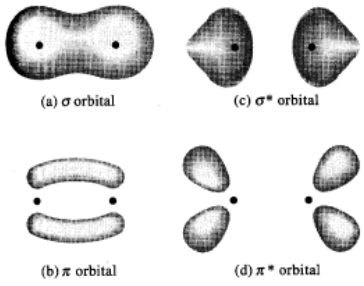
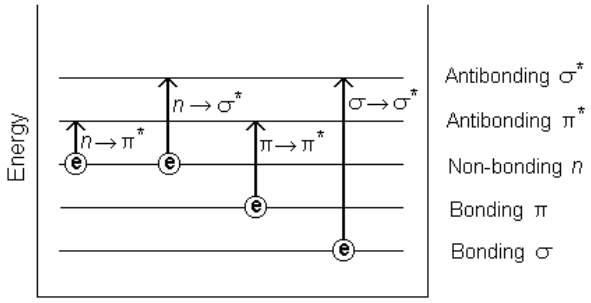
UV-VIS photometry



1. Identification by UV-VIS photometry
2. Effect of pH on absorption spectrum of acid-base indicators

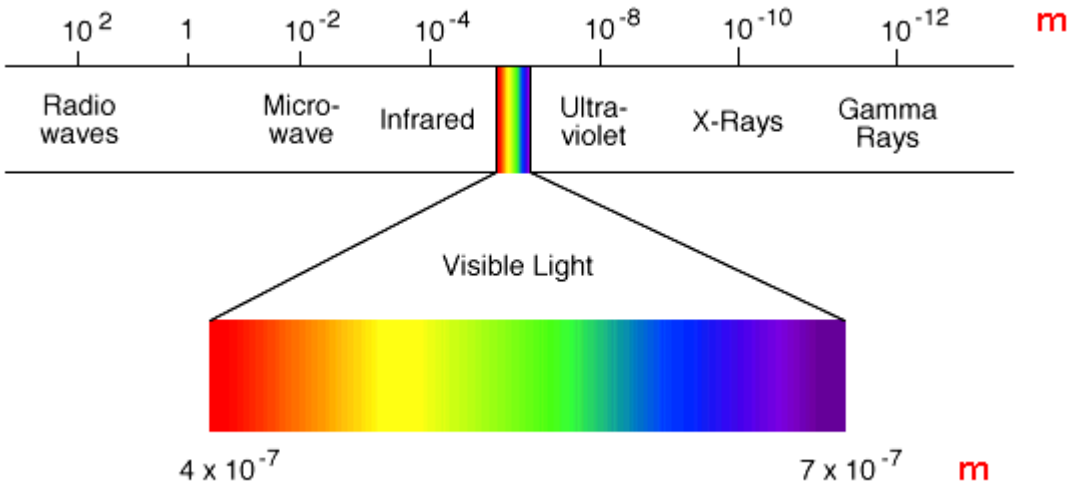
1. Theory

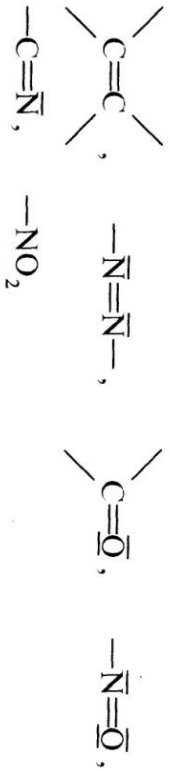


Molecules can absorb photons of suitable wavelength λ, i. e. those corresponding to the energy of electronic transition. In UV-VIS the photons exhibit energy sufficient for outer electrons to be excited into higher energy levels (200-600 kJ/mol).
Parts of the molecules where this effect occurs are called *chromophores.* Absorption of UV-radiation occur by transitions into antibonding σ* or π* orbitals in compounds with σ, π and n (nonbonding) electrons (single or multiple bonds including nonbonding electron pairs). Absorption maxima are shifted into visual (VIS) region if higher number of π-π* transitions is possible (e.g. due to conjugation of double bonds). Visual absorption (transition to π*) can occur with π or nonbonding electrons.
Note: this text will not deal with energy transitions into d nor f orbitals (coordinate-covalent bonding).

σ→σ*	ΔE large (λ<150 nm) ε=10-10,000 L/mol·cm
n→σ*	(halogens, N, O, S) ΔE smaller (λ=150-250 nm) ε=200-2000 L/mol·cm
π→π* n→π*	ΔE small (λ=200-700 nm) ε=10-10,000 L/mol·cm

FIG. 1 Wavelengths of VIS electromagnetic radiation

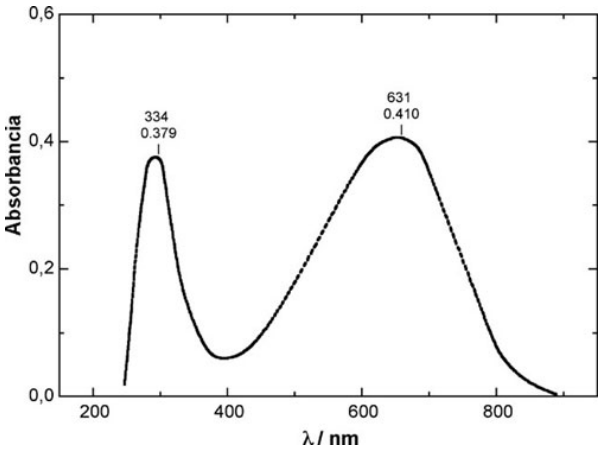




Absorption Characteristics of Some Common Organic Chromophores

Chromophore	Example	Solvent	λ_{max}, nm	ε_{max}
Alkene	$C_6H_{13}CH{=}CH_2$	*n*-Heptane	177	13,000
Conjugated alkene	$CH_2{=}CHCH{=}CH_2$	*n*-Heptane	217	21,000
Alkyne	$C_5H_{11}C{\equiv}C{-}CH_3$	*n*-Heptane	178	10,000
			196	2,000
			225	160
Carbonyl	CH_3CCH_3 (O)	*n*-Hexane	186	1,000
			280	16
	CH_3CH (O)	*n*-Hexane	180	Large
			293	12
Carboxyl	CH_3COH (O)	Ethanol	204	41
Amido	CH_3CNH_2 (O)	Water	214	60
Azo	$CH_3N{=}NCH_3$	Ethanol	339	5
Nitro	CH_3NO_2	Isooctane	280	22
Nitroso	C_4H_9NO	Ethyl ether	300	100
			665	20
Nitrate	$C_2H_5ONO_2$	Dioxane	270	12
Aromatic	Benzene	*n*-Hexane	204	7,900
			256	200

FIG.2 Maxima shifts of a conjugated system



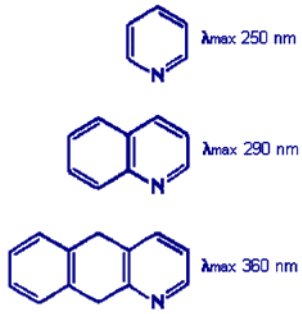


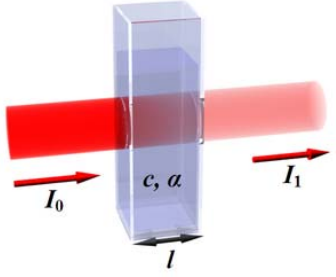
FIG.3 En example of absorption spectrum with two maxima in VIS range

Definition of Transmitance, Absorbance and Lambert-Beer´s law

$$T = \frac{I}{I_0}$$

$$A = -\log\frac{I}{I_0} = \log\frac{I_0}{I}$$

$$A = \varepsilon bc$$

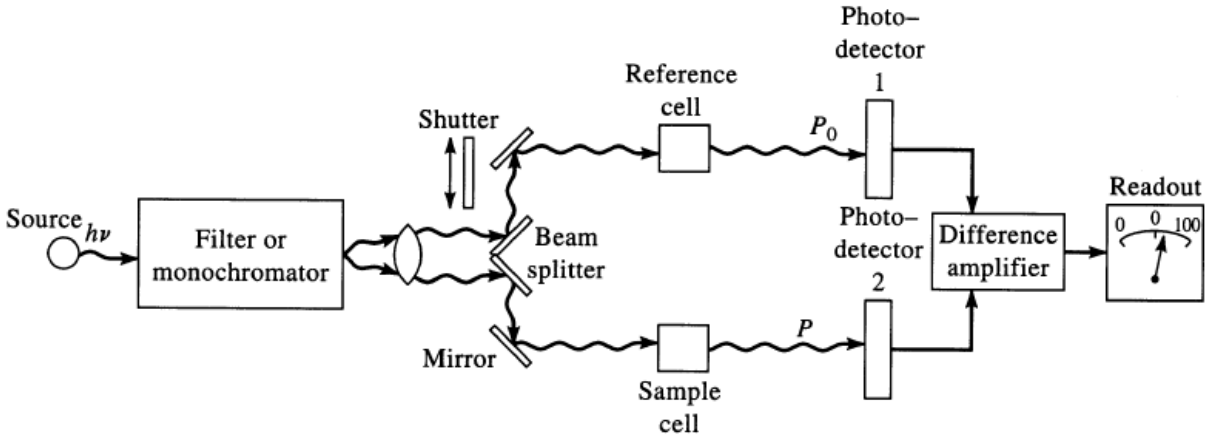


b=thickness of the cuvette [cm], ε=molar absorption coefficient [l/mol.cm], c=molar concentration [mol/l], I_0 and I=intensity of the incident light and the transmitted light, respectively.

INSTRUMENTATION

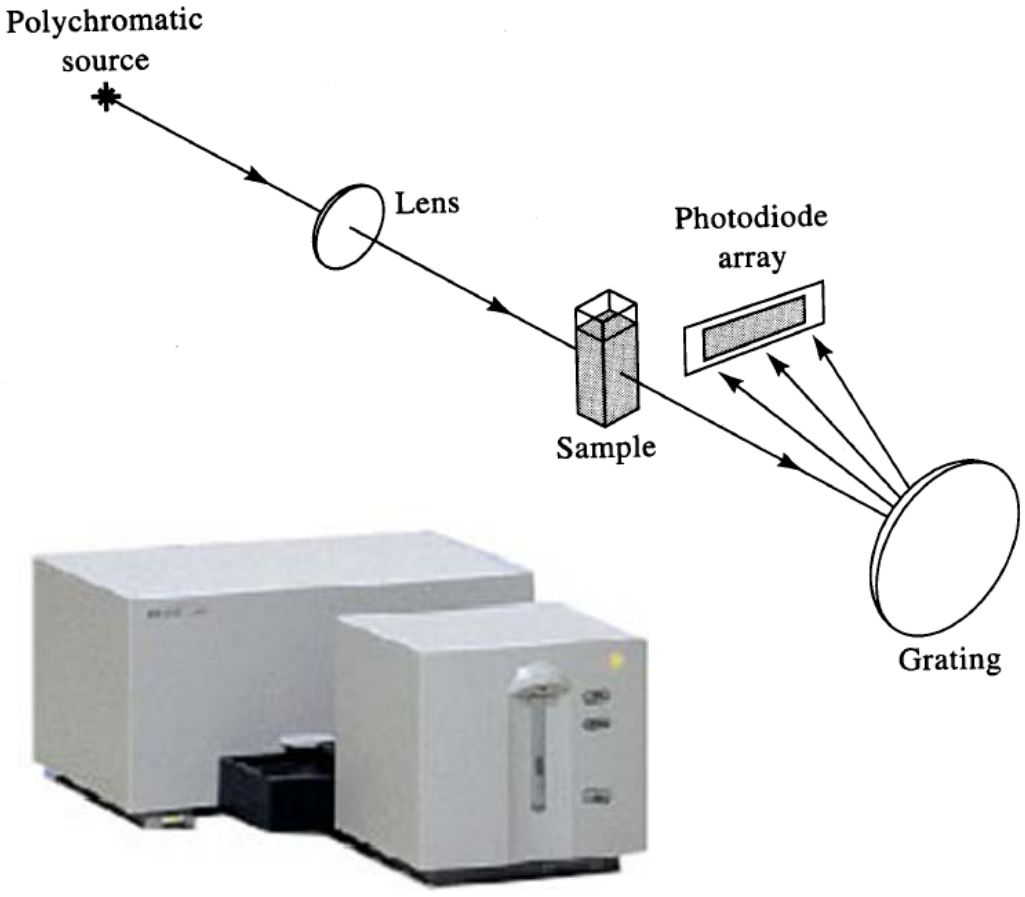
Classical Double-Beam Photometer with Monochromator

Monochromator selects (filtrates) from the polychromatic source the wavelength that is in use. In double beam photometers there are two cuvettes (cells) so that blank sample can be always measured.



Multichannel Spectrophotometer (single beam)

There is grating that decomposes a polychromatic beam after it passes through the cuvette! The transmitted light is dispersed and measured "all wavelengths at ones". **This principle is used at the photometer HP 8543 utilized in this exercise.**



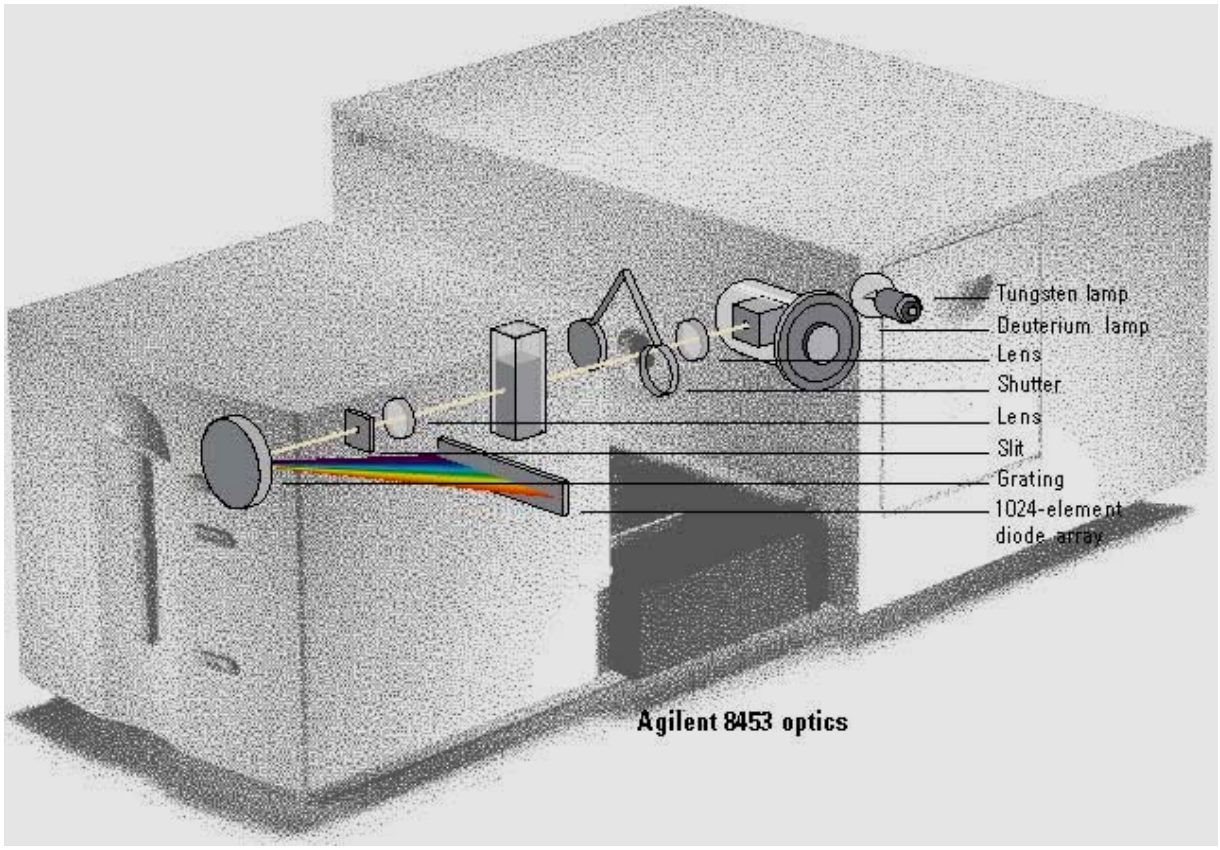
2. Acid-base indicators

What is an acid-base indicator?
An acid-base indicator is a weak acid or a weak base. The undissociated form of the indicator is **a different color** than the iogenic form of the indicator. An indicator does not change color from pure acid to pure alkaline at specific hydrogen ion concentration, but rather, color change occurs over a range of hydrogen ion concentrations. This range is termed the color change interval. It is expressed as a pH range:

Indicator	pH Range	Quantity per 10 ml	Acid	Base
Thymol Blue	1.2-2.8	1-2 drops 0.1% soln. in aq.	red	yellow
Pentamethoxy red	1.2-2.3	1 drop 0.1% soln. in 70% alc.	red-violet	colorless
Tropeolin OO	1.3-3.2	1 drop 1% aq. soln.	red	yellow
2,4-Dinitrophenol	2.4-4.0	1-2 drops 0.1% soln. in 50% alc.	colorless	yellow
Methyl yellow	2.9-4.0	1 drop 0.1% soln. in 90% alc.	red	yellow
Methyl orange	3.1-4.4	1 drop 0.1% aq. soln.	red	orange
Bromphenol blue	3.0-4.6	1 drop 0.1% aq. soln.	yellow	blue-violet
Tetrabromphenol blue	3.0-4.6	1 drop 0.1% aq. soln.	yellow	blue
Alizarin sodium sulfonate	3.7-5.2	1 drop 0.1% aq. soln.	yellow	violet
α-Naphthyl red	3.7-5.0	1 drop 0.1% soln. in 70% alc.	red	yellow
p-Ethoxychrysoidine	3.5-5.5	1 drop 0.1% aq. soln.	red	yellow
Bromcresol green	4.0-5.6	1 drop 0.1% aq. soln.	yellow	blue
Methyl red	4.4-6.2	1 drop 0.1% aq. soln.	red	yellow
Bromcresol purple	5.2-6.8	1 drop 0.1% aq. soln.	yellow	purple
Chlorphenol red	5.4-6.8	1 drop 0.1% aq. soln.	yellow	red
Bromphenol blue	6.2-7.6	1 drop 0.1% aq. soln.	yellow	blue
p-Nitrophenol	5.0-7.0	1-5 drops 0.1% aq. soln.	colorless	yellow
Azolitmin	5.0-8.0	5 drops 0.5% aq. soln.	red	blue
Phenol red	6.4-8.0	1 drop 0.1% aq. soln.	yellow	red
Neutral red	6.8-8.0	1 drop 0.1% soln. in 70% alc.	red	yellow
Rosolic acid	6.8-8.0	1 drop 0.1% soln. in 90% alc.	yellow	red
Cresol red	7.2-8.8	1 drop 0.1% aq. soln.	yellow	red
α-Naphtholphthalein	7.3-8.7	1-5 drops 0.1% soln. in 70% alc.	rose	green
Tropeolin OOO	7.6-8.9	1 drop 0.1% aq. soln.	yellow	rose-red
Thymol blue	8.0-9.6	1-5 drops 0.1% aq. soln.	yellow	blue
Phenolphthalein	8.0-10.0	1-5 drops 0.1% soln. in 70% alc.	colorless	red
α-Naphtholbenzein	9.0-11.0	1-5 drops 0.1% soln. in 90% alc.	yellow	blue
Thymolphthalein	9.4-10.6	1 drop 0.1% soln. in 90% alc.	colorless	blue
Nile blue	10.1-11.1	1 drop 0.1% aq. soln.	blue	red
Alizarin yellow	10.0-12.0	1 drop 0.1% aq. soln.	yellow	lilac
Salicyl yellow	10.0-12.0	1-5 drops 0.1% soln. in 90% alc.	yellow	orange-brown
Diazo violet	10.1-12.0	1 drop 0.1% aq. soln.	yellow	violet
Tropeolin O	11.0-13.0	1 drop 0.1% aq. soln.	yellow	orange-brown
Nitramine	11.0-13.0	1-2 drops 0.1% soln in 70% alc.	colorless	orange-brown
Poirrier's blue	11.0-13.0	1 drop 0.1% aq. soln.	blue	violet-pink
Trinitrobenzoic acid	12.0-13.4	1 drop 0.1% aq. soln.	colorless	orange-red

Because of the color change, the indicators are utilized for visual indication of pH change, typically at acidobasic titrations.

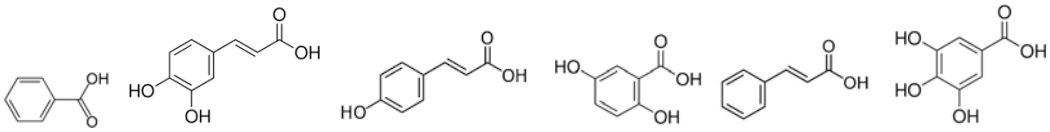
FIG. 4 Sheme of Hewlett Packard 8453



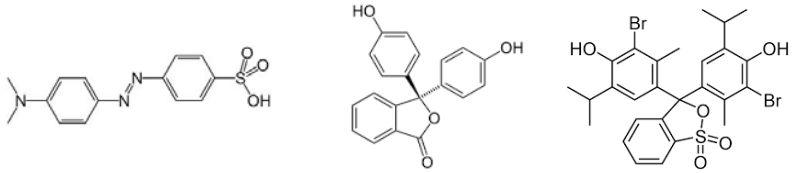
http://www.p-forster.com/english/themes/Spectroscopy/BASICS/

Instrument
Single-beam photometer HP8453

Chemicals and dish
Acids: benzoic a., caffeic a., *p*-cumaric a., gentisic a., cinnamic a., gallic a.



Methyl orange / phenolphthalein / bromthymol blue (or other indicators)



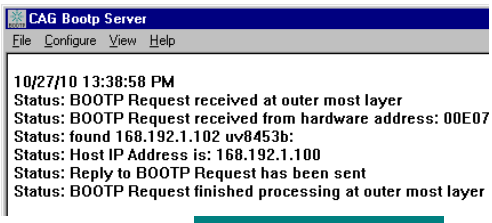
6x25 ml, 6x100 ml volumetric flasks, ethanol / methanol.
Quartz cuvette

http://www.unco.edu/nhs/chemistry/Instrument/uvvis.html

Operating Instructions of Diode Array Spectrophotometer HP8453

Turning on the Instrument:

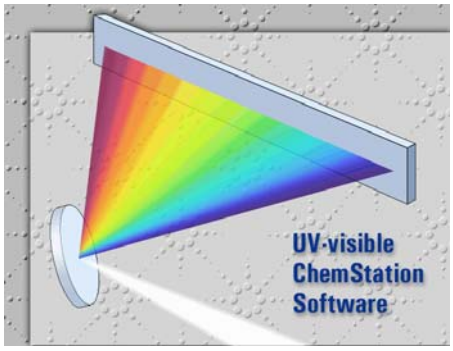
1. Switch on PC. Wait until CAG-Bootp window appears



2. Turn on the HP 8453 Diode Array. (Power button directly below model plate.) Wait for the yellow light on the front of the instrument to turn green. (This takes a few minutes.) CAG-Bootp window shows some lines like this:

3. Double click on the HP Diode Array icon (on-line). When the window UV-Visible ChemStation appears, click on Cancel, and the software program will continue to load.

Taking Spectrophotometric Data:



1. For data to be taken, a task must be selected. Your selections are found by clicking on the down arrow u of the Task box: (The following selections appear.)

Fixed Wavelengths (Default)
Spectrum/Peaks
Ratio/Equation
Quantification

Determination of λ_{max} (Spectrum/Peaks Task)

1. To determine a solution's wavelength of maximum absorption (λ_{max}), click on Spectrum/Peaks. The Spectrum/Peaks Parameters window will appear.

2. For normal use, the following Spectrum/Peaks parameters should be utilized:
   Peak/Valley find
   Find and annotate up* to 1 peaks (default is on X and 3)
   Find and annotate up* to 3 valleys (default is on X and 3)
   Prompt for sample information* (default is off)
   *(to turn parameter off _ or on X click on parameter)
   Data type* Display spectrum**
   Absorbance From: 190 nm To: 1100 nm

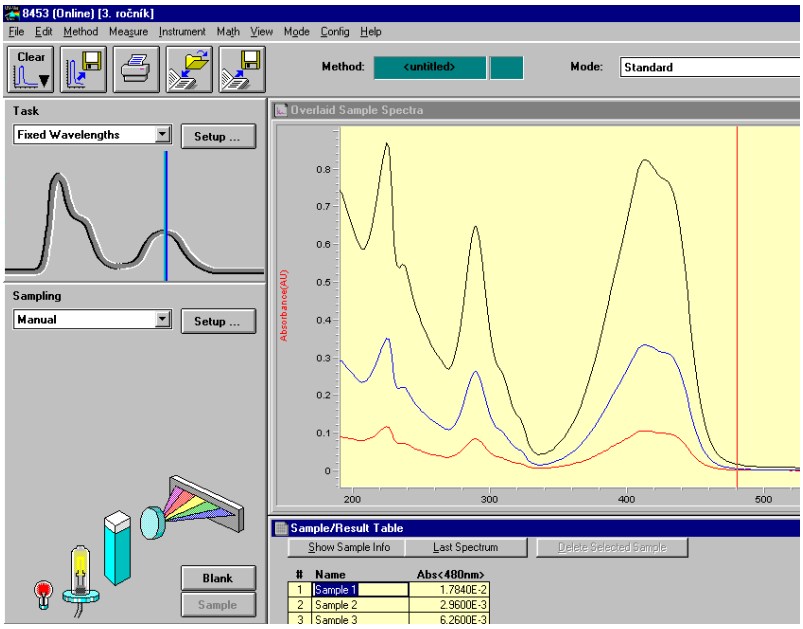
3. Click on OK when finished, if you need to change any parameters click on Setup, and enter the new parameters.

4. You are now ready to take data.
   a. Taking a spectrum of your blank: Place your blank in a clean cuvette, inspect for bubbles, and then carefully wipe the exterior surface. Place the cuvette

into the holder in the diode array 8453 (make sure cuvette is positioned correctly), and secure the locking mechanism by gently pressing down on the lever. Locate the Sampling box (left side of screen), and confirm the Manual setting. Click on Blank. This spectrum displays where the cuvette or blank is absorbing.

b. Taking a spectrum of your sample: Rinse the cuvette well, and fill it with sample. Then, inspect for bubbles, and carefully wipe the exterior surface (with a Kimwipe). Place the cuvette into holder, and secure it. Click on Sample .

c. Printing your sample spectrum: Locate the print icon (upper left corner of screen), and click on it.



Shutting Down the Instrument:

1. Click on File, and then click on Exit ChemStation.

2. When the window, Close HP 845x UV-Visible, appears, make sure you DO NOT Save Configuration, and then click on OK.

3. Click on CAG Bootp Server (lower left corner of screen), click on File, then Exit.

4. Click on Start (lower left corner of screen), then Shut Down. When the window Shut Down Windows appears click on Yes .

6. Turn off the HP 8453 Diode Array.

Sample preparation and measurement

1. Qualitative analysis of spectra
   - Set the spectrum range to 210-410 nm
   - Prepare solutions of standards of suitable concentration (20 mg/l)
   - Measure blank
   - Take spectra of all the standards and record the spectra characteristics (record maxima and minima wavelengths, maxima intensities)
   - Take a spectrum of an unknown sample

2. Spectral change of an acid-base indicator

   - Set the spectrum range to 210-700 nm
   - Prepare a solution of a selected indicator(s) – find a suitable concentration by step-wise dilution so that the absorbance does not exceed 2
   - Measure (adjust) pH of the solution and take the spectrum
   - Adjust pH (addition of a drop of a strong acid or a strong base) until a visual color change occurs and take the spectrum again

Evaluation of the data

- Comparing the spectrum of the unknown sample to spectra of standards confirm the unknown
- After exporting the data from your experiments (USB-flash), construct the first derivative spectrum and decide what an advantage the derivative spectrum brings
- Describe a color change of the indicator by shifts of absorption maximum. Draw the indicator formula in both the colored forms and note the indicator pH-range.