

UV-Vis spectrophotometry and determination of Ca²⁺

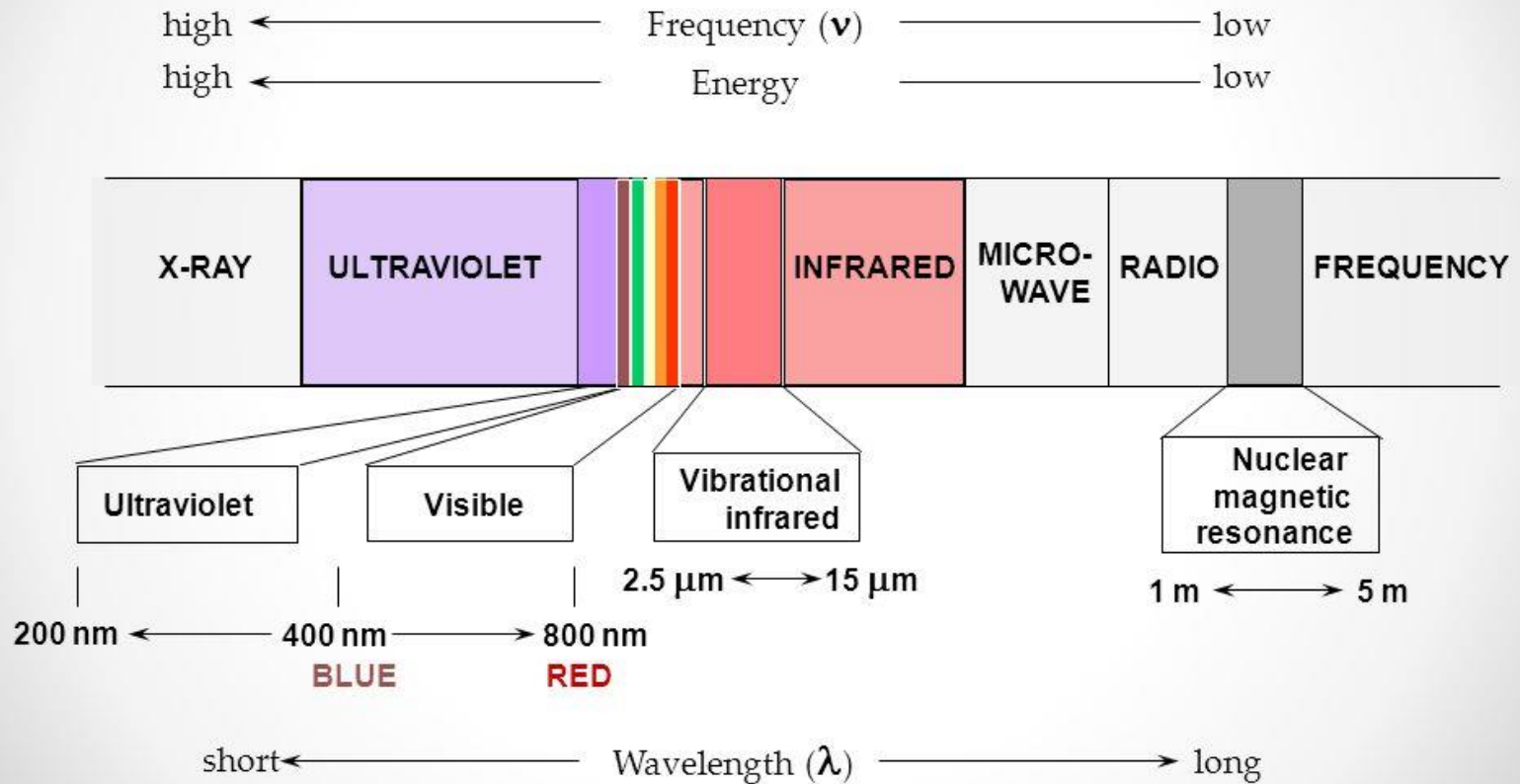
September 17, 2018

Reference literature

**As a reminder of absorption
UV-vis spectrophotometry...**

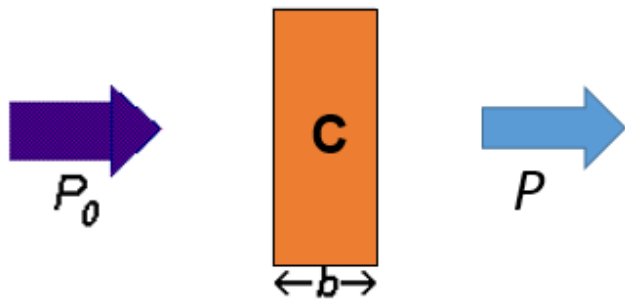
**(See: CHEM 2470 notes, Module 8
Textbook 6th ed., Chapters 13-14)**

The Electromagnetic Spectrum

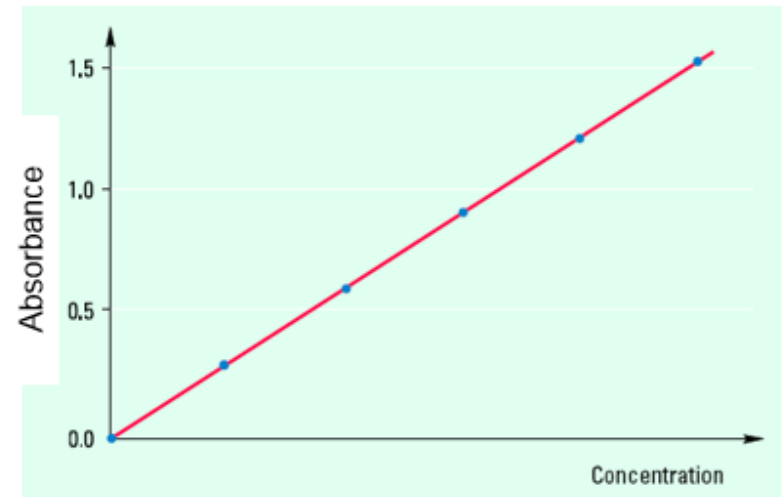


Spectroscopy	Usual Wavelength	Type of Quantum Transition
Gamma-ray emission	0.005 – 1.4 Å	Nuclear
X-ray absorption, emission, fluorescence, and diffraction	0.1 – 100 Å	Inner electron
Vacuum UV absorption	10-180 nm	Bonding electrons
UV-visible absorption, emission and fluorescence	180 – 780 nm	Bonding electrons and Electrons in “n” transfer bonds
IR absorption and Raman scattering	0.78 – 300 μm	Rotation/vibration of molecules
Microwave absorption	0.75 – 3.75 mm	Rotation of molecules
Electron spin resonance	3 cm	Spin of electrons in a magnetic field
Nuclear magnetic resonance	0.6 – 10 m	Spin of nuclei in a magnetic field

UV absorption: Beer's Law (quantitative)



$$A = \epsilon b C$$



where A = the *absorbance*

b = the light path of the sample (cm)

c = the concentration of the analyte (mol/L)

ϵ = the molar absorbivity (L/mol·cm).

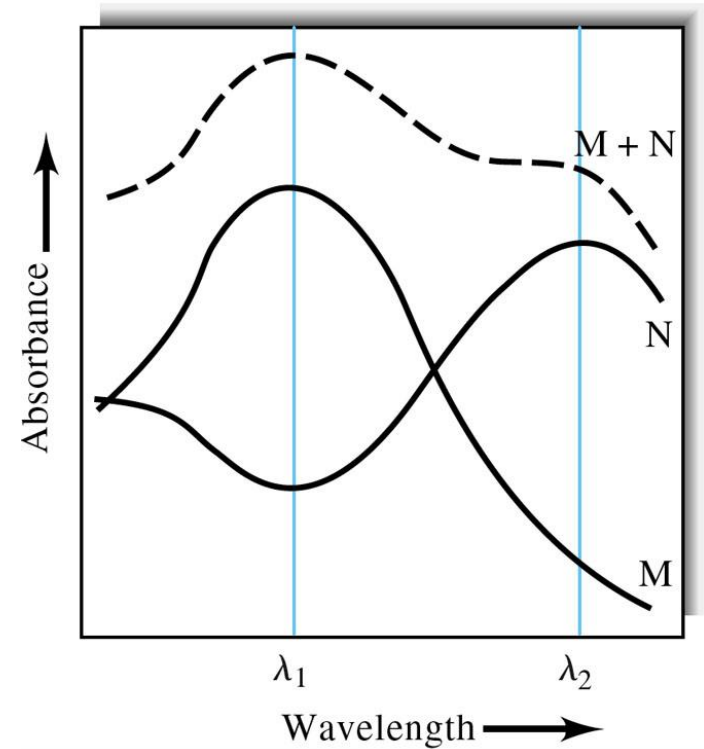
**Linearity does not always hold, particularly when C is too high
(A is preferred to be < 0.6)**

Absorbance is additive:

$$A = \epsilon bc$$

$$\begin{aligned} A^1_{M+N} &= A^1_M + A^1_N \\ &= \epsilon^1_M bc_M + \epsilon^1_N bc_N \end{aligned}$$

$$A^2_{M+N} = \epsilon^2_M bc_M + \epsilon^2_N bc_N$$

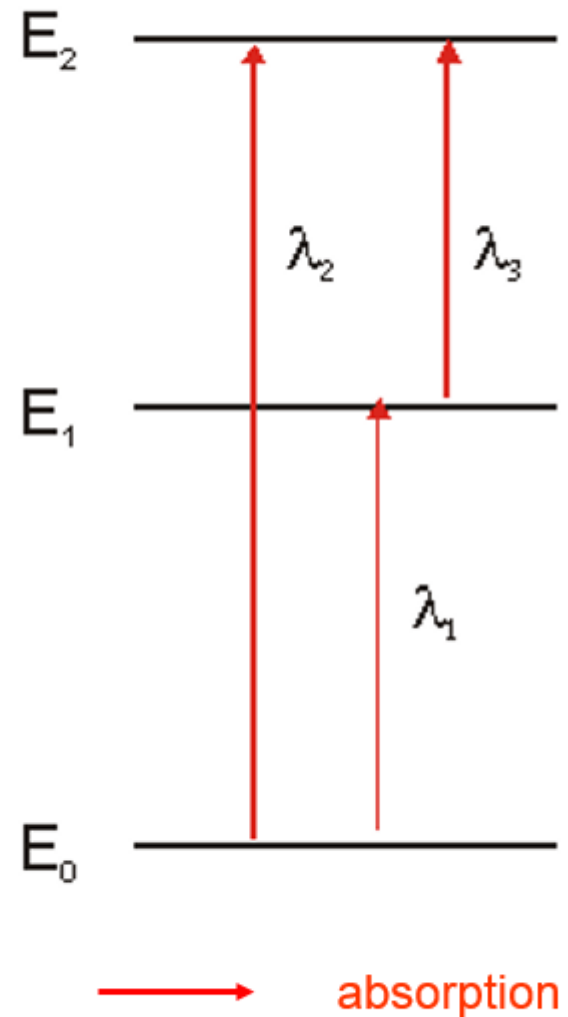


Absorption of Radiation: electronic transitions

$$\lambda_1 = \frac{hc}{E_1 - E_0}$$

$$\lambda_2 = \frac{hc}{E_2 - E_0}$$

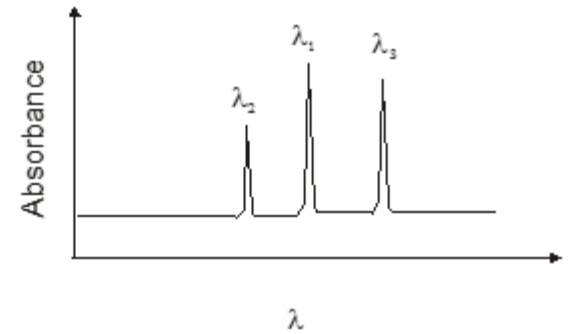
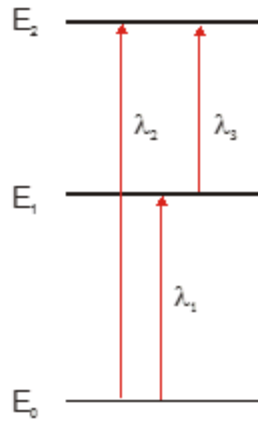
$$\lambda_3 = \frac{hc}{E_2 - E_1}$$



● Line Spectrum

Atoms

Electronic transition only

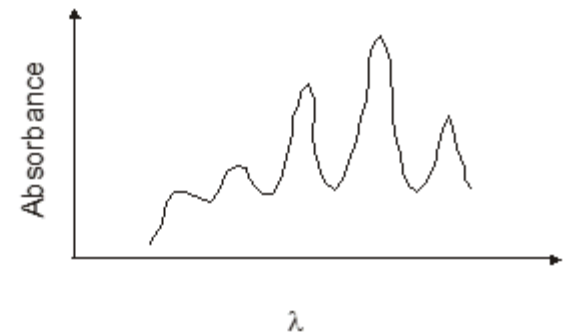
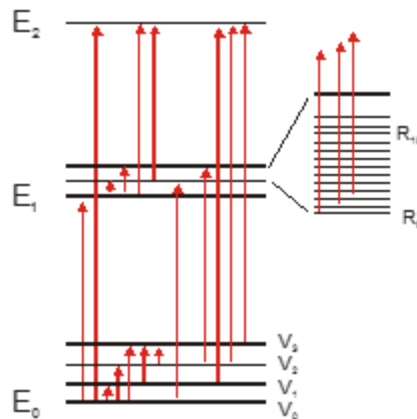


A. Atomic absorption spectrum

● Band Spectrum

Molecules

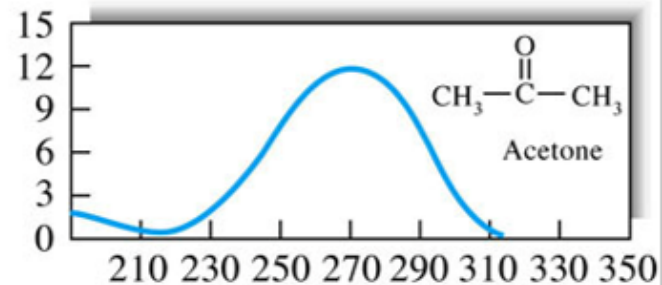
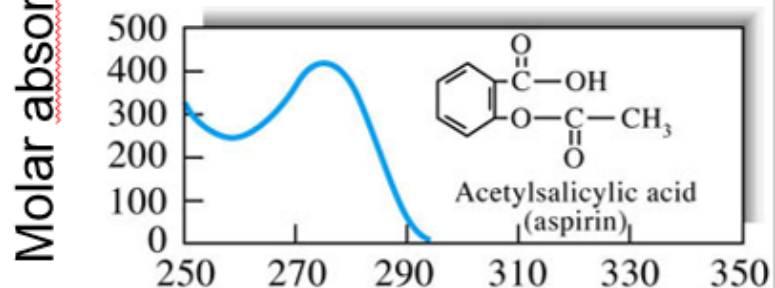
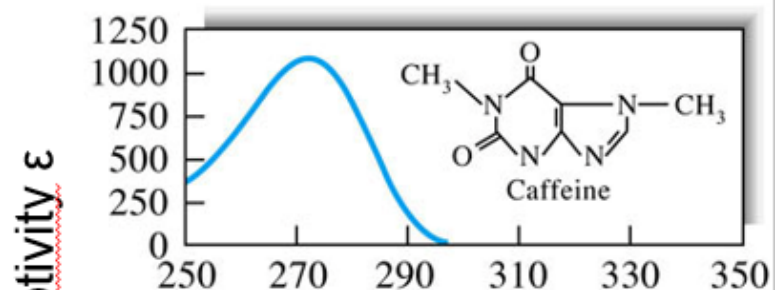
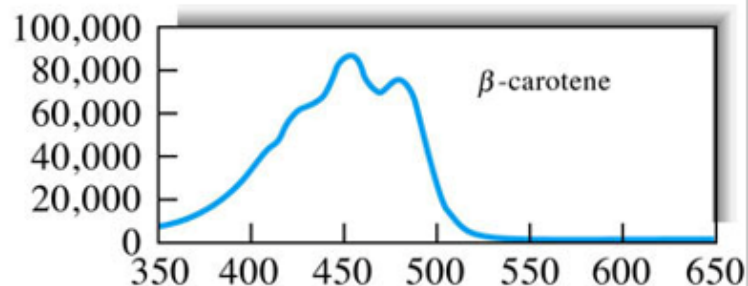
Electronic, rotational and vibrational transitions



B. Molecular absorption spectrum

Most UV-vis spectra of organic compounds are complex:

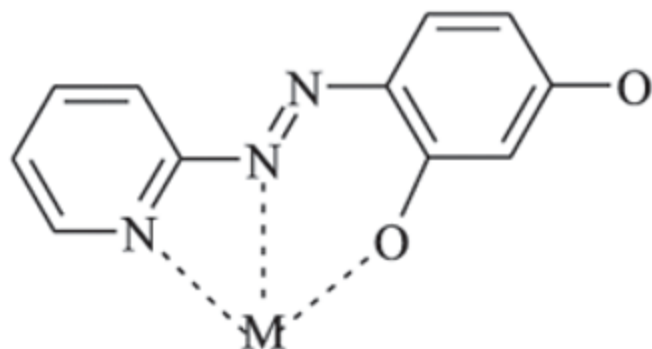
- Electronic and vibration transitions superimposed: absorption bands usually broad
- Detailed theoretical analysis not possible, but semi-quantitative or qualitative analysis of types of bonds is possible.
- Solvent effects



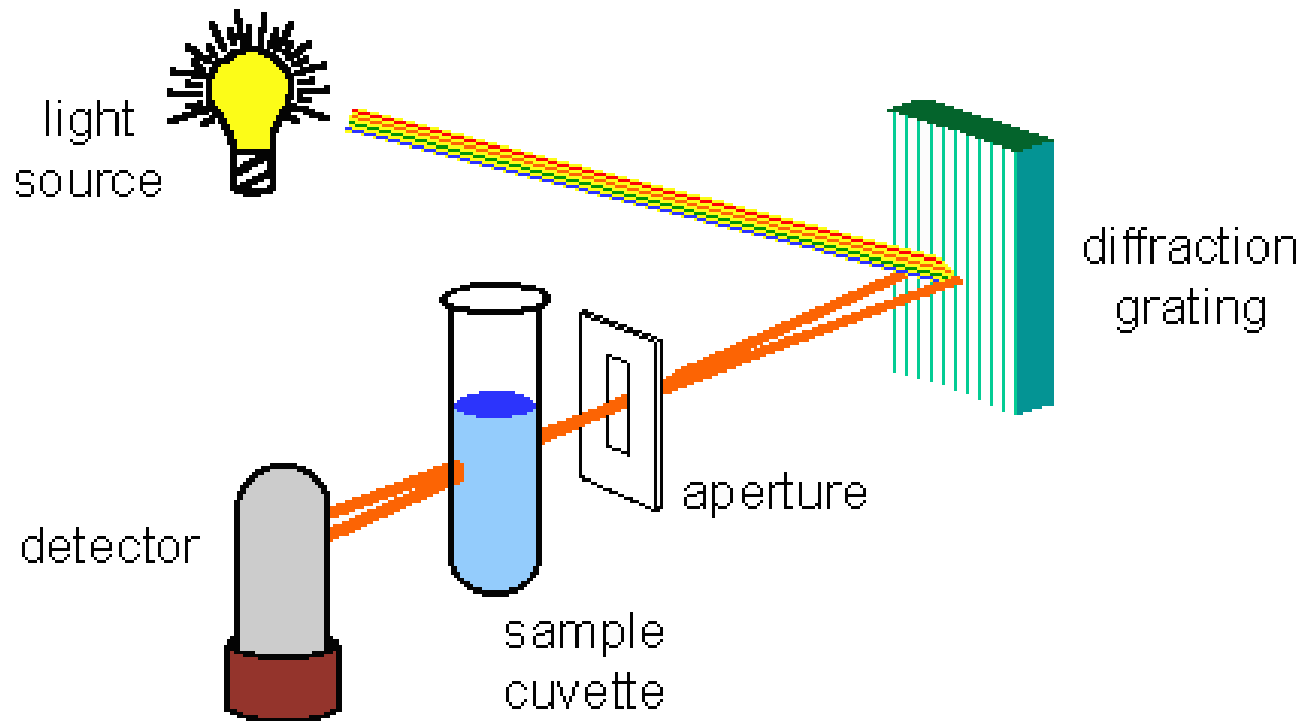
➤ UV-Vis Absorption Involving Charge-Transfer Electrons

Absorption of UV-Vis involves the transfer of an electron from the electron-donor to an orbital associated with the electron-acceptor (usually, but not always, a metal ion).

Important analytically because of large ϵ ($> 10,000$)



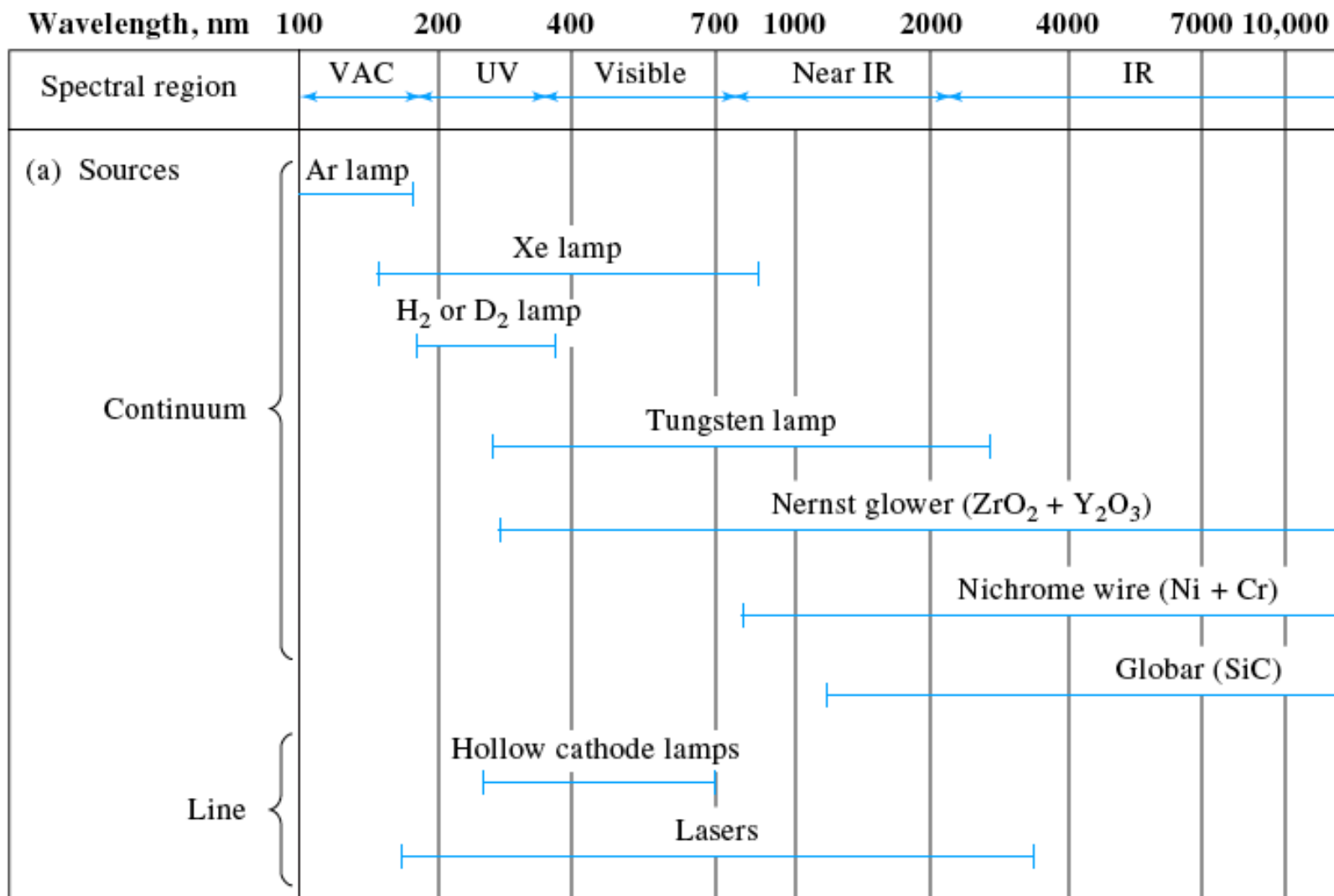
Instrumentation for spectroscopy



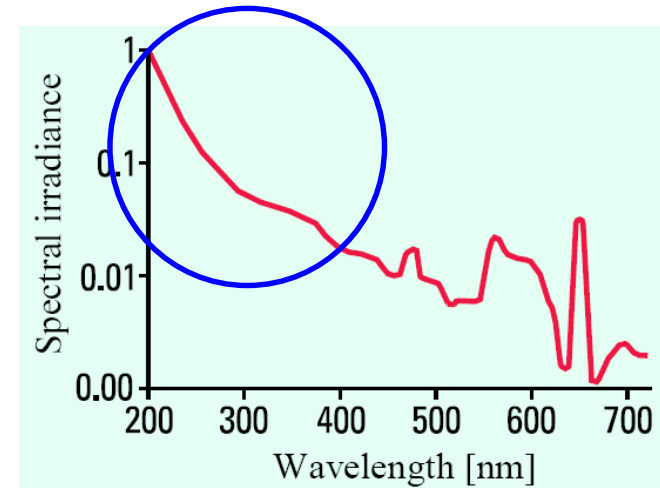
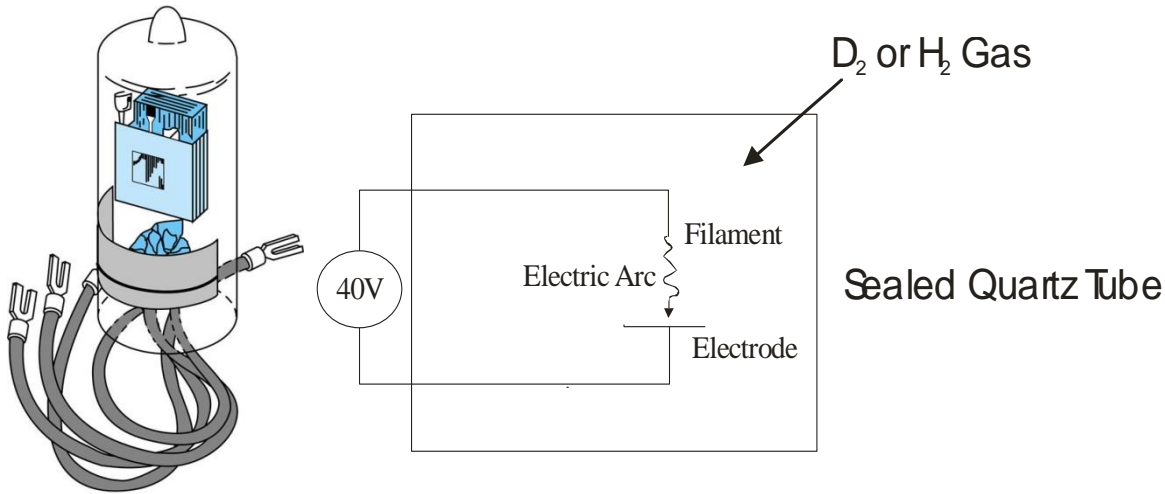
Instrumentation for Spectroscopy

Most spectroscopic instruments are made up of five key components:

- **Radiation source**
- **Wavelength selector**
to isolate a restricted wavelength region
- **Sample container(s)**
- **Radiation detector or transducer**
to converts radiant energy to a measurable signal (usually electrical)
- **Signal processor and readout**



Examples of Continuum Radiation Sources: Deuterium Lamp

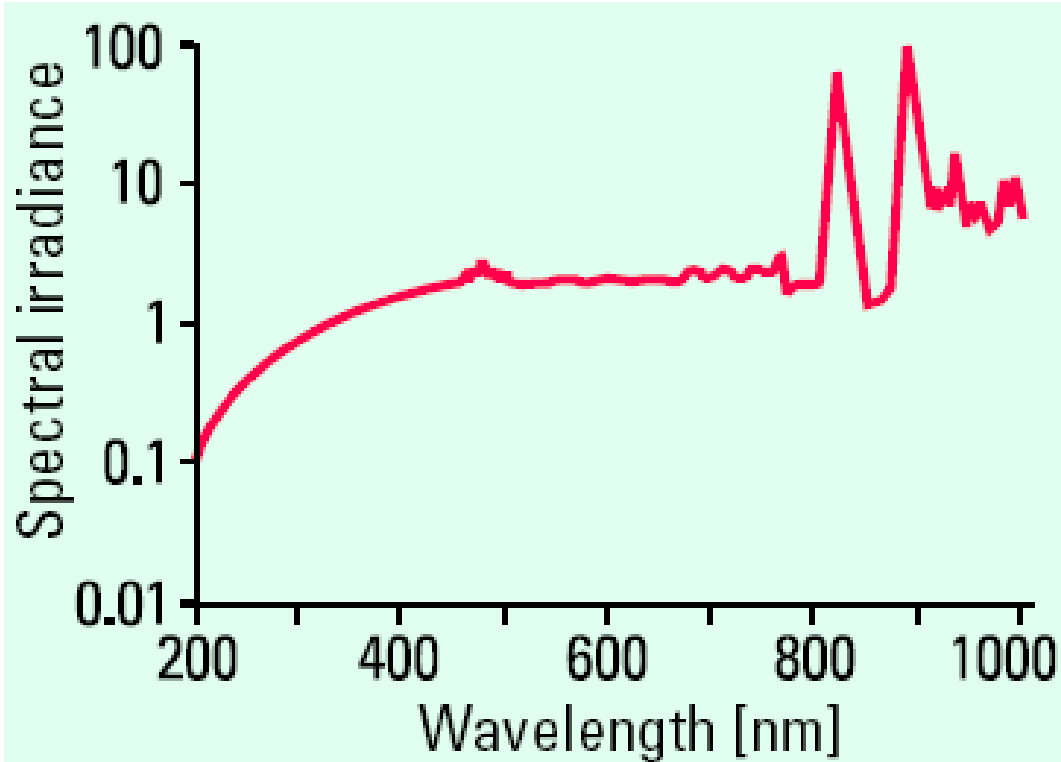


Deuterium Lamp



Based on electric excitation of D₂ at low pressure
Good for ~200-500 nm; short intensity half-life (~1000 hr)

Examples of Continuum Radiation Sources: Xenon Lamp



Good for the 250 nm - visible regions
higher noise

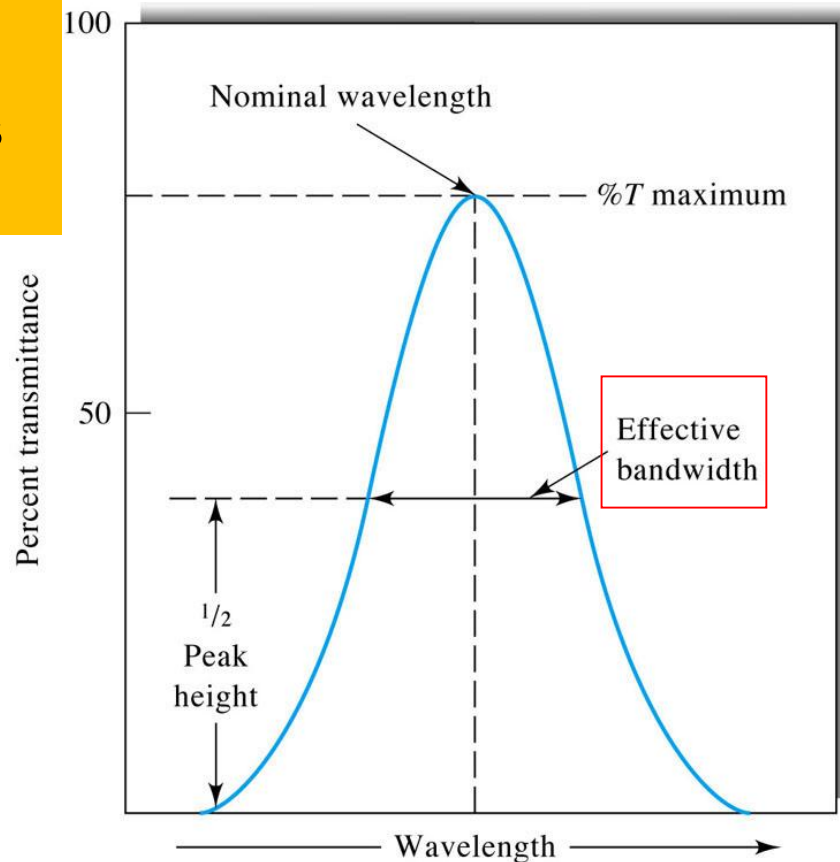
Wavelength Selectors

To restrict the wavelength to a narrow band that is absorbed or emitted by the analyte, in order to enhance the selectivity and sensitivity of the instrument.

Ideally a wavelength selector should produce a single wavelength; in reality, it always produce a narrow band.

➤ **Filters**

➤ **Monochromators**

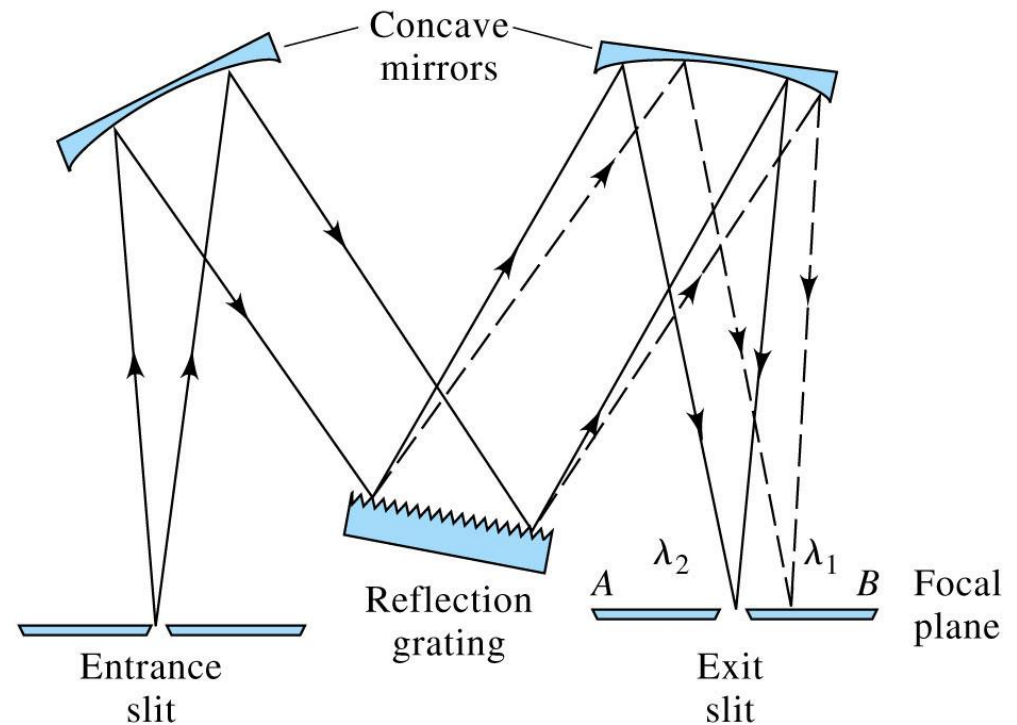


Wavelength selection: Monochromators

Separating wavelengths *continuously (scanning)* from polychromatic light in time or space to allow only certain λ 's to be selected and used.

A monochromator typically consists of:

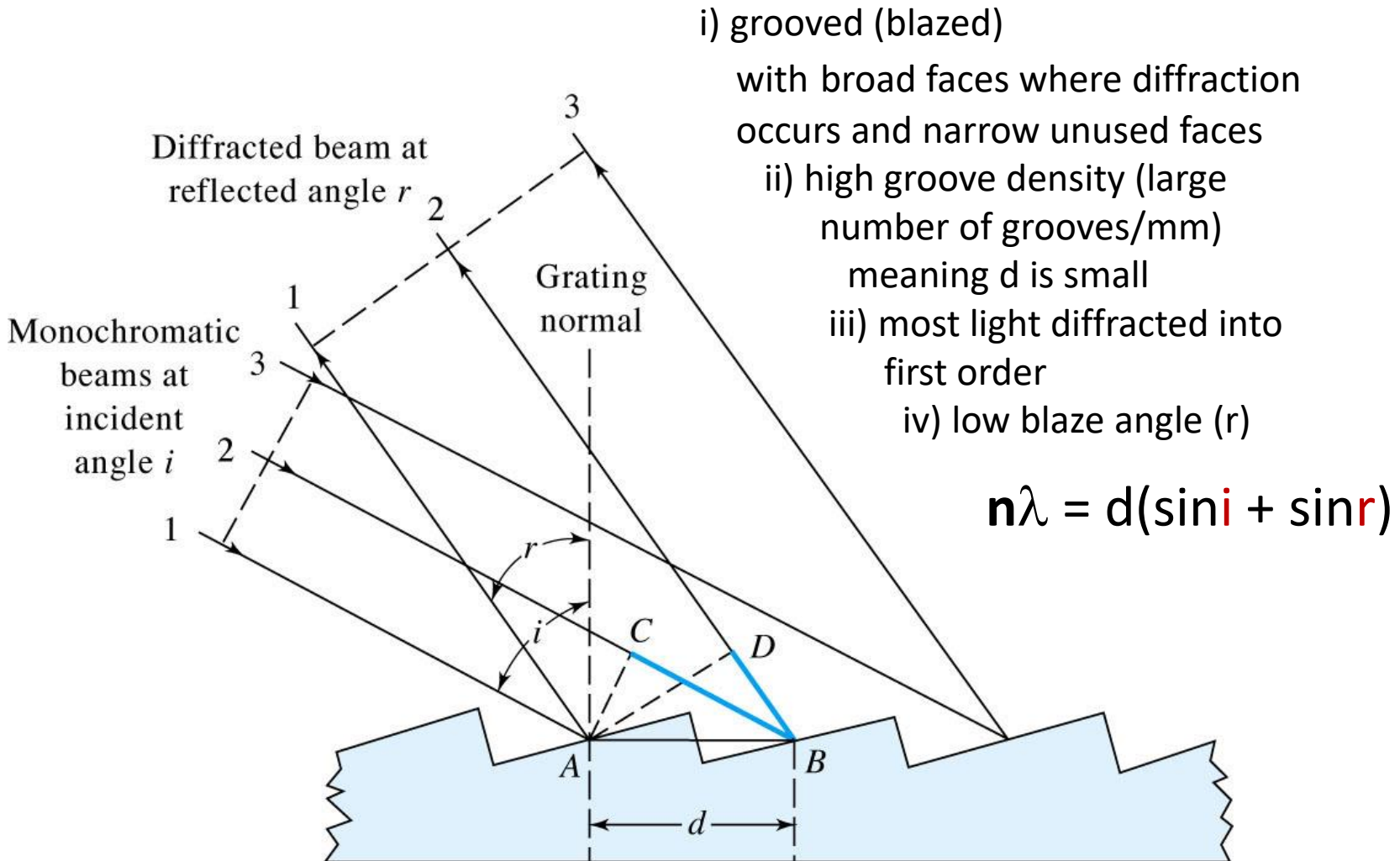
- Prisms or gratings
- Slits
- Lenses
- Mirrors
- Windows



(a)

Echellette-type grating (conventional)

Light is reflected off the **longer side of the blazes** in the grating.



Sample Holders

The sample holders (known as cells or cuvettes) must:

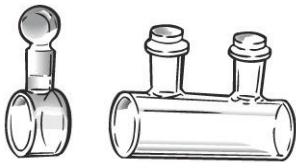
- Not be reactive with the sample
- Be transparent to the wavelengths of in use
- Be readily cleaned for reuse
- Fit the specific instrument of interest

Wavelength, nm	100	200	400	700	1000	2000	4000	7000	10,000	
Spectral region	← VAC →		← UV →		← Visible →		← Near IR →		← IR →	
(a) Materials for cells, windows, lenses, and prisms			Lithium fluoride							
			Fused silica or quartz							
			Corex glass							
			Silicate glass							
					NaCl					
					KBr					
					TlBr or TlI					
							ZnSe			

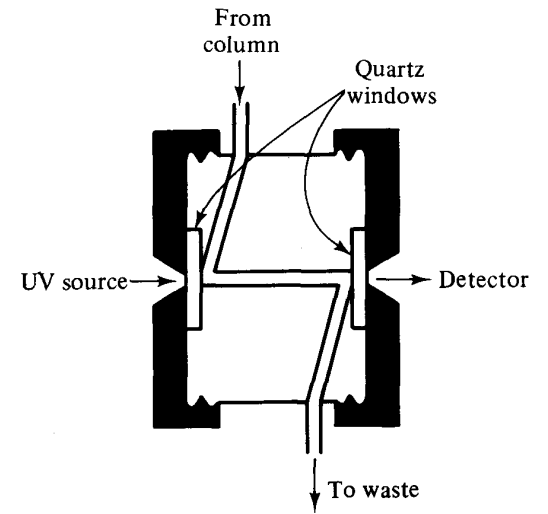
Standard
1-cm path



Cylindrical



Micro cells



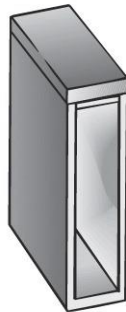
5-mm
path



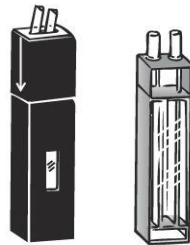
1-mm
path



20-mm path



Flow



Thermal



Detectors:

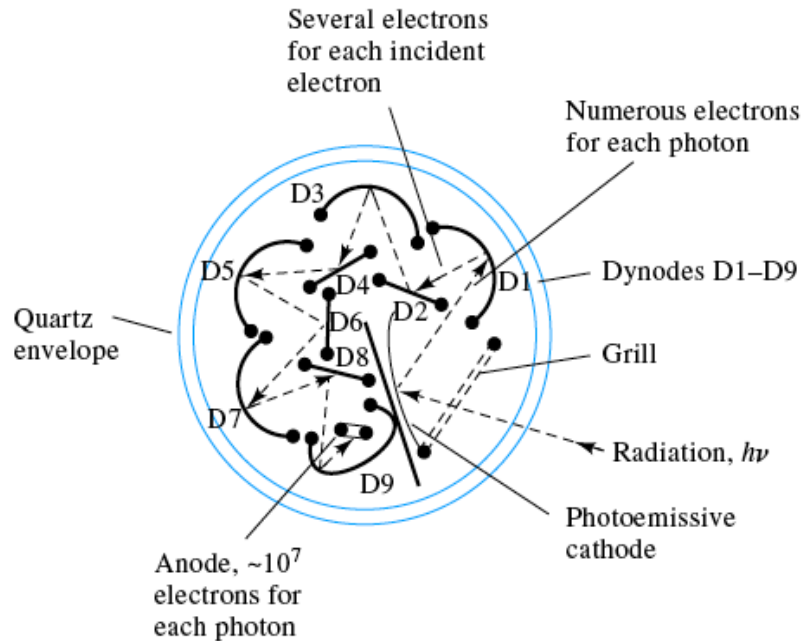
Devices that convert light to electrical signal. Ideally, they should have:

- High and fast conversion yield (sensitivity)
- Good S/N
- Constant response over λ range of interest
- Signal **proportional to** light intensity
- Little or no signal in absence of light (**dark current**)

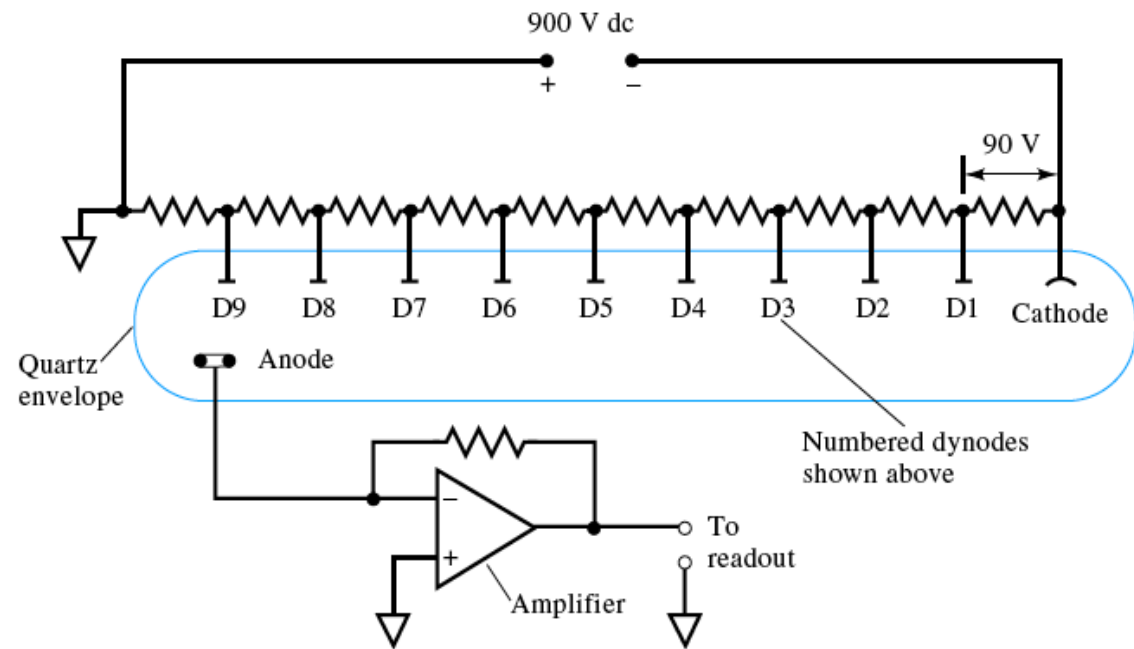
Photomultiplier tubes



(a)



(b)



(c)

Process:

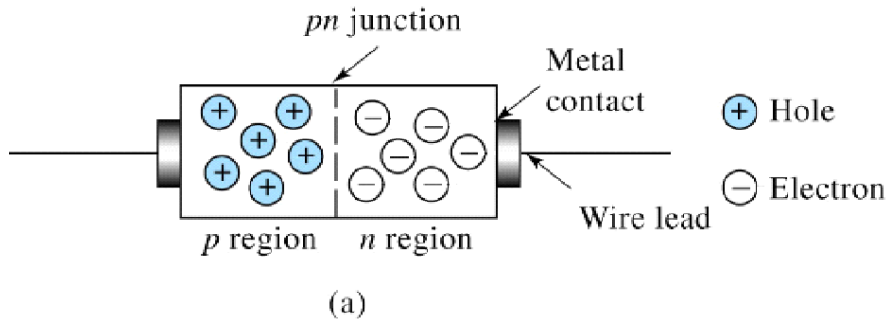
- a) light hits cathode and e^- emitted.
- b) an emitted e^- is attracted to electrode #1 (dynode 1), which is 90V more positive. Causes several more e^- to be emitted.
- c) these e^- are attracted to dynode 2, which is 90V more positive than dynode 1, emitting more e^- .
- d) process continues until e^- are collected at anode after amplification at 9 dynodes.
- e) overall voltage between anode and cathode is 900V.
- f) One photon produces $10^6 - 10^7$ electrons, and as such current is amplified and measured.

Advantages: very sensitive to low intensity, very fast response.

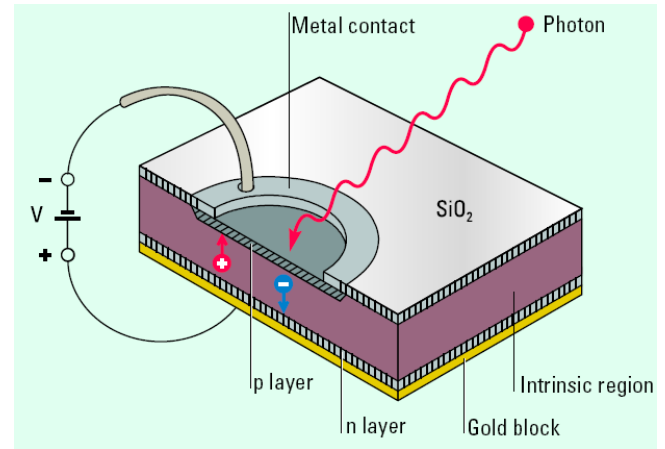
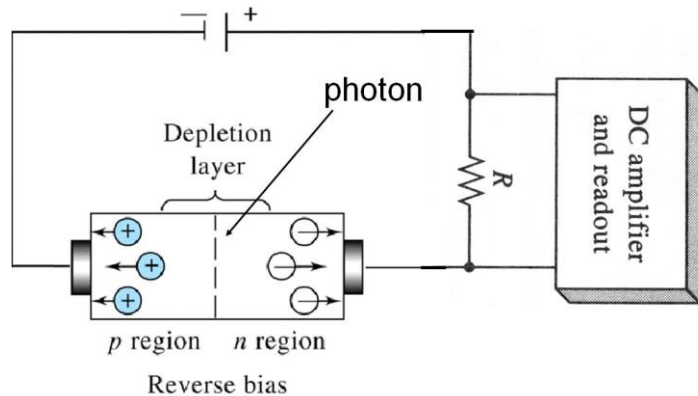
Disadvantages: need high voltage power supply; intense light damages at high power radiation

Silicon Photodiode Detectors

Semiconductor material – conducts current only under certain conditions



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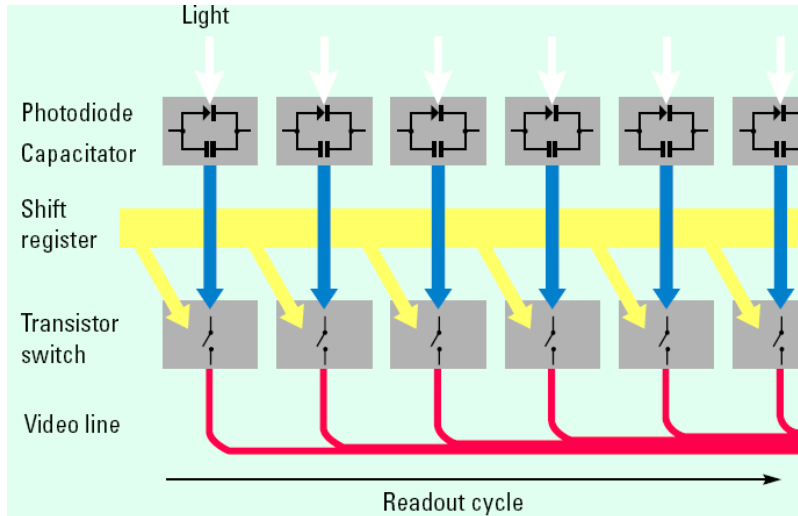
Process:

- A reverse-biased pn junction silicon (190-1100 nm) or germanium (800-1700 nm) diode.
- No current when there is no light due to the non-conducting depletion layer.
- When light strikes, it produces electrons and holes in the depletion layer and thus produces a current that is proportional to radiant power.

Solid-state detector; more reliable and robust.
Sensitivity a little lower than that of PMTs

Photodiode Arrays (PDAs)

Consisting of a series (200-1000) of photodiodes each of which consists of a reverse-biased pn junction, positioned side by side on a single silicon crystal.



Solid-state detector;
Measuring the entire
wavelength range
essentially
simultaneously.

Process:

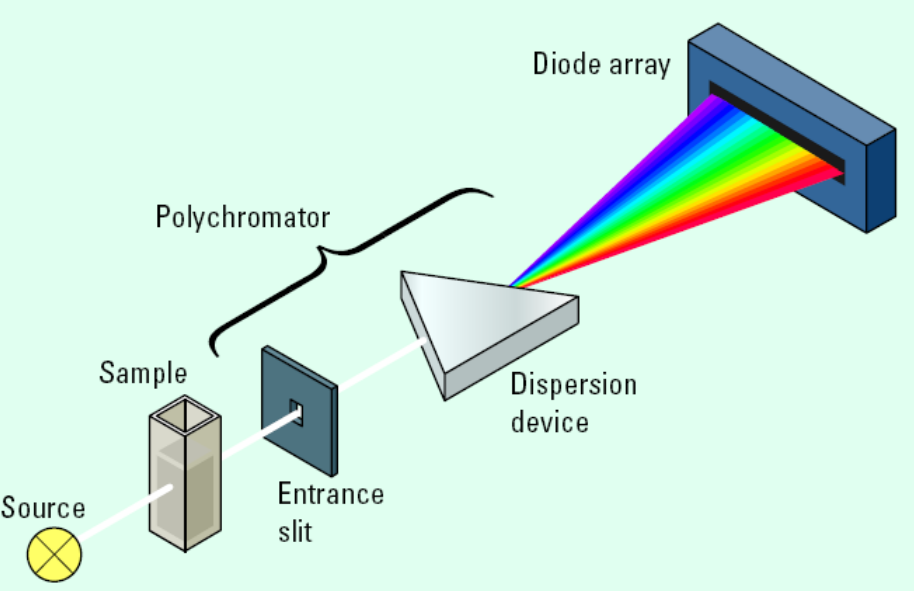
- Each diode has a dedicated capacitor and is connected by a solid-state switch to a common output line. The switches are controlled by a shift register.

- Initially, the capacitors are charged to a specific level.

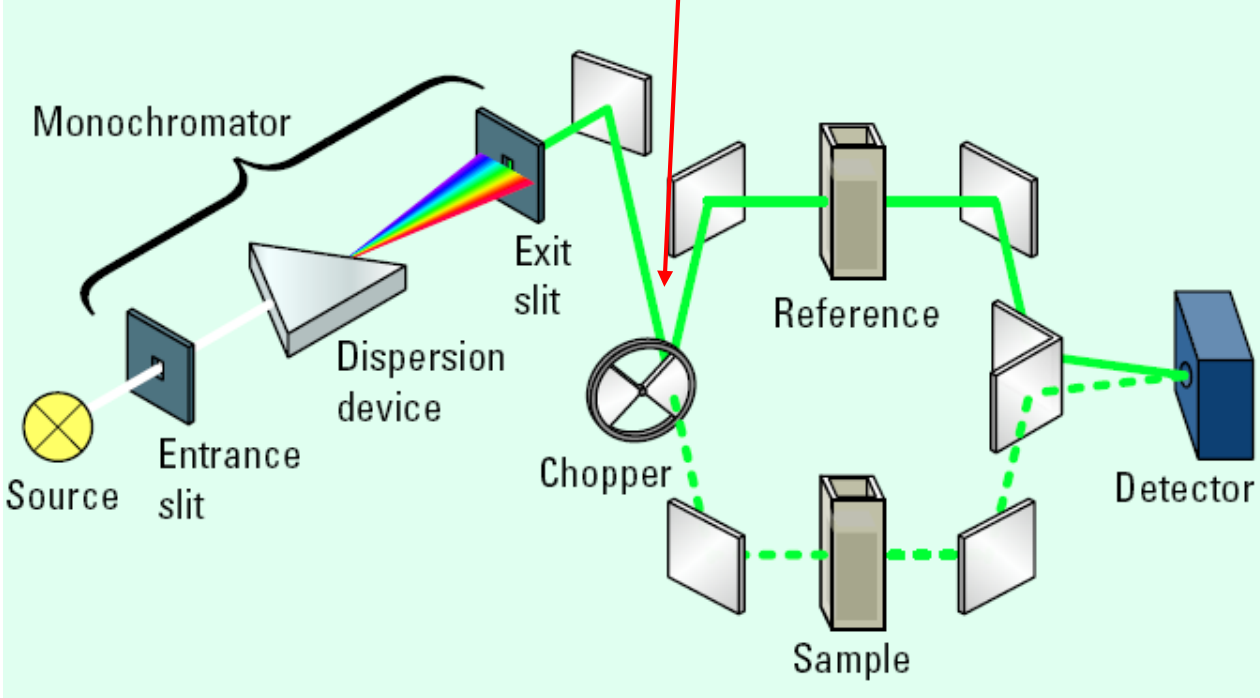
- When photons penetrate the silicon, free electrical charge carriers are generated that discharge the capacitors. The capacitors are recharged at regular intervals that represent the measurement period for each scanning cycle.

- The amount of charge needed to recharge the capacitors is proportional to the number of photons detected by each diode, which in turn is proportional to the light intensity.

Single vs. double beam UV-Vis absorption spectrophotometers



uses a beam chopper to separate the reference beam from the sample beam.



Limitations to Beer's Law

- **Chemical Deviations:**

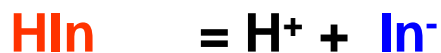
- (1) Real deviations due to high concentrations**

When c is too high (e.g., $> 0.01\text{ M}$), the distance between analyte molecules decreases to the extent that they change each other's charge distribution, modifying radiation is absorbed.

(2) Apparent deviations due to chemical reactions

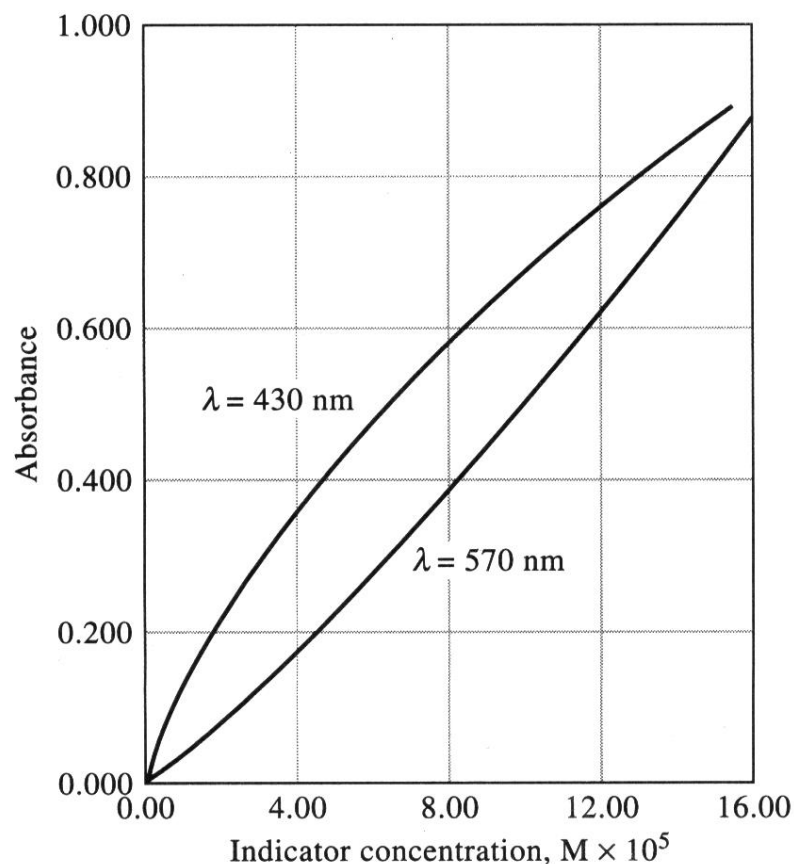
If the analyte associates, dissociates, or reacts with the solvent or other components in the solution, deviations from Beer's Law will occur due to the formation of molecules with different ϵ .

Example:



ϵ_{430}	6.30×10^2	7.12×10^3
ϵ_{570}	2.06×10^4	9.61×10^2

“Apparent” deviation since can be accounted for by chemical equilibrium



Simultaneous spectrophotometric determination of calcium and magnesium in water

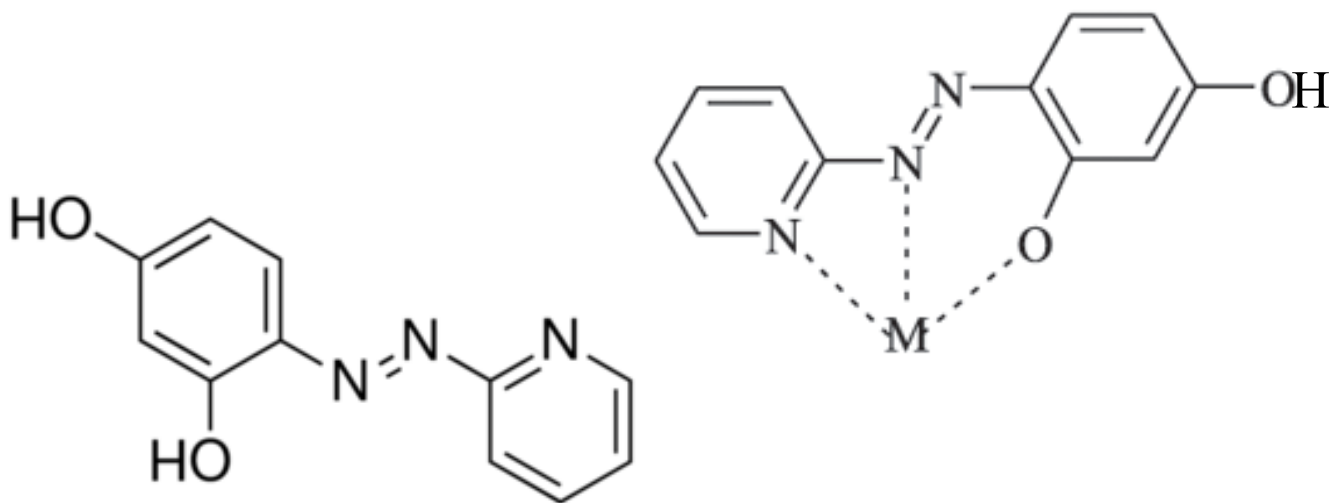
E. Gómez, J.M. Estela and V. Cerdà *

Department of Chemistry, Faculty of Sciences, University of the Balearic Islands, 07071 Palma de Mallorca (Spain)

- Method based on the formation of complexes with 4-(2-pyridylazo)resorcinol.
- The absorbance band yielded by the mixture is resolved by applying multilinear regression to the corrected, standardized spectrum of each metal ion as standard.
- Provides a linear determination range of 0.10-4.0 µg/mL for Ca and 0.15-2.5 µg/mL for Mg and can be applied to the analysis of various types of water.
- Based on the use of a diode array detector.



ϵ of complexes formed between metal ions and 4-(2-pyridylazo)resorcinol (PAR) are on the order of $10^4 \text{ Lmol}^{-1}\text{cm}^{-1}$



Method:

1) For background subtraction:

Measure 3 spectra of PAR at 6×10^{-4} M, average and use as PAR spectrum to subtract from other spectra. Range: 470-650 nm.

2) Calibration curves:

0.10-4.0 pg/mL for Ca and 0.15-2.5 pg/mL for Mg, with 6×10^{-4} M PAR.

Spectra were recorded and subtracted with 1) and calibration curves were obtained at each λ (J).

3) Unknowns:

Spectra were recorded and subtracted for PAR with 1). Under the assumption that Beer's law is obeyed and additive, a matrix corresponding to N absorbing components at J wavelengths was created.

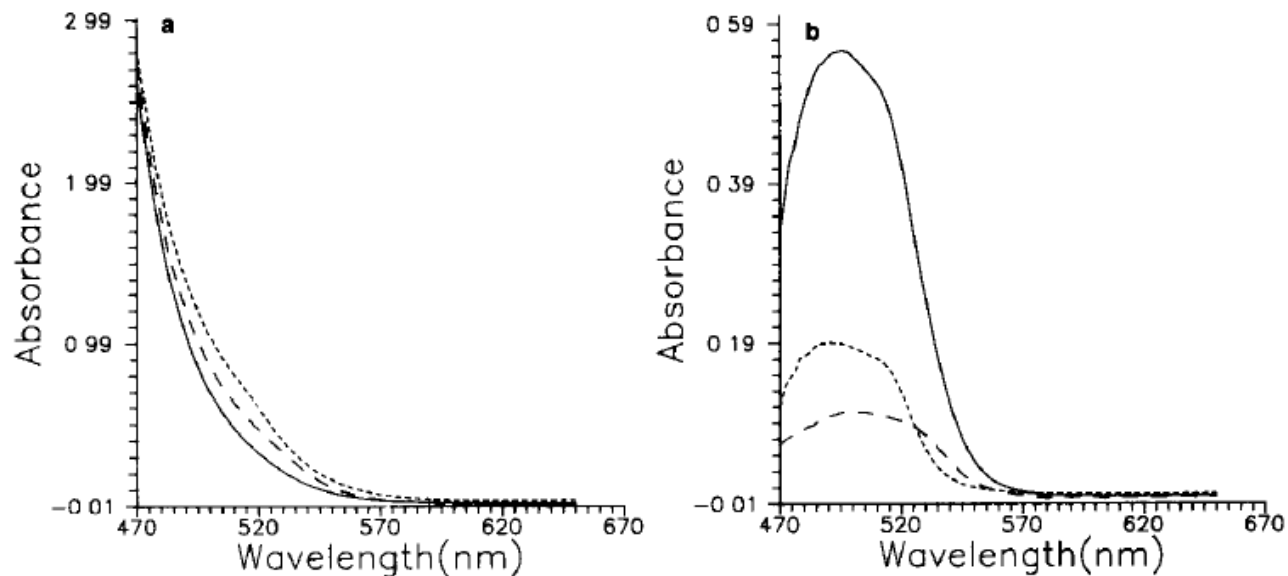


Fig. 1 Absorbance bands yielded by (a) the PAR complexes formed from 6×10^{-4} M PAR and $1 \mu\text{g ml}^{-1}$ Ca(II) and Mg(II); (b) Ca(II) and Mg(II) standards and a mixture of Ca and Mg containing $2 \mu\text{l ml}^{-1}$ of each. (a) ———, PAR; - - -, PAR-Ca(II), - - - - -, PAR-Mg(II). (b) ———, Ca-Mg, - - -, Ca(II) standard; - - - - -, Mg(II) standard.

Maxima: 490 nm (Mg, $i = 1$) and 502 nm (Ca, $i = 2$).

Wavelength range for multi-regression: 470-650 nm ($j = 490, 491, 492 \dots 502$).

TABLE 1

Calibration graphs obtained by the proposed method

Element	No. of points	Intercept ($a \pm 95\%$ confidence limit)	Slope ($b \pm 95\%$ confidence limit)	r^a	$s_{y/x}^a$
Ca	6	0.0223 ± 0.0086	0.0927 ± 0.0038	0.9996	0.0043
Mg	6	0.0036 ± 0.0122	0.1868 ± 0.0080	0.9996	0.0057

r = Correlation coefficient; $s_{y/x}$ = standard deviation of the fitting

$$A_j = \sum_{i=1}^N \epsilon_{ij} b c_i + k_0$$

A_j : absorbance of the mixture at wavelength j

ϵ_{ij} : molar absorptivity of species i at wavelength j

c_i : concentration of species i

The ϵ_{ij} values were obtained from the standard spectra of the i components

k_0 : experimental error term (residual)

The matrix was solved by using a multilinear regression treatment based on determining the concentrations of the *i* components of the unknown sample with a minimum experimental error by matrix calculus.

TABLE 4
Results and 95% confidence limits obtained in the simultaneous determination of Ca and Mg in real water samples

Sample	Proposed method ($\mu\text{g ml}^{-1}$)			AAS reference method ($\mu\text{g ml}^{-1}$)	
	Dilution factor	Ca	Mg	Ca	Mg
Mineral waters:					
Zone 1 (Gerona)	1 : 10	26.8 \pm 0.4	4.4 \pm 0.2	23.1	4.6
Zone 2 (Mallorca)	1 : 50	100 \pm 2	26 \pm 1	88	31
Well waters (Mallorca):					
Zone 1	1 : 25	45 \pm 1	27.2 \pm 0.4	55	25.7
Zone 2	1 : 50	110 \pm 1	22.8 \pm 0.9	104	26.7
Tap waters (Palma de Mallorca):					
Zone 1	1 : 50	112 \pm 2	21.3 \pm 0.9	92	19.4
Zone 2	1 : 50	122 \pm 2	21.0 \pm 0.7	110	23.0
Zone 3	1 : 50	138 \pm 2	23 \pm 1	135	22
Zone 4	1 : 100	250 \pm 4	120 \pm 2	250	110
Sea waters (Mallorca):					
Zone 1 (Palma Bay)	1 : 500	450 \pm 22	1450 \pm 20	440	1400
Zone 2 (Alcudia Bay)	1 : 1000	374 \pm 30	1470 \pm 27	400	1410

Conclusions on UV-vis section

- Relatively inexpensive
- Covers a broad range of analytes (chemical modification may be necessary)
- Not as sensitive as fluorescence (by 100-1000 x)
- Often used in preliminary analysis of samples

Questions

1. The absorbance of a solution is 0.37. If the transmittance value of the blank sample (T_0) is 97%, what is the transmittance T of the solution?

2. In analytical chemistry, a spectrophotometer is mainly used :
 - a) To measure the concentration of a mixture of unknown compounds
 - b) To measure the concentration of a known light absorbing compound.
 - c) To identify compounds by their retention time.
 - d) To determine the extinction coefficients of unknown compounds.
 - e) To study the relationships between absorption bands and structure.