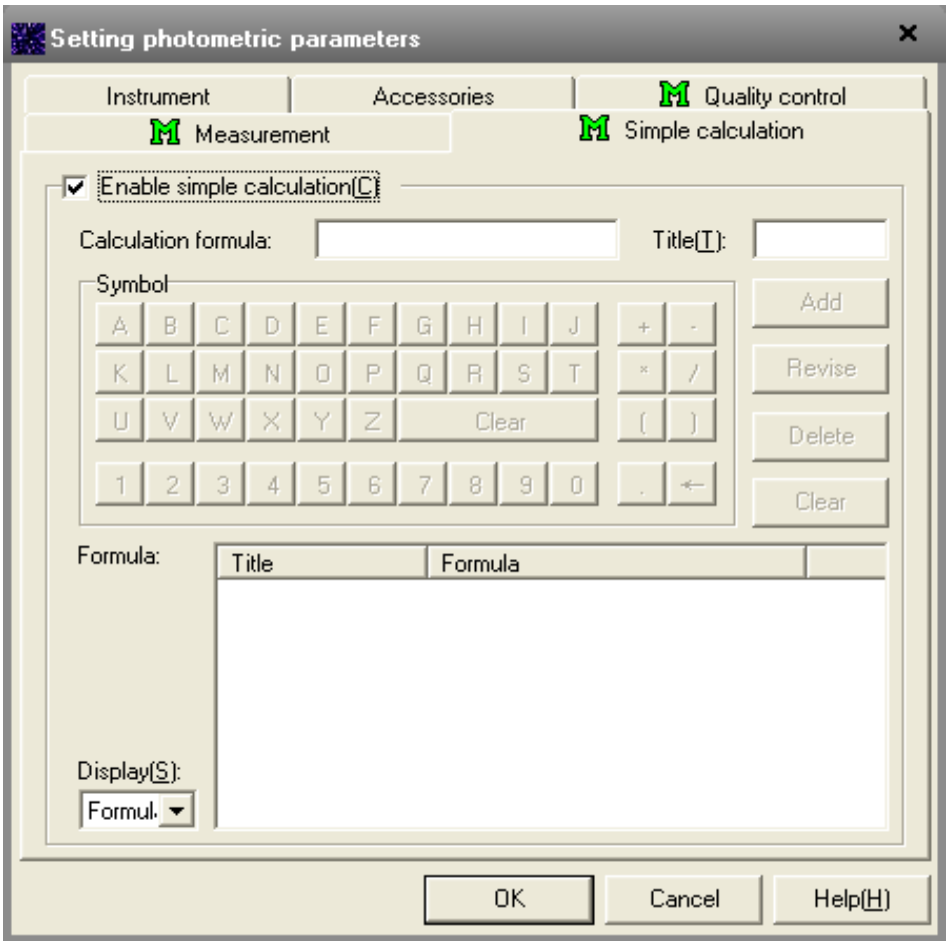


Figure 1.2; Photometric settings "Simple Calculation Tab"

• ***Calculation formula:***

In "Calculation formula" edit box, input required formula for results calculation. In formula, letters of A, B, C, and D...represent measured data of corresponding wavelengths. For example, input two wavelength points in Wavelength list of Measurement Tab, which are 600nm and 500nm. When calculate the ratio of measured data of these two wavelength points, so can input AM in Formula Edit Box of Simple Calculation Tab. Then press Add Button. The default title of calculation formula is "Result 1", "Result2"... If want to specify a title, input it, at same time when input calculation formula, in Title Edit Box. If need to amend a

formula, select it correspondingly in formula list, and revise it in formula edit box. Press Revise Button to make it effective. If require to delete or clear the content of formula list, press Delete Button or Clear Button. Number of Formula can be up to 10.

• ***Characters;***

The role of Characters here is for input imitating as keyboard. Press a character button so as to input the corresponding character equivalently.

• ***Display***:

The role of Display option here is for selection of different display modes for calculation formula. Pull-down box offers two choices, formula and title. Formula here means calculation formula will be displayed in result table as title. Title here means the default title or user set title will be displayed.

• ***Instrument Tab;***

The content of Instrument Tab is same as that of Instrument Performance Settings.

• ***Accessories Tab***;

The content of Accessories Tab is same as that of Accessories Settings.

• ***Quality Control***

For example, The scientific may think of what a product's quality is about. For example, the instruments get a problems. So he can think its quality is not good, as our judgment criterion is that its quality is not good if a new instrument gets problems shortly. so, quality is used to describe merchandise good or not. And quality is also able to be used to describe data good or not. For example, measured a datum of 0.1, while its normal value should be around 1, and at the utmost it should be not less than 0.8. Obviously, the measured result is not right, or is out of request. And can say its quality is not good, as our judgment criterion is not less than 0.8 at the utmost.

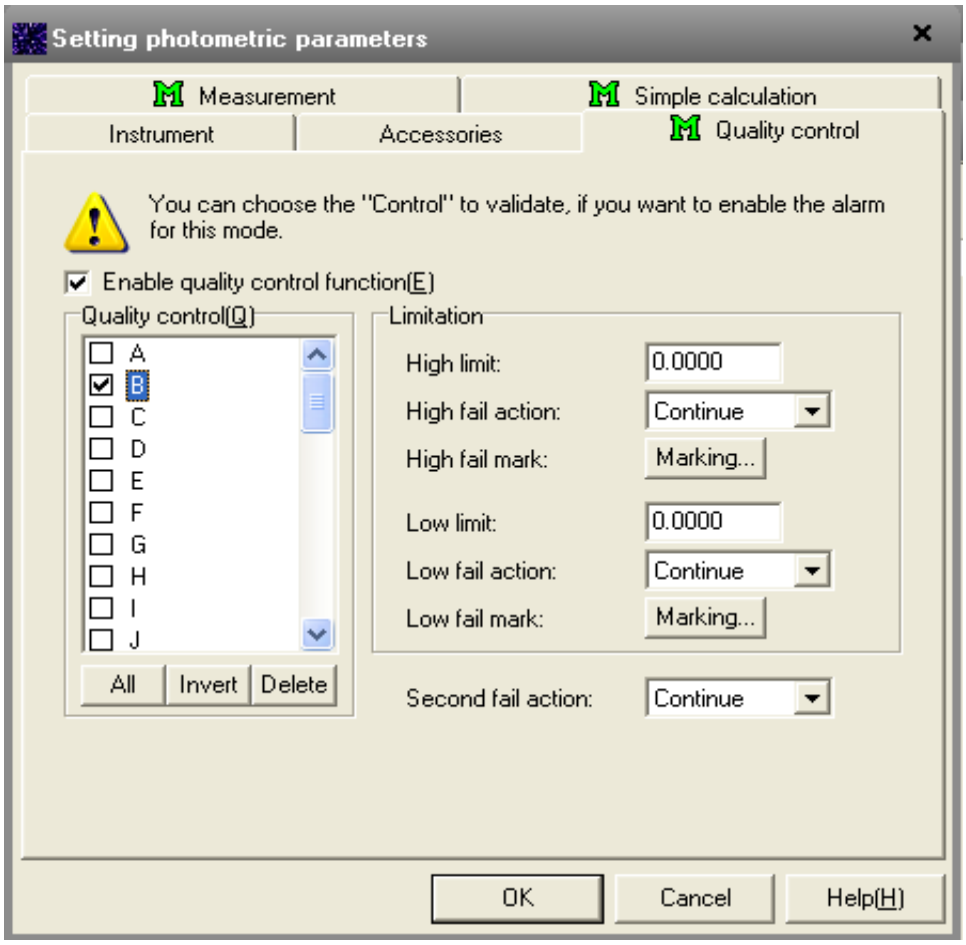


Figure 1.3 Quality control settings

In case there are abnormal data measured, System will prompt or act according to our preset. Of cause, judgment way for data can be preset. Shown as in Figure

1-3. In the quality control window, it can set Quality Control on or off through "Enable quality control function" option.

• ***Quality Control List***

Set items for quality control. Letters of A, B, C, D... express columns of measuring wavelength points, resultl, result2, result3..., and calculating results. Click at All Button to select all items, while click on Invert Button invert selection of all items. Click on Clear Button to clear all selections.

• *Limitation*

In limitation box, input High Limit and Low Limit for control of selected items. Fail Action could be set for System to act when data out of limits occur. The optional actions include: Continue—continue to measure, Stop—stop measurement, and Re-measure — Measure current sample again. If need to make a mark on result out of limits in result table, click on Marking Button to set mark type, as shown in Figure 1-4.

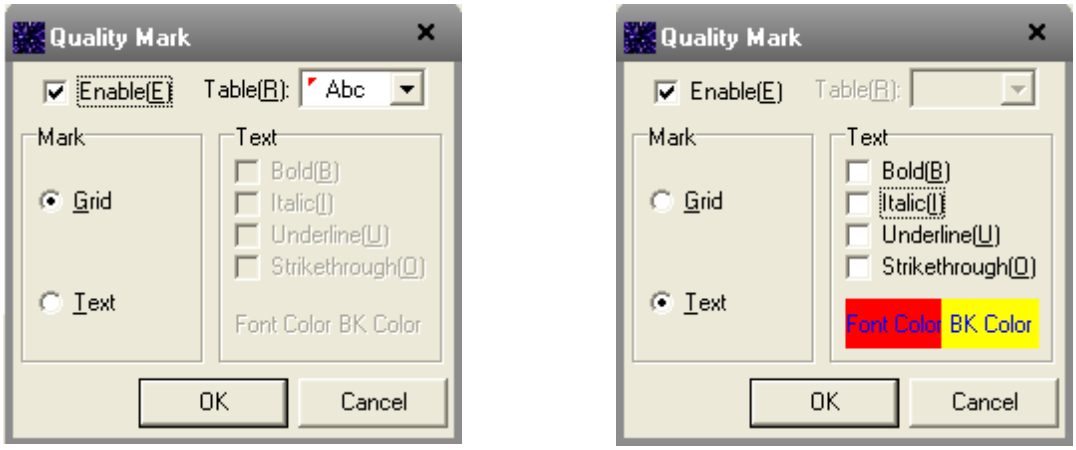


Figure 1.4 Quality mark Marking Grid "left" **Marking Text "Right"**

Check the Enable Option to open marking function. In Mark box, select marking mode for those data out of limits. "Grid" is marking the grid data located in. The optional marks can be selected from pull-down list of Grid. choose "Text", color and font of text can be setup.

• *Second fail action*

Second fail action means that System would take what action when there are successively twice of out of limits. The optional actions include "Continue" and "Stop".

1.3 Photometric Measurement

The measuring procedure of Photometric Measurement is very simple. Just click on *START* button to finish a measure. The measured result is displayed in result table. If want to delete a measured result, use mouse to click on it and select Delete

submenu under Edit Menu to delete it. If need to recall the deleted results, can click right button of mouse in result table. In popup menu, select the Cancel Deleting Submenu under the Delete Menu to recall the deleted results. If want to hide the deleted results, uncheck the Display Deleted Sample under the Delete Menu.

1.4 Saving and Printout

For measured results, they are not only able to be saved to a file, but also be able to be printed out. After finish analytic measurement, select Save submenu under File Menu, or click on *SAVE* button. System would pop up the Save File Window, as shown in Figure 1-5. Input a filename to save to and press Save button to save files to a specified location.

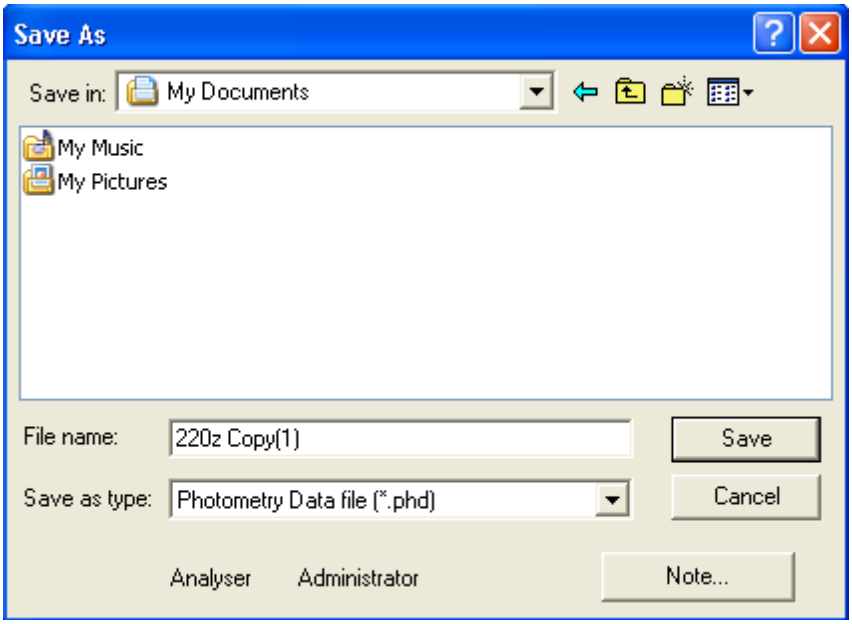


Figure 1.5 Save File window

In lower part of window, It shows current analyzer. Click on Note Button to make a note on measured results. Like as figure 1.6.

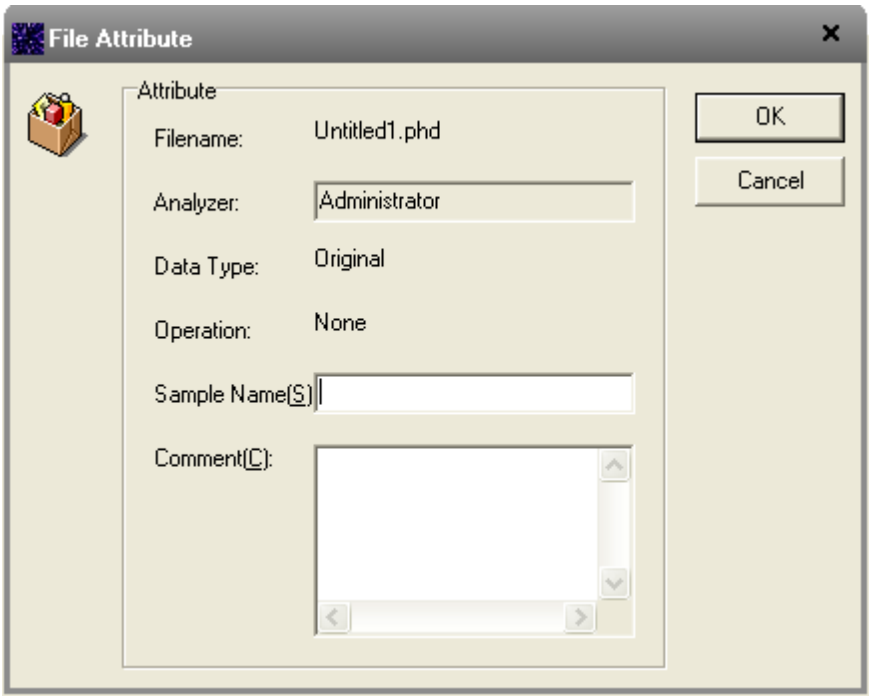


Figure 1.6 Note Window

We are able to input measuring sample name in Sample Name edit box, input notes on measured results in Comments edit box. All info above will be saved to measuring files for future reference. If want to print out measured results, also can select Print submenu under File Menu, Naturally, An addition, if want to modify printout format, select Page Settings submenu under File Menu to open the Page Setting Window. Shown as Figure 1-7.

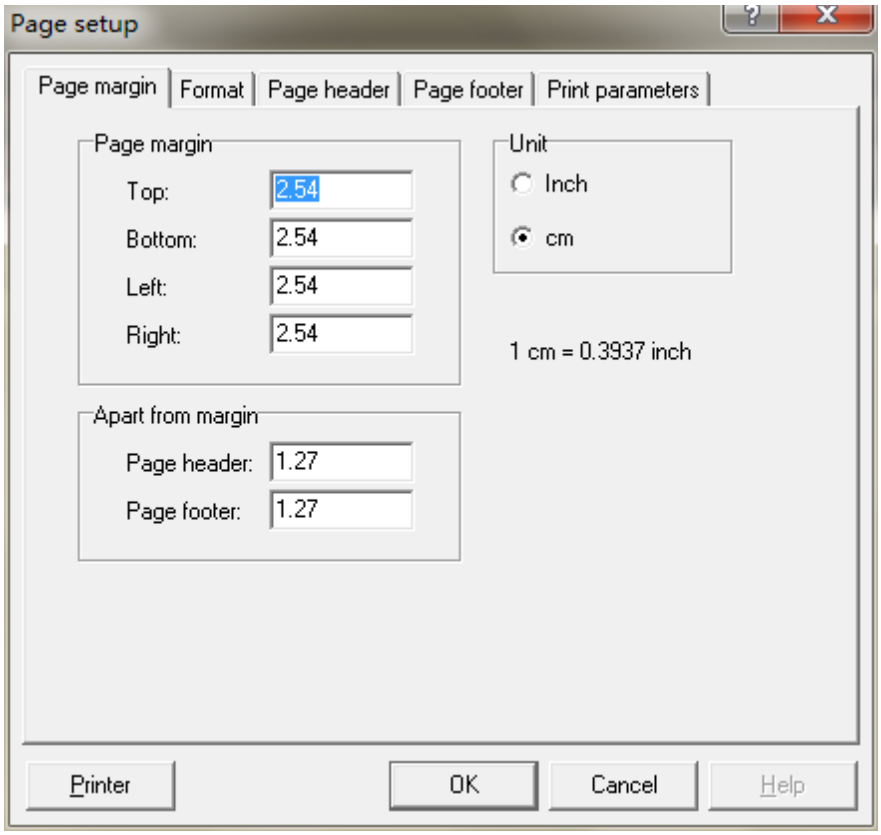


Figure 1.7 Page settings window

- ***Page Margin Tab***

The contents of Page Margin Tab are mainly setting parameters relating to interval. Shown as the above figure. First you are able to set the unit of margin, which has two choices of Inch and Centimeter. Then input four margins of top, bottom, left, and right in Margin Box. That is, the interval of text apart from paper edges. Apart from margin box is used to set the interval of page header and page footer apart from paper edges.

- ***Format Tab***

The contents of Format Tab are mainly setting the format, font, table, and direction of page output.

- ***Text***

Setting text printing format. The optional formats include Normal, Simple, and Table. "Normal" means printing out in text, without any decoration. "Simple" is to

underline sort headers, to highlight headers. "Table" means printing out in table. In addition, also able to click on Font Button to set print fonts.

• Line Width

Setting the thickness of table lines. The optional line widths include "Thin" and "Thick".

• Date/Time Format

Setting time format and date format for printout. The optional formats include "Weekday, MM DD, YYYY HH:MM:55", "MWDD/YY HH:MM:SS", "MM/DEVYY", and "HH:MM:SS". Except the first option, the year of date format is two digits.

• Page Number Format

Setting page number for printout. The optional page number formats include "1, 2, 3... The meaning of these formats is respectively: direct page number printout, adding hyphens to both sides of page number, adding angle brackets to both sides of page number, and adding parentheses to both sides of page number.

• Direction

Setting direction of printing paper as Portrait (vertical print) or Landscape (horizontal print).

"Date/Time": To print current date and time. The format of date and time can be set in Format Tab.

"Filename": To print the filename of measurement file.

"Module Name": To print the name of current measurement module, such as Photometric, Spectrum Scan, etc.

Instrument Name/Number": To print the name and serial number of current instrument, such as UV-VIS Spectrophotometer/01-1901-01-0001.

"Analyser": To print current login username.

"Page Number": To print current page number.

"Custom": To print custom text. If choose this option, bellow the pull-down menu, there is an Edit Box, in which can input custom text. If input relevant contents, click on "Font" Button to set its font. In addition, also can select Separation Line to draw separation lines among Header, Footer, and Text.

• Print Parameters Tab

Print Parameters Tab to select parameters relating to measurement to be printed out. The optional parameters include "Instrument Performances" and "Note". Instrument performances include instrument name, serial number, bandwidth, and etc, while Note includes analyzer, sample name and note. If want to see the result of page settings, or to preview the print result, can select Print Preview Submenu under File Menu, to perform print preview. Shown as Figure 1.8.

- **Instrument Performance**
  Model : T10CS
  Number : 12-1012-01-0001
  Spectral Bandwidth : 2.00 nm
- **File Information**
  Data File : C:\Documents and Settings\Administrator\Desktop\T10 12U1012-01-0001\T10 12U1012-01-0001\220z.phd
  Create Date/Time : Friday, April 20, 2012 4:13:22 PM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :
-

No.	ID	Mode	A 220.00 nm
1	1-1	T%	0.0
2	1-2	T%	0.0
3	1-3	T%	0.0
4	1-Avg	T%	0.0

Figure 1.8 Print Preview

1.5- Calibration Curve:

• Prepare a series of standard solution with known concentration.

• Measure the absorbance of the standard solutions.

• Plot the graph Abs vs concentration of std.

• Find the "best' straight line.

Procedure:

1- Prepare stock solution by using simple equation: N1V1=N2V2.
2- Prepare 10 different Conc. in 50 ml volumetric flask 50ml. by using equation: N1V1=N2V2.
3- Run your samples in UV device
4- Draw curve and find the better λ_{max} for your solution , after which the unknown is measured and focused by dropping it on the straight line.

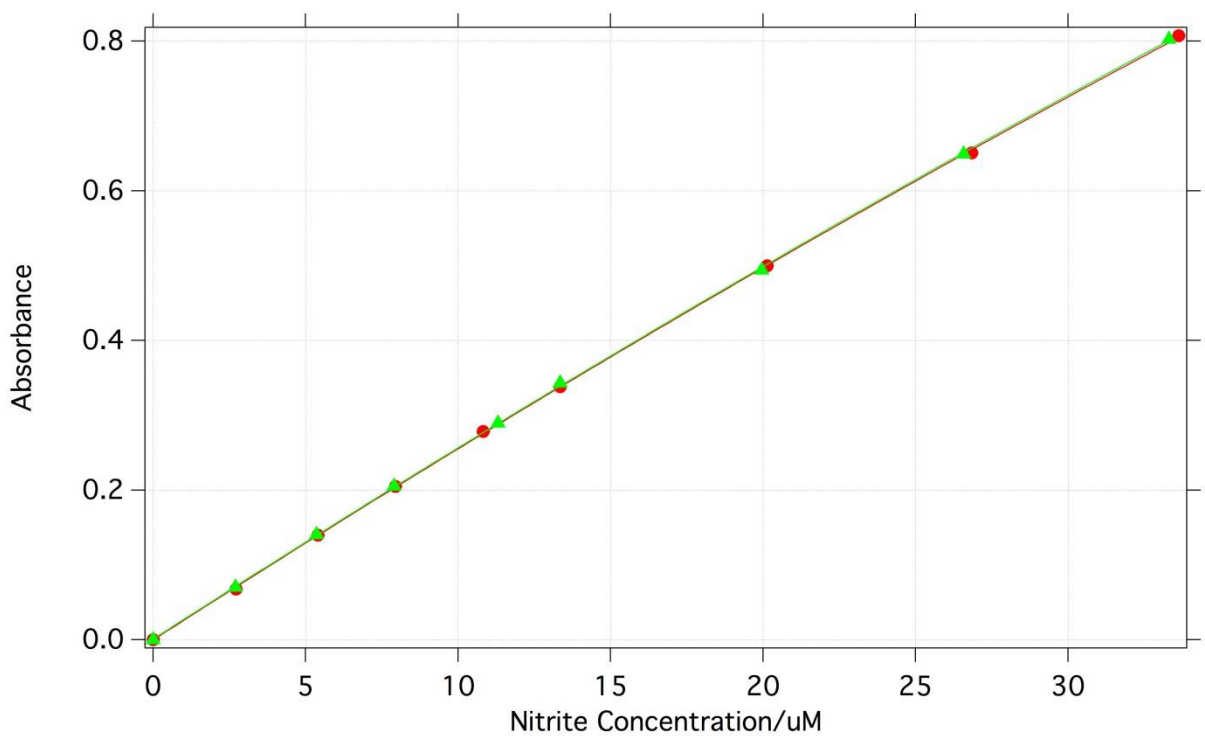


Figure 1.9 Calibration Curve for Nitrite Sol.

Path length / cm	0	0.2	0.4	0.6	0.8	1.0
%T	100	50	25	12.5	6.25	3.125
Absorbance	0	0.3	0.6	0.9	1.2	1.5

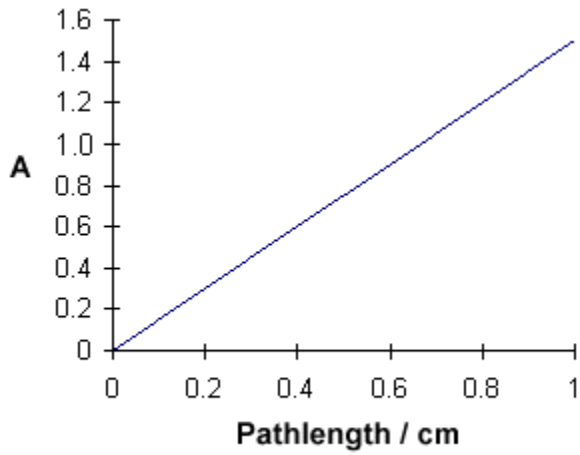


Figure 1.10 Calibration Curve Example

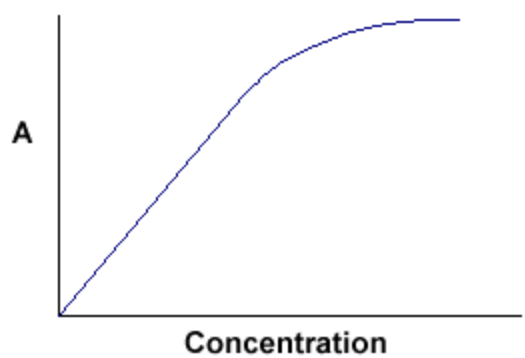


Figure 1.11 deviation Curve for High Conc.

Note that the Law is not obeyed at high concentrations. This deviation from the Law is not dealt with here.

The linear relationship between concentration and absorbance is both simple and straightforward, which is why we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T.

THANKS: A.L. Abdulkareem Hamad