### PERKIN ELMER LAMBDA 35 UV/VIS SPECTROMETER

INSTRUMENT SUPPORT: David J. Chesney					
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### INTRODUCTION

This instrument is generously provided and supported by the Department of Chemistry. Any MTU faculty, staff or students may use it if they receive proper training from the person in charge of instrument support. This person will ensure you know how to properly use and care for the instrument without damaging it.

#### **OPERATION INFORMATION**

### I. GENERAL INSTRUMENT, SAMPLE PREP & WORKSPACE MAINTENANCE

- A. Keep all solvents and chemicals on the prep bench across from the spectrometer.
- B. Sign in and out on the instrument log sheet before and after using it.
- C. Polystyrene cuvettes are available in drawer #11 and may be used in the visible region (350-800 nm). Organic solvents should not be used in these cuvettes as they may dissolve the polystyrene.
- D. Quartz cuvettes are available in drawer #11 and may be used in both the visible and UV regions (200-800 nm).
- E. Use lens paper, not Kimwipes to clean and wipe the outer surfaces of the cuvettes.
- F. When finished, polystyrene cuvettes may be thrown into the garbage.
- G. Clean quartz cuvettes with distilled water and return them to drawer #11.
- H. Wipe up spills, throw away used lens paper, remove your sample to a student locker or remove from 408.

#### II. STARTING

- A. Turn on the instrument. The switch is in the top right corner of the spectrometer box. (NOTE: ONCE THE LAMPS ARE ON, YOU MUST USE PROPER SHUT-DOWN PROCEDURE TO SHUT THEM OFF – PROCEDURE FOLLOWS).
- B. Turn on the computer and monitor.
  - i. USERNAME: administrator
  - ii. PASSWORD: <a></a> <a></a><
  - iii. <OK>
- C. Select the *Perkin Elmer UV-VIS* icon.
  - i. USERNAME: <u>analyst</u>
  - ii. <OK>

Definitions: A **Method** in the Lambda 35 is analogous to a "procedure". A **Method** defines the wavelength range, the slit width, and other instrument parameters.

A **Task** is an experiment run using a **Method**. For example, you might take a series of spectra, changing the polarity of the samples each time. Or, you might have a series of spectra, each of a different concentration of analyte. You use the same **Method**, but store the data as a **Task**.

- D. Create a new Method using the New Method Wizard.
  - i. Click File  $\rightarrow$  New  $\rightarrow$  Method

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- ii. New Method Wizard window
  - a) Select Type of Instrument <Medium Performance> <Next>
  - b) Select the Instrument <Lambda 35> <Next>
  - c) Select a Method Type
    - <Scan> Scans the spectrum between two specified wavelengths.
    - <Wavelength Program> Obtains absorption data at one specified wavelength (for producing calibration curves) <Next>

- d) Choose Accessory Type <Make no selection> <Next>
- e) Properties of Method <Save/Finish>
- iii. Save Method Window

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- a) Create a folder specific to your use. Please do not use the default "Method" directory it gets full very quickly of random files of indeterminate ownership.
- b) Specify your method name. Give it a descriptive name the generic names given by the software differ by date/time of creation only making it very difficult to go back and find a specific method.

# III. DATA COLLECTION/SAVING DATA

## A. Scan Method

i. From the Data Collection Window, specify <u>Start (nm)</u> wavelength, <u>End (nm)</u> wavelength, <u>Ordinate mode</u> & <u>Slit width (nm)</u>

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- ii. Fill reference and sample cuvettes with your blank. Insert both in the instrument.
- iii. Click the blue triangle to collect a background.
- iv. Wait for instrument response: "Remove sample(s) and then press OK to perform a 100%T/0A correction (Autozero)."
- v. Click OK to perform Autozero.

NOTE: DO NOT REMOVE THE REFERENCE OR SAMPLE CUVETTES. You are correcting here for any mis-match in your reference and sample cuvettes. This background scan is recorded and then used as the baseline for all subsequent measurements using this method.

vi. When prompted, remove the sample cuvette, fill with your sample, return to sample cuvette holder, and click OK.

#### October, 2012

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vii. To collect more than one spectrum, click <u>Sample Info</u> in the <u>Folder List</u>

- a) Change the number of samples, rename samples with meaningful names
- b) When you click the blue triangle, the software will automatically prompt you to change samples until all samples in the table have been run.

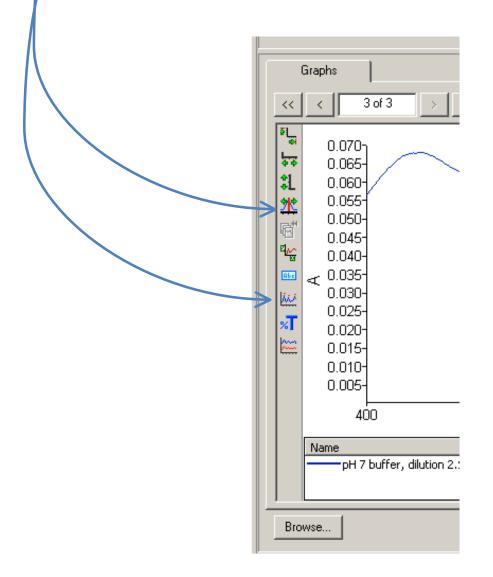
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viii. <u>To change default display</u>: see buttons along left side of spectrum

a) **Full range** – displays entire spectrum between specified wavelengths & normalizes to maximum absorbance

b) **Autorange x** – adjusts x-axis to include spectrum between specified wavelengths

- c) Autorange y adjusts y-axis to include all peaks
- d) Vertical cursor allows placement of cursor on peak or other feature
- e) **Previous range** reverts to previous range
- f) Format graph launches dialog box to format axes, titles, etc.
- g) Add text adds a text box
- h) Label peaks depress button, right click on spectrum, Label Peaks . . .
- i) %T converts to percent transmittance
- j) Split display toggles between overlaid spectra and split spectra



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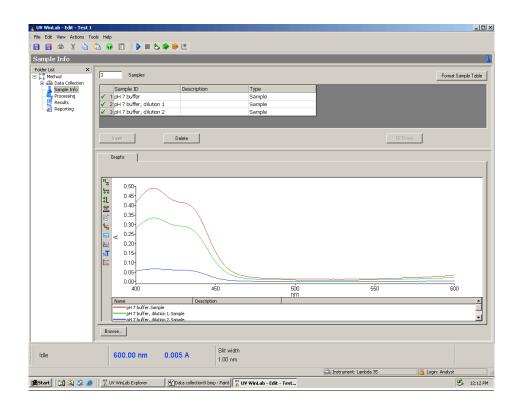
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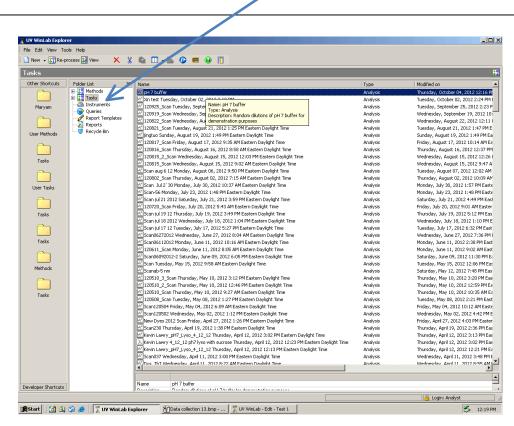
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- ix. Overlaying Spectra (all spectra must be in the same Task)
   a) Tools --> Options --> Click button for <u>All samples on one graph</u>



x. <u>To save task</u>: File --> Save (or Save As) --> Task . . .

a) Edit your file name. Give it a descriptive name – the generic names given by the software differ by date/time of creation only making it very difficult to go back and find specific spectra.

NOTE: The Task file is ALWAYS stored in the "Tasks" directory of UV WinLab Explorer. This is a default in the software that cannot be changed. To move your task to a directory, you must cut and paste the file into the selected directory.



#### B. Wavelength Method

- i. From the Data Collection Window, specify wavelengths to be measured using <u>Add</u> and **Remove** buttons.
- ii. Click **Sample Info** in the **Folder List** to setup a sample table.
- iii. Fill reference and sample cuvettes with your blank. Insert both in the instrument.
- iv. Click the blue triangle to collect a background. Click OK to perform Autozero.

NOTE: DO NOT REMOVE THE REFERENCE OR SAMPLE CUVETTES. You are correcting here for any mis-match in your reference and sample cuvettes. This background scan is recorded and then used as the baseline for all subsequent measurements using this method.

- v. When prompted, remove the sample cuvette, fill with your sample, return to sample cuvette holder, and click OK.
- vi. The software will automatically prompt you to change samples until all samples in the table have been run. In the "Sample Info" window, a green check mark will appear as

each sample is done.

- vii. Click "Reporting" to get a printable report.
  - a) Click on printer icon in the toolbar to initiate print.
- viii. To save task: File --> Save As --> Task . . .

a) Edit your file name. Give it a descriptive name – the generic names given by the software differ by date/time of creation only making it very difficult to go back and find specific spectra.

NOTE: The Task file is ALWAYS stored in the "Tasks" directory of UV WinLab Explorer. This is a default in the software that cannot be changed. To move your task to a directory, you must cut and paste the file into the selected directory.

#### IV. HANDLING FILES

- A. **Moving files** to a personal folder (this will help keep the files organized)
  - i. When you save a Method or Task, they are saved to a common directory.
    - ii. Select the UV WinLab Explorer window.
  - iii. After saving, find your Task file, right click, select Cut.
  - iv. Within the Task folder list, find or create a file folder for yourself.
  - v. Open that folder, right click, select Paste.

#### B. Opening files

- i. Find your task or method in the folder list and select it.
- ii. Along the top button bar, you have the option of choosing View or Reprocess. Re-process allows you to make changes to your spectrum/data, View only allows printing and viewing.

#### C. Printing files

- i. Spectra are printed automatically when saved.
- ii. To re-print, View or Re-process your task, click Reporting, File--> Print

#### D. ASCII export

- i. Right click on a spectrum.
- ii. Click **Export as asc**.

### V. SHUT-DOWN PROCEDURE: MANDATORY TO PROTECT LAMP LIFE

A. <u>With an active Method or Task window open</u>, perform the UV-VIS Shut Down Procedure below (also in a separate sheet protector in drawer #7). to turn off the UV and Visible lamps.

- i. Add a sample to your list under **Sample Info**
- ii. In the <u>Folder List</u> pane on the left-hand side of the window, select the following path: Method/Data Cellection/Instrument. A gray window will appear to the right of the Folder List.

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	on the blue tria				
vi. Afte	r the lamps hav	<b>'e <mark>shut off</mark></b> , clic	k the blue s	square near the	e top of the
SC	creen to stop the	program.			
vii. Exit					
viii. Turn	off power to spe	ectrophotomete	er.		
	t down computer				
inti eritat					
🔥 UV WinLab - Edit - 🗤 est				_D×	
	Tools Help	T			
Inst whent				<u></u>	
Fold X X	Scan Settings	ate mode Slit width (nm)			
Accessory	600 400 A				
Corrections Sample Info	Advanced Scan Settings Scan speed (nm/min)	a interval (nm)			
Results Reporting	480 💌 🚺	_			
	Cycles Number of cycles C (	lycle as fast as possible			
		)ycle time			
	Lamps				
	Visible lamp on 326	p change at (nm)			
	Visible lamp on				
	Graphs			1	
	N. 0.001				
	<b>1</b> ≪ 028 020 @ff 0.15	X			
		á á			
	DH 7 buffer, diution 1. Sample 0.334	r Description 99 A			
	pH 7 buffer, dilution 2.Sample 0.068	Slit width			
Idle	600.00 nm 0.005 A	1.00 nm			
🔀 Start 🛛 🔀 🖄 🤞	👂 📉 UV WinLab Explorer 🛛 🚰 Shut down 1.bm	p - Paint VV WinLab - Edit - Test	🖄 Instrument: Lambda 35	📙 Login: Analyst 🥢	

Your comments/corrections to these procedures are welcome. Please make comments directly on this document or email suggestions to: <u>djchesne@mtu.edu</u>