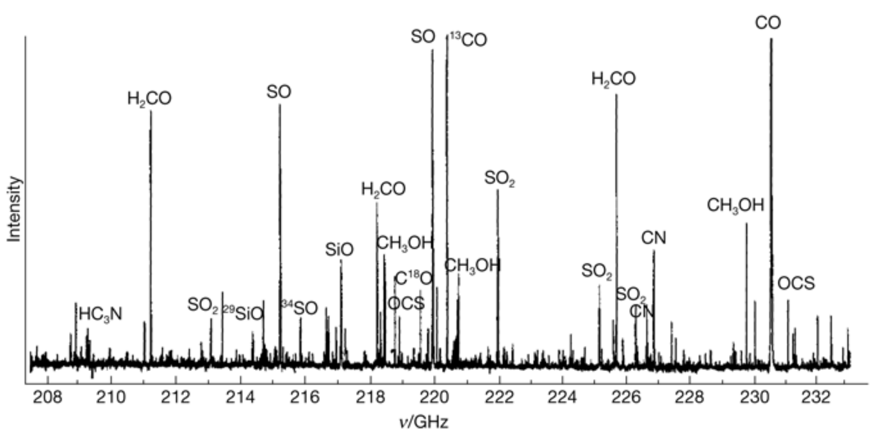
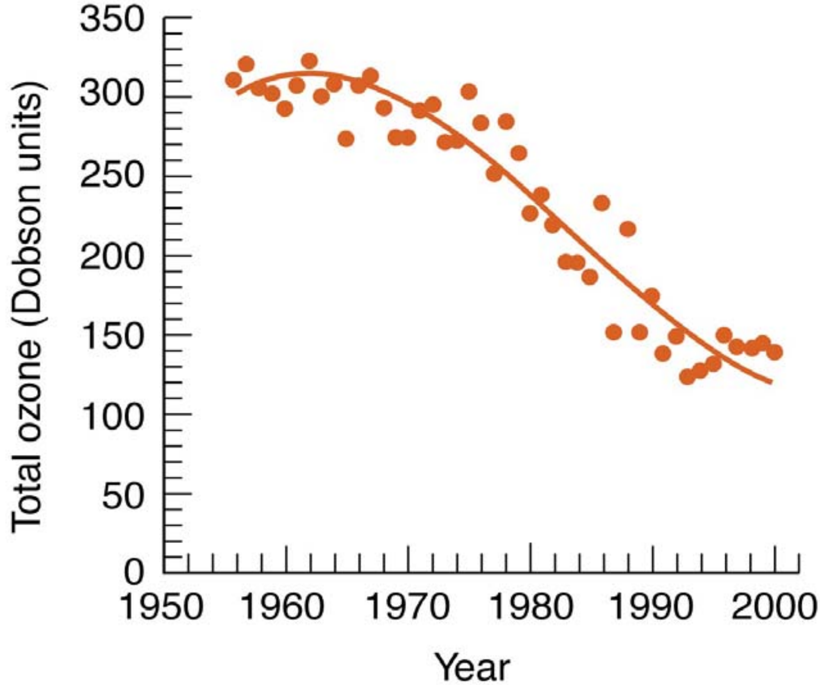
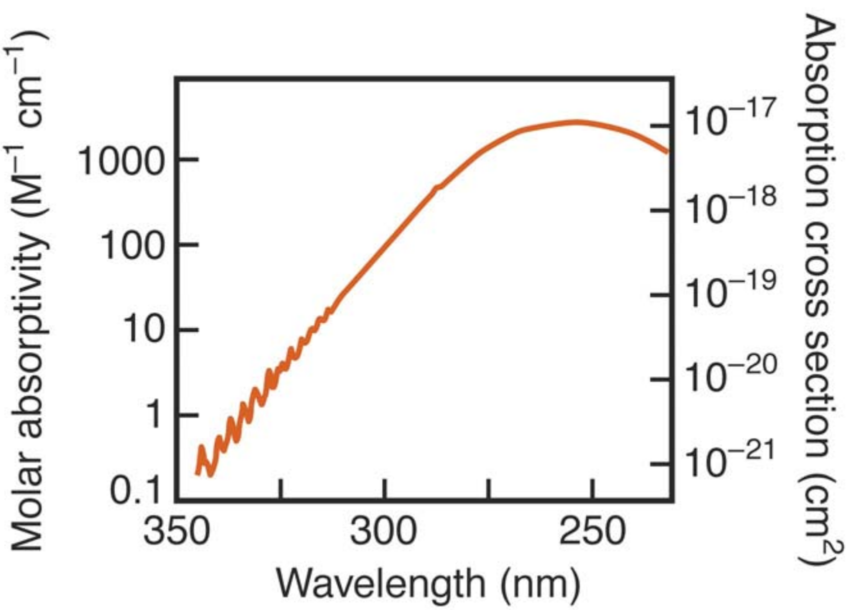
Optické metody

k stanovení analytu se využívá interakce elektromagnetického záření se zkoumanou látkou (vzorkem).

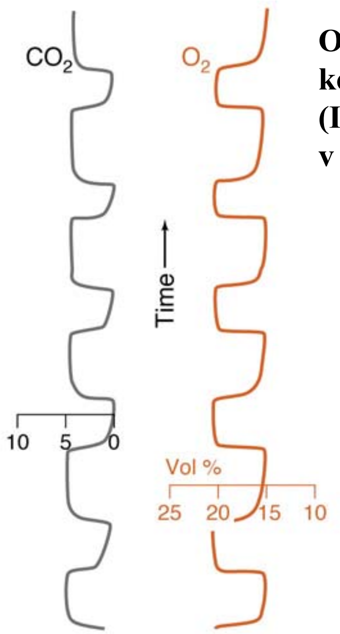


Rotační spektrum mlhoviny v Orionu



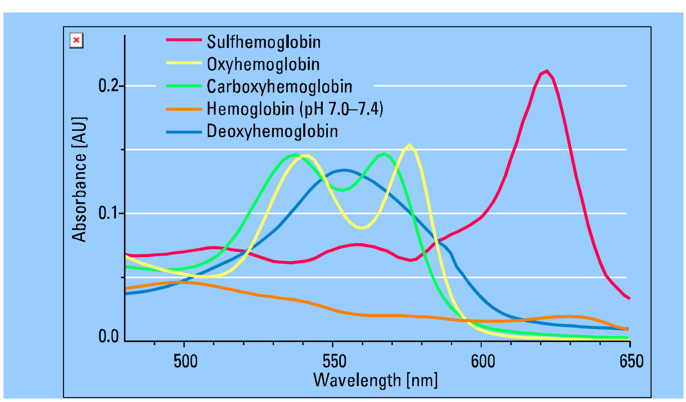


Molekulové absorpční spektrum ozonu

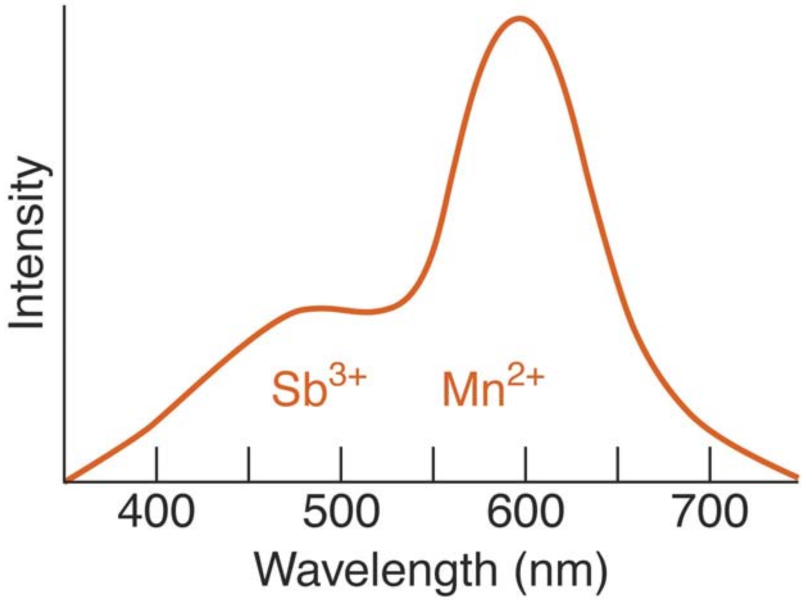


On-line kontinuální měření koncentrace oxidu uhličitého (IR, 2300 cm^{-1}) a kyslíku (UV, 147 nm) v dechu

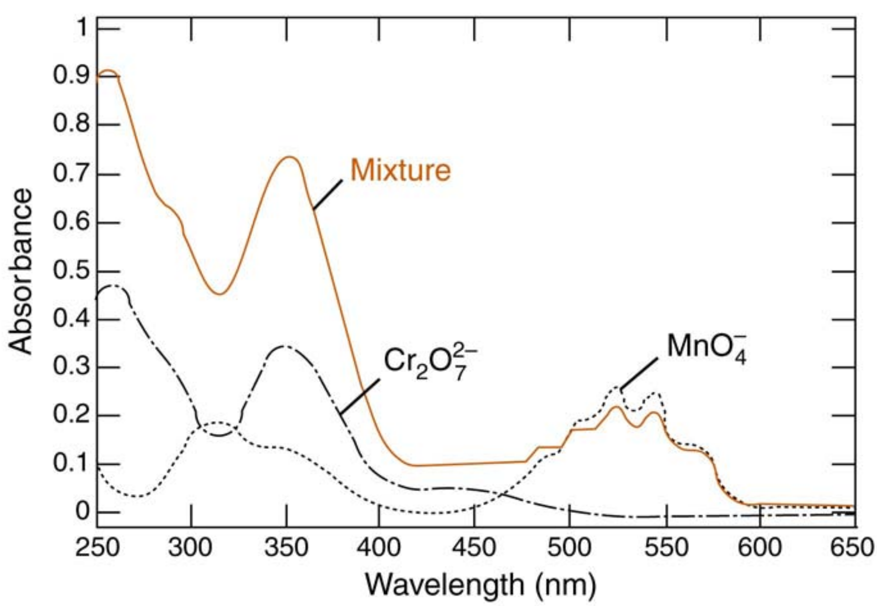
Absorption Spectra of Hemoglobin Derivatives



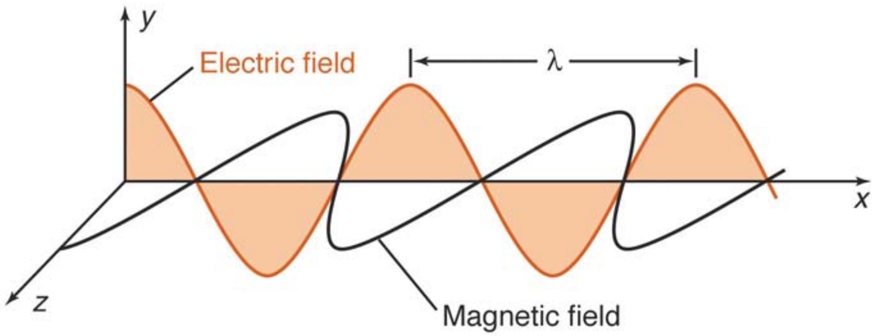
Vícesložková luminiscenční analýza

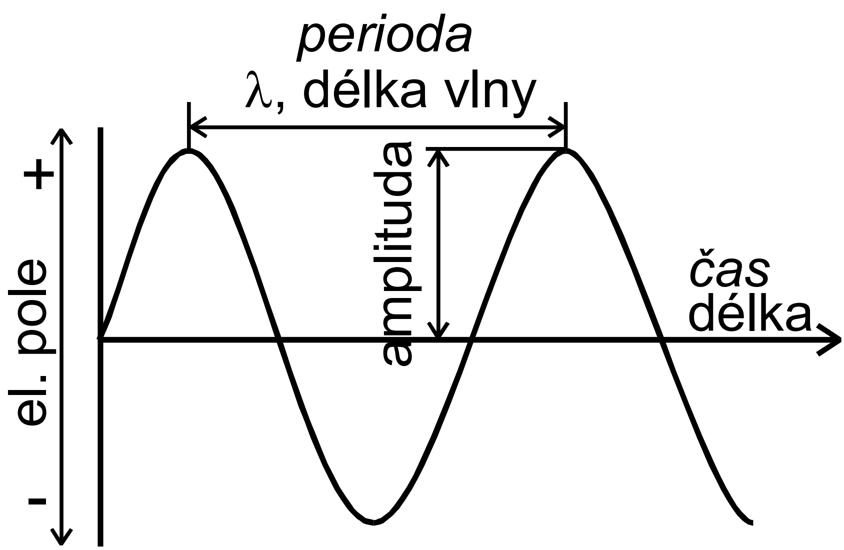


Vícesložková analýza



Polarizované elmg záření





Duální charakter elmg záření

Parametry charakterizující vlnové vlastnosti:

λ – vlnová délka, [μm, nm]

υ – rychlost šíření v určitém prostředí [m/s]

(rychlost ve vakuu **c** = 2,9979.108 m/s)

ν – frekvence, [Hz] $\nu = \frac{\upsilon}{\lambda}$

vztah mezi základními parametry:

$\tilde{\nu} = \frac{1}{\lambda}$ – vlnočet [cm^{-1}]

Parametry charakterizující korpuskulární vlastnosti:

$E = h \cdot \nu$ – energie [J]

(*h* = Planckova konstanta, 6,6.10-34 J.s)

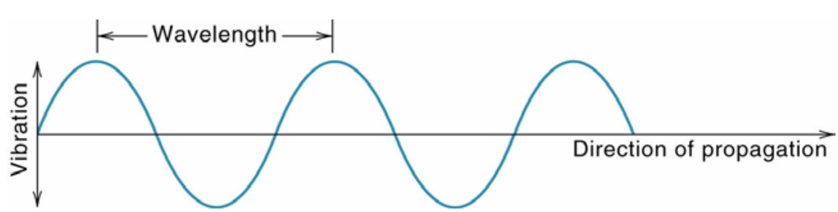
The distance of one cycle is the wavelength (λ).

The frequency (ν) is the number of cycles passing a fixed point per unit time.

$\lambda = c/\nu$ **(c = velocity of light, 3 x 10^{10} cm s^{-1}).**

The shorter the wavelength, the higher the energy: $E = h\nu = hc/\lambda$

This is why UV radiation from the sun burns you.



We see only a very small portion of the electromagnetic spectrum .

In spectrochemical methods, we measure the absorption of UV to far IR radiation.

UV = 200-380 nm, VIS = 280-780 nm, IR = 0.78 μm-300 μm

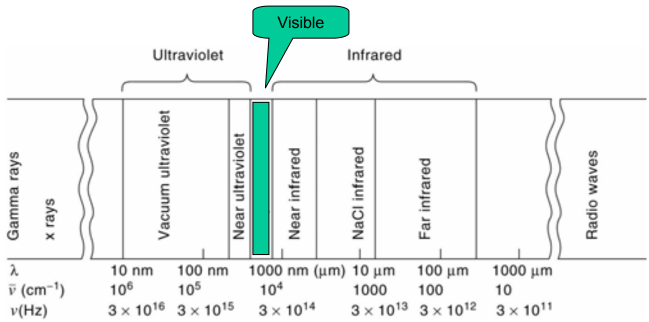
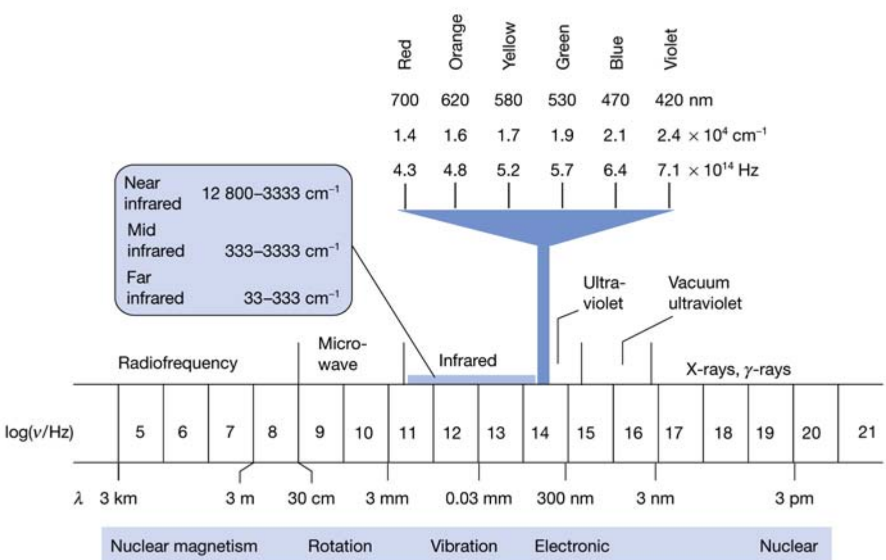
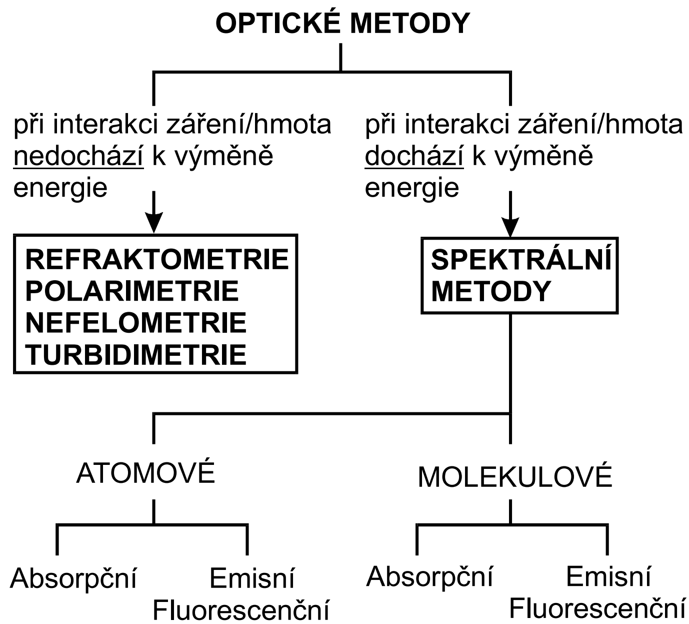


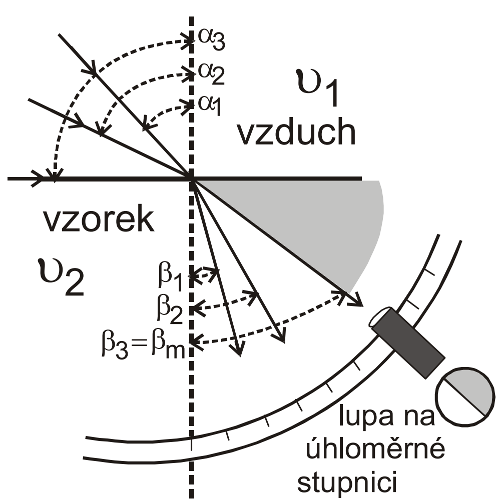
Fig. 16.2. Electromagnetic spectrum.





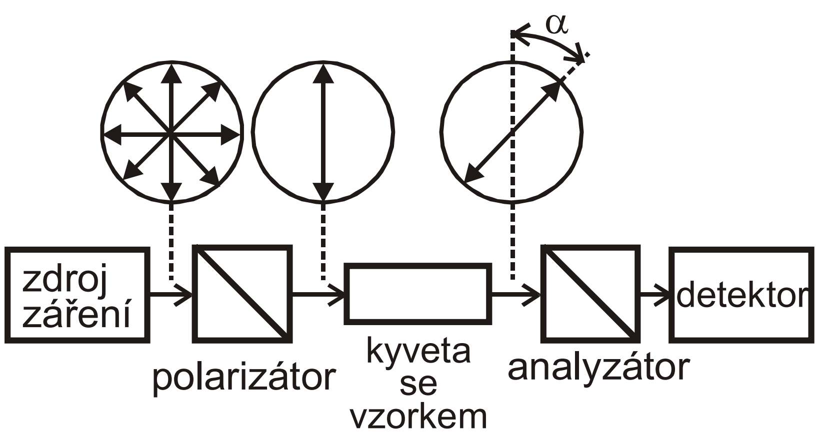
Refraktometrie

$$n = \sin\alpha / \sin\beta = v_1 / v_2$$



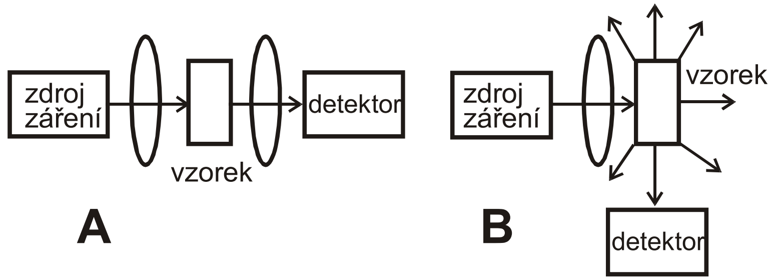
Polarimetrie

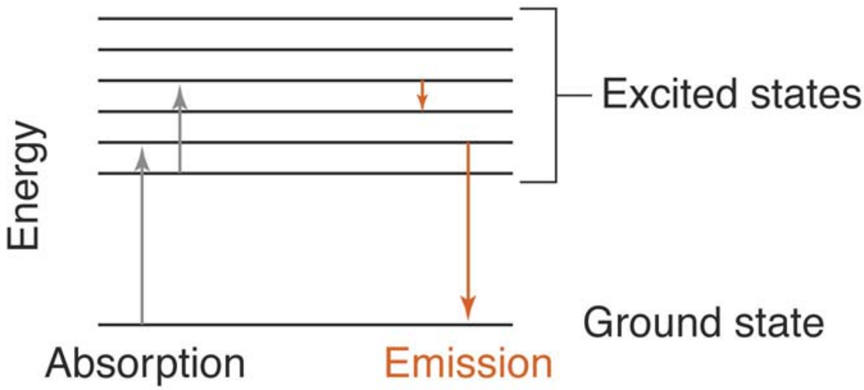
$\alpha = [\alpha]_{\lambda}^{t}\ L\ c$



Nefelometrie a turbidimetrie

(A-turbidimetrie, B-nefelometrie)

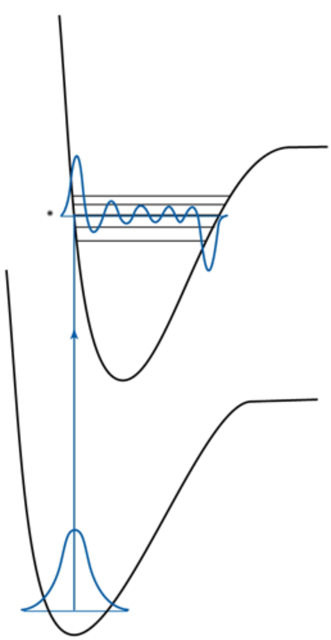




Příjem energie – absorpce
Ztráta energie – emise (luminiscence)

- atomy a molekuly mohou měnit svůj energetický stav přijmutím nebo vyzářením energie, přičemž jak přijatá, tak i vyzářená energie může nabývat pouze určitých diskrétních hodnot;
-v atomech přijímají nebo vyzařují energii pouze elektrony, v molekulách jsou elektronové energetické hladiny rozštěpeny na podhladiny vibrační a rotační; ***pro energetické rozdíly mezi hladinami platí:*** $\Delta E_{rot} << \Delta E_{vibr} << \Delta E_{el}$;

$\Delta E = \Delta E_{rot} + \Delta E_{vibr} + \Delta E_{el} + \Delta E_{spin} + \Delta E_{core}$

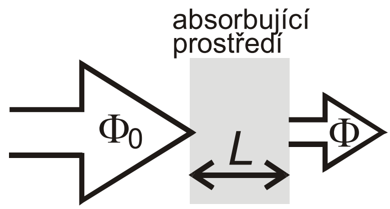


Franck-Condonův princip

Výběrová pravidla

Spektrální metody absorpční a emisní – společný základ

Absorpce záření, $X + h\nu \rightarrow X^*$

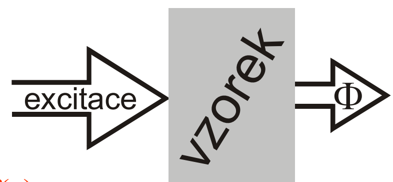


$$A = \log \frac{\Phi_0}{\Phi} = \varepsilon L c$$

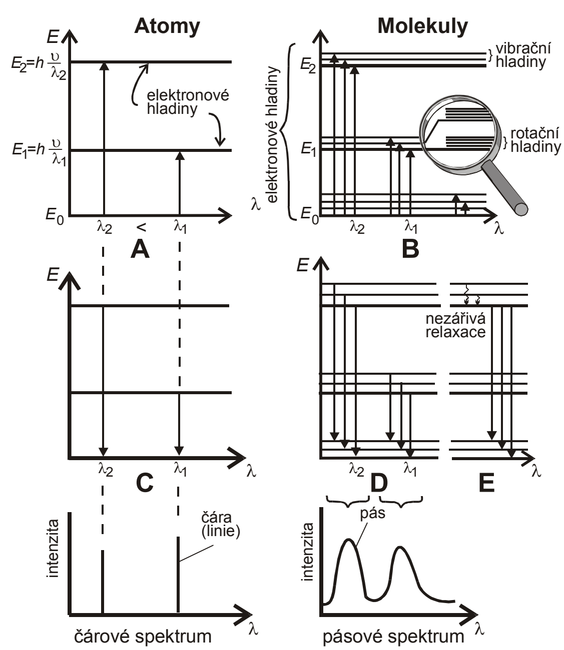
Emise záření, $X^* \rightarrow X + h\nu$
(excitace absorpcí záření či dodáním tepla)

$$\Phi = a.c^b$$

Fluorescence, $X^* \rightarrow X + h\nu' +$ teplo
(excitace absorpcí záření),
Excitovaný atom či molekula ztrácí část energie nezářivým způsobem, $\nu' < \nu$;



Tok fluorescenčního záření: $\Phi_F = f(c)$



Absorption of a photon causes electronic transition from the ground state to a higher energy state.

The electron relaxes to the lowest energy level of the first excited state.

The wavelengths of emitted radiation are independent of the wavelength of excitation.

But intensities are not.

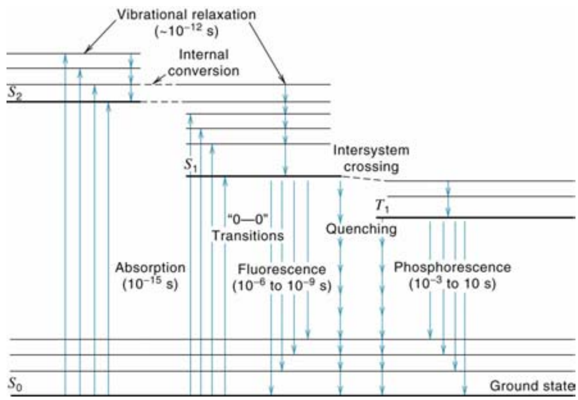


Fig. 16.29. Energy level diagram showing absorption processes, relaxation processes, and their rates.

The excitation spectrum corresponds to the absorption spectrum.

In larger molecules, the vibrational spacings of excited states are similar to those in the ground state.

So the emission spectrum may be a mirror image of the excitation spectrum.

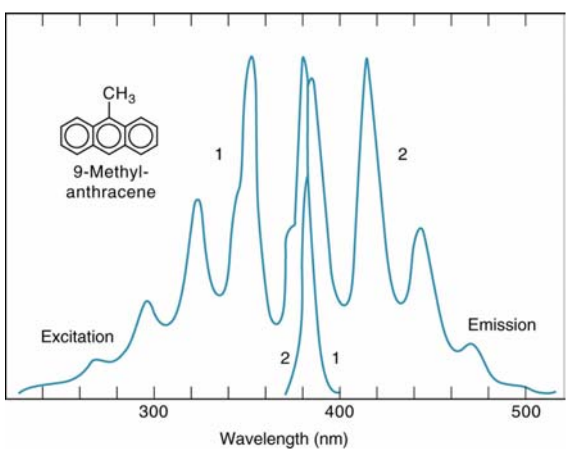
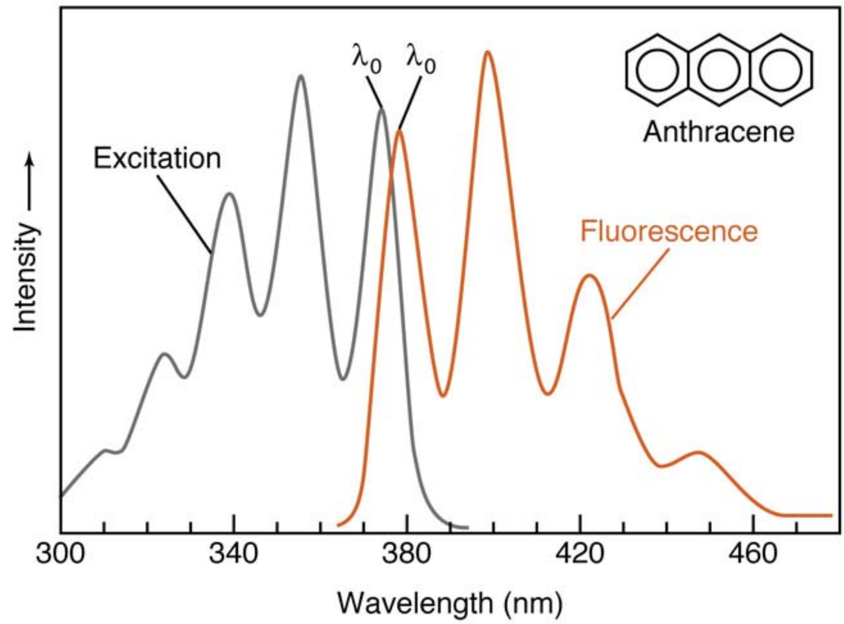


Fig. 16.30. Excitation and emission spectra of a fluorescing molecule.



- <u>**Spektrum**</u> – uspořádaný soubor absorbovaných či emitovaných vlnových délek

  počet a hodnoty λ - kvalitativní údaj

  intenzita abs./emit. záření – kvantitativní údaj.

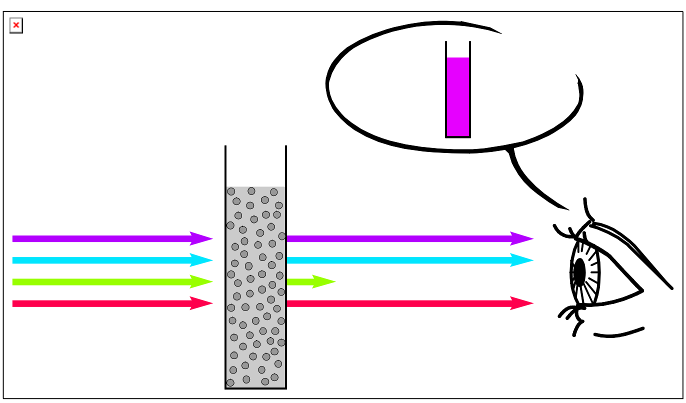
Spektrum

• **absorpční**	**atomová**
• **emisní**	**molekulová**

Metody molekulové a atomové spektrometrie – společný základ

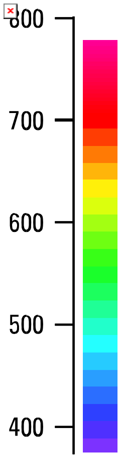
Molekulová spektrometrie	Atomová spektrometrie
informace o přítomnosti molekul, vazeb, funkčních skupin	informace o přítomnosti atomů
využití k identifikaci a stanovení	využití k důkazu a stanovení
vyžadují malých excitačních energií, UV, VIS, IČ, μ-vlny, RF	vyžadují vyšších excitačních energií, VIS, UV, RTG
vzorek v kyvetě	vzorek ve formě oblaku atomů
analyticky se využívá absorpce a fluorescence	analyticky se využívá absorpce, emise, fluorescence
spektra jsou pásová	spektra jsou čárová

Transmission and Color



The human eye sees the complementary color to that which is absorbed

Absorbance and Complementary Colors



Wavelength [nm]	Absorbed color	Complementary color
650-780	red	blue-green
595-650	orange	greenish blue
560-595	yellow-green	purple
500-560	green	red-purple
490-500	bluish green	red
480-490	greenish blue	orange
435-480	blue	yellow
380-435	violet	yellow-green

The complement of the absorbed light gets transmitted.

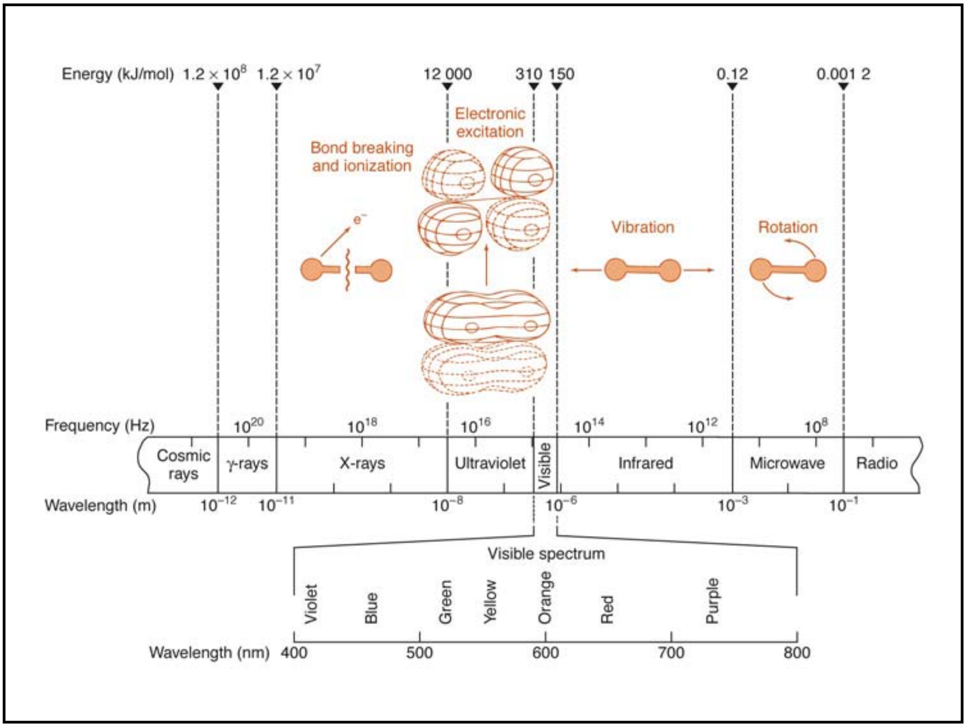
The color of an object we see is due to the wavelengths transmitted or reflected. Other wavelengths are absorbed.

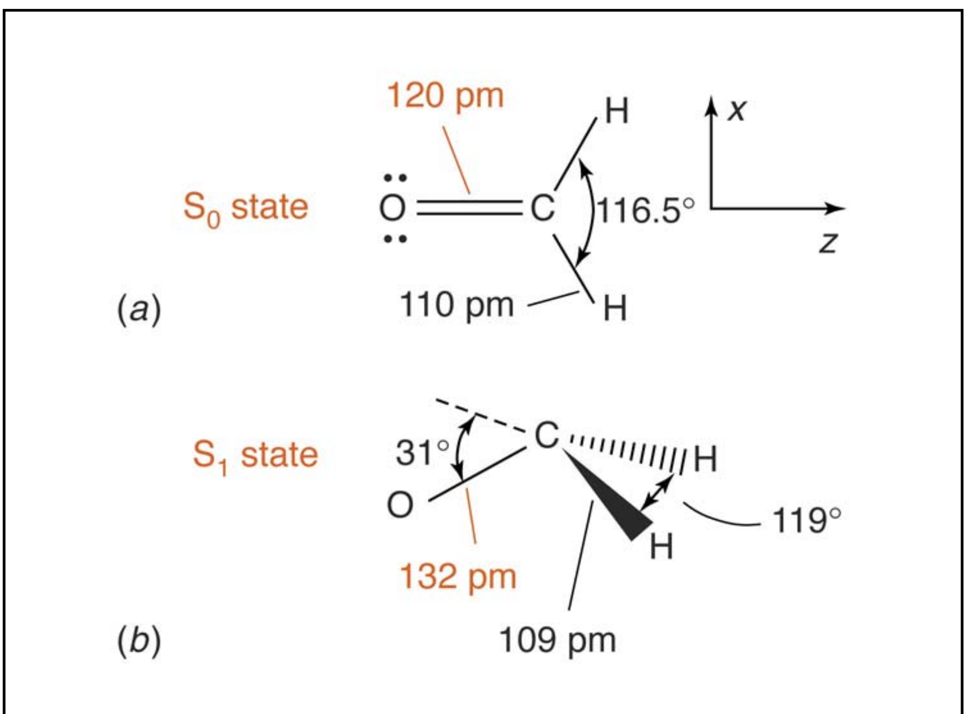
The more absorbed, the darker the color (the more concentrated the solution).

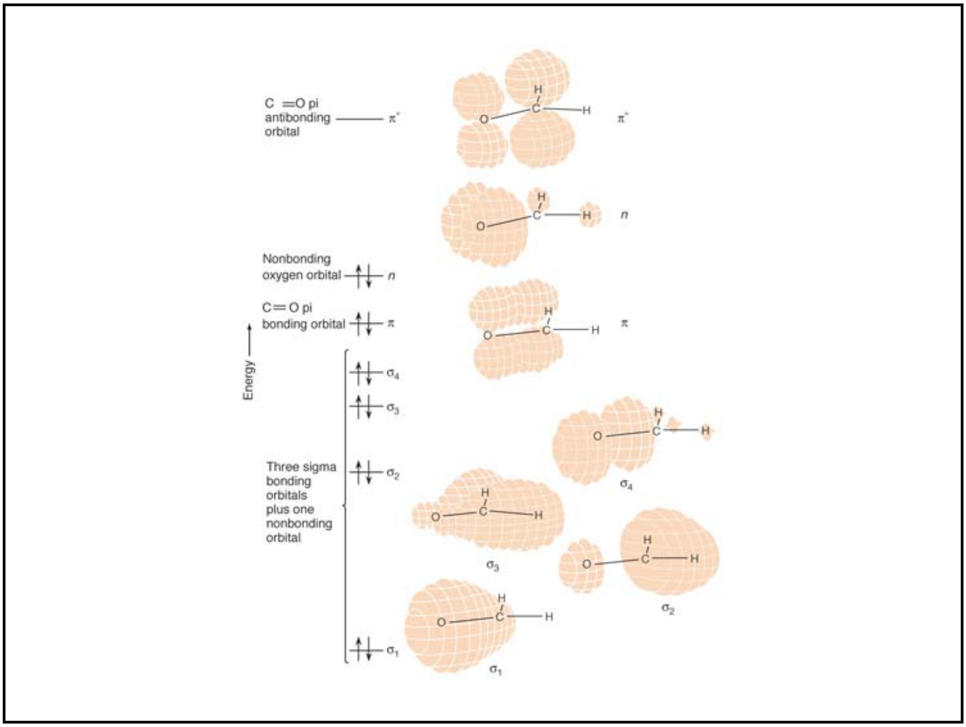
In spectrochemical methods, we measure the absorbed radiation.

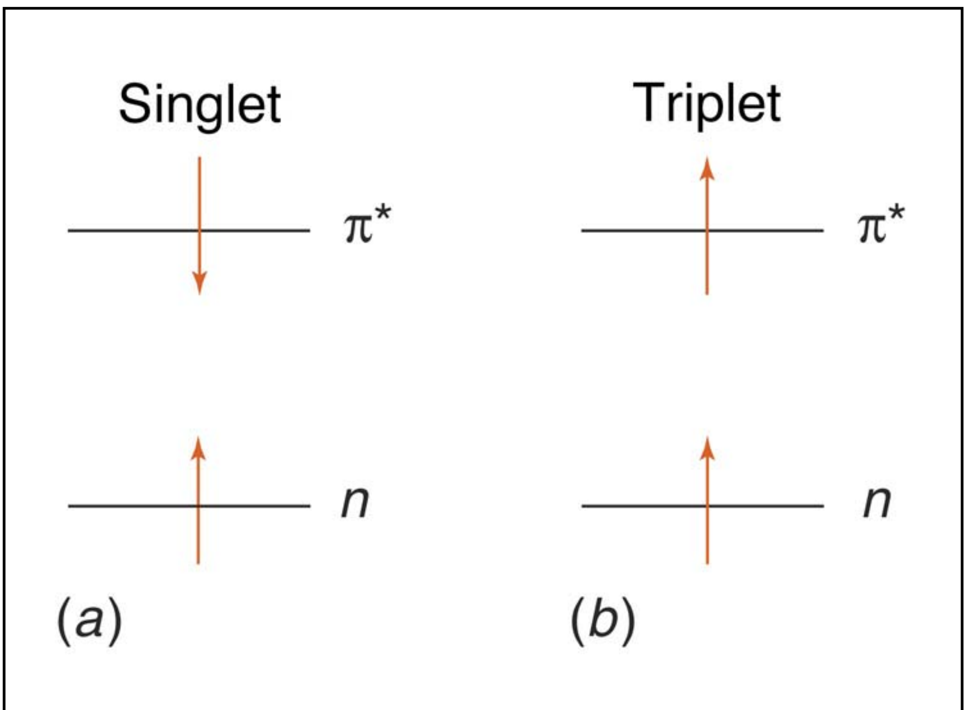
Table 16.1
Colors of Different Wavelength Regions

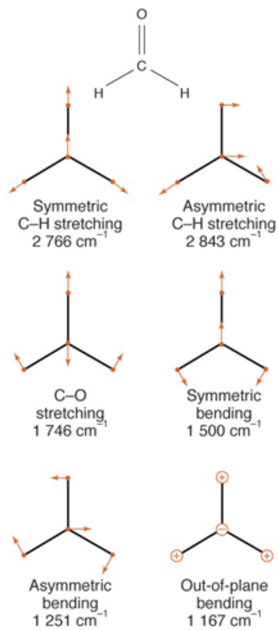
Wavelength Absorbed (nm)	Absorbed Color	Transmitted Color (Complement)
380–450	Violet	Yellow-green
450–495	Blue	Yellow
495–570	Green	Violet
570–590	Yellow	Blue
590–620	Orange	Green-blue
620–750	Red	Blue-green


Energy (kJ/mol) 1.2 × 10^8 1.2 × 10^7 12 000 310 150 0.12 0.001 2
Electronic excitation
Bond breaking and ionization
e⁻
Vibration
Rotation
Frequency (Hz) 10^20 10^18 10^16 10^14 10^12 10^8
Cosmic rays
γ-rays
X-rays
Ultraviolet
Visible
Infrared
Microwave
Radio
Wavelength (m) 10^−12 10^−11 10^−8 10^−6 10^−3 10^−1
Visible spectrum
Violet
Blue
Green
Yellow
Orange
Red
Purple
Wavelength (nm) 400 500 600 700 800


120 pm
H
x
S0 state
O
C
116.5°
z
110 pm
H
(a)
S1 state
31°
C
H
O
119°
H
132 pm
109 pm
(b)


C =O pi antibonding orbital
π*
Nonbonding oxygen orbital
n
C=O pi bonding orbital
π
Energy
σ4
σ3
σ2
σ1
Three sigma bonding orbitals plus one nonbonding orbital


Singlet
Triplet
π*
n
(a)
(b)



A - pure rotational changes (far IR).

B - rotational-vibrational changes (near IR).

C - rotational-vibrational-electronic changes (visible and UV).

These transitions are all quantized.

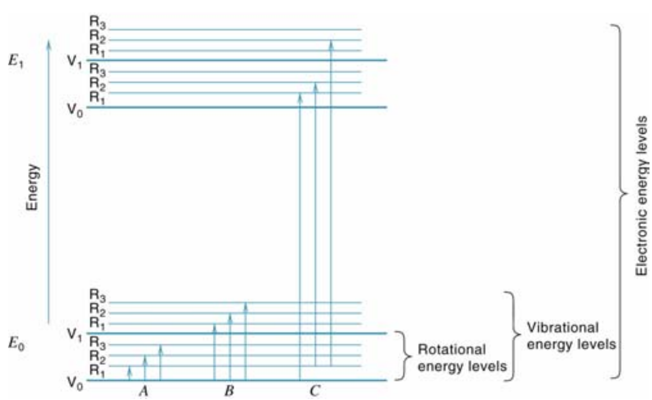


Fig. 16.3. Energy level diagram illustrating energy changes associated with absorption of electromagnetic radiation. E_0 is electronic ground state and E_1 is first electronic excited state.

The peaks are associated with vibrational modes within the molecule. (More in Fig. 16.8 on types of bonds that give peaks.)

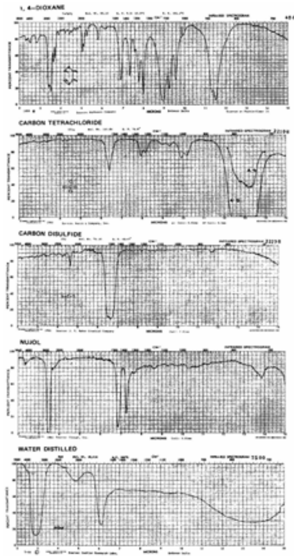
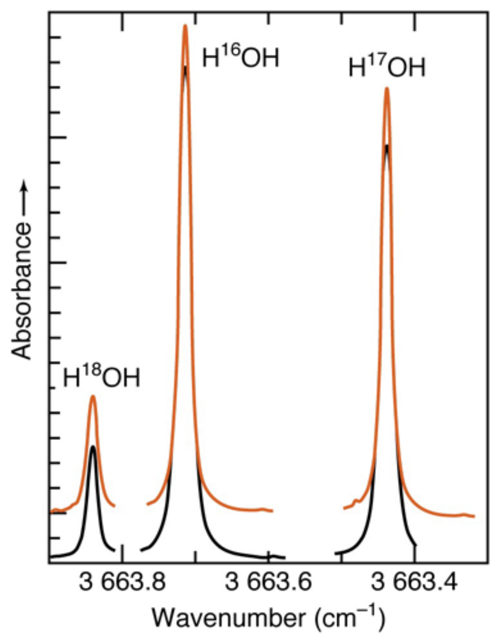


Fig. 16.4. Typical infrared spectra.



Electronic transitions (at higher energy – shorter wavelengths) are superimposed on rotational and vibrational trasitions.

These many discrete transitions result in a broad band of unresolved fine structure.

π (double or triple bonds) and n (outer shell) electrons are responsible for most UV and Vis electronic transitions.

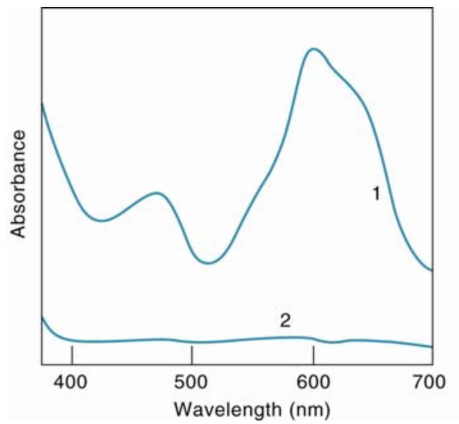


Fig. 16.5. Typical visible absorption spectrum. 1, Sample; 2, blank.

These are similar in structure to visible spectra.

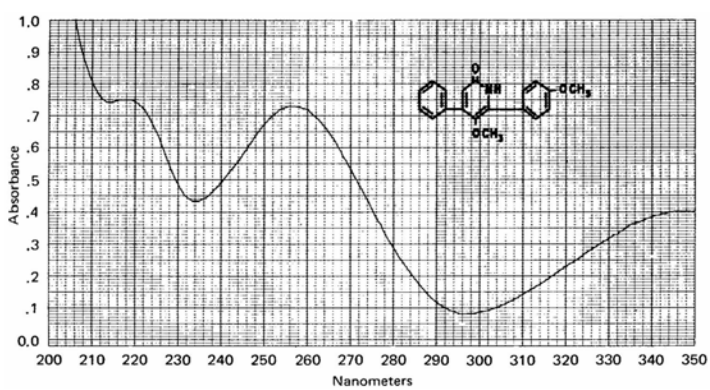


Fig. 16.6. Typical ultraviolet spectrum.

These groups absorb in the UV or visible regions.

Table 16.2

Electronic Absorption Bands for Representative Chromophores[a]

Chromophore	System	λ_{max}	ϵ_{max}
Amine	—NH_2	195	2,800
Ethylene	—C=C—	190	8,000
		195	1,000
Ketone	>C=O	270–285	18–30
Aldehyde	—CHO	210	Strong
		280–300	11–18
Nitro	—NO_2	210	Strong
Nitrite	—ONO	220–230	1,000–2,000
		300–400	10
Azo	—N=N—	285–400	3–25
Benzene		184	46,700
		202	6,900
		255	170
Naphthalene		220	112,000
		275	5,600
		312	175
Anthracene		252	199,000
		375	7,900

[a]From M. M. Willard, L. L. Merritt, and J. A. Dean, *Instrumental Methods of Analysis*, 4th ed. Copyright © 1948, 1951, 1958, 1965, by Litton Educational Publishing, Inc., by permission of Van Nostrand Reinhold Company.

Aromatic compounds are good absorbers of UV radiation.

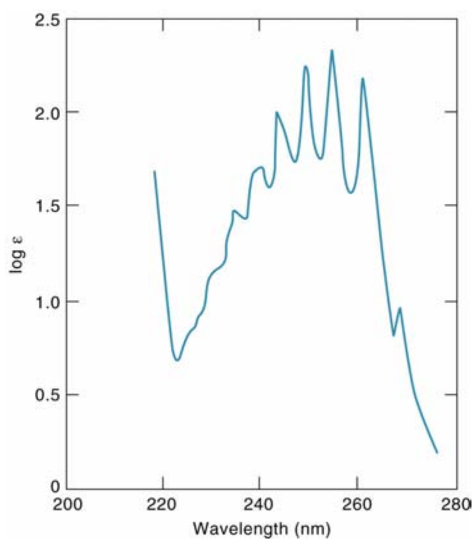


Fig. 16.7. Ultraviolet spectrum of benzene.

Absorption in the 6- to 15-μm region is very dependent on the molecular environment. This is called the fingerprint region.

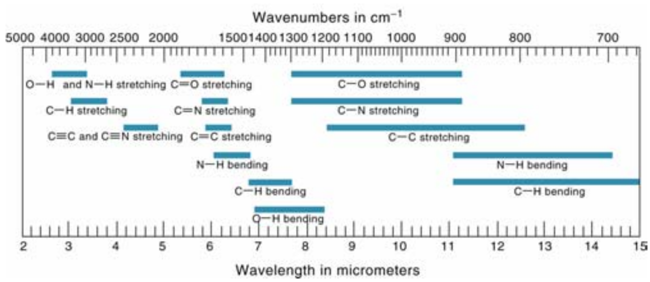


Fig. 16.8. Simple correlation of group vibrations to regions of infrared absorption.

Organic substances measured in the UV must usually be dissolved in organic solvents.

The solvent may affect the spectrum due to solute-solvent interactions.

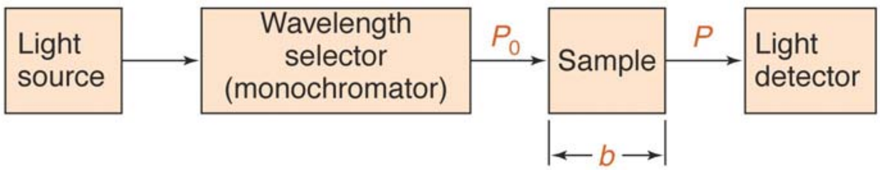
A polar solvent may cause loss of fine structure.

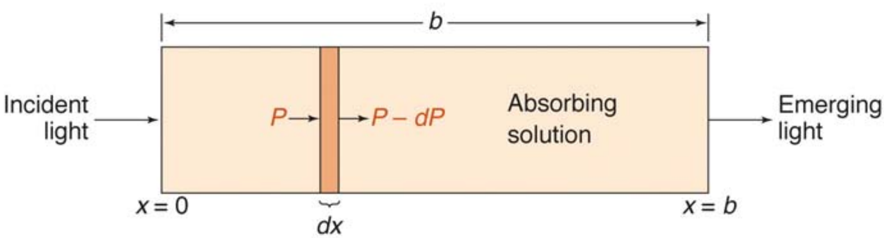
Table 16.3

Lower Transparency Limit of Solvents in the Ultraviolet Region

Solvent	Cutoff Point (nm)	Solvent	Cutoff Point (nm)[a]
Water	200	Dichloromethane	233
Ethanol (95%)	205	Butyl ether	235
Acetonitrile	210	Chloroform	245
Cyclohexane	210	Ethyl proprionate	255
Cyclopentane	210	Methyl formate	260
Heptane	210	Carbon tetrachloride	265
Hexane	210	*N*,*N*-Dimethylformamide	270
Methanol	210	Benzene	280
Pentane	210	Toluene	285
Isopropyl alcohol	210	*m*-Xylene	290
Isooctane	215	Pyridine	305
Dioxane	220	Acetone	330
Diethyl ether	220	Bromoform	360
Glycerol	220	Carbon disulfide	380
1,2-Dichloroethane	230	Nitromethane	380

[a] Wavelength at which the absorbance is unity for a 1-cm cell, with water as the reference.





$$dP = -\beta P c \, dx$$

$$-\frac{dP}{P} = \beta c \, dx$$

$$-\int_{P_0}^{P} \frac{dP}{P} = \int_{0}^{b} \beta c \, dx$$

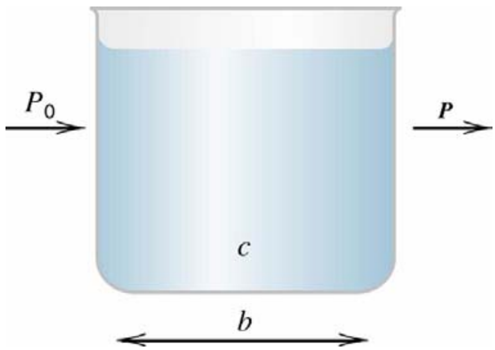
$$\ln\left(\frac{P_0}{P}\right) = \beta c b$$

$$\log\left(\frac{P_0}{P}\right) = \left(\frac{\beta}{\ln(10)}\right) c b$$

Transmittance = $P/P_0 = 10^{-abc}$

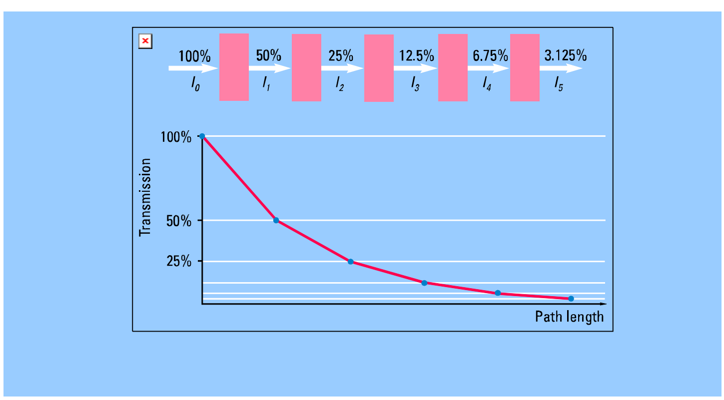
a = proportionality constant = absorptivity

$-\log T = abc = A$ = absorbance



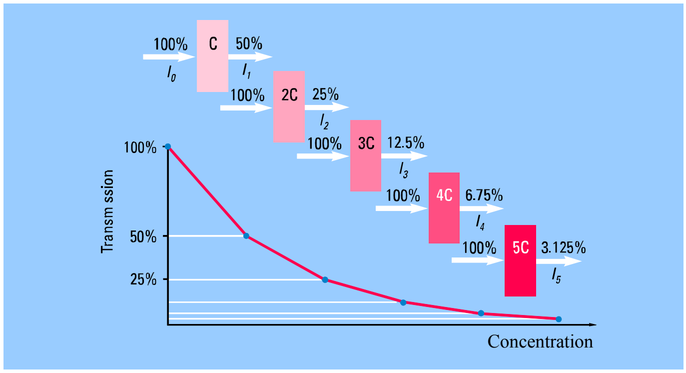
Absorption of radiation. P_0 = power of incident radiation,
P = power of transmitted radiation, c = concentration, b = pathlength.

Transmittance and Concentration
The Bouguer-Lambert Law



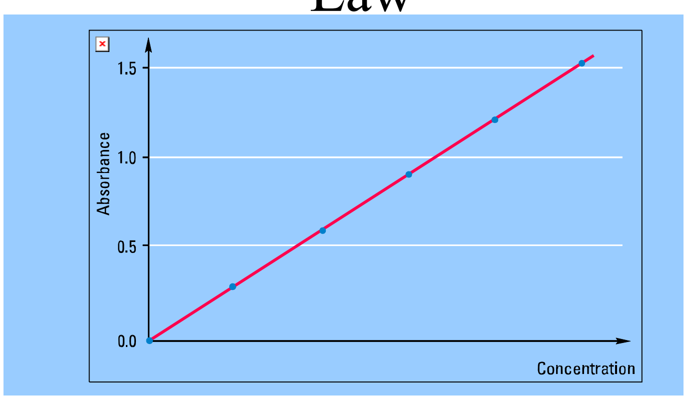
$$T = I / I_0 = e^{-Const \cdot Pathlength}$$

Transmittance and Path Length
Beer's Law

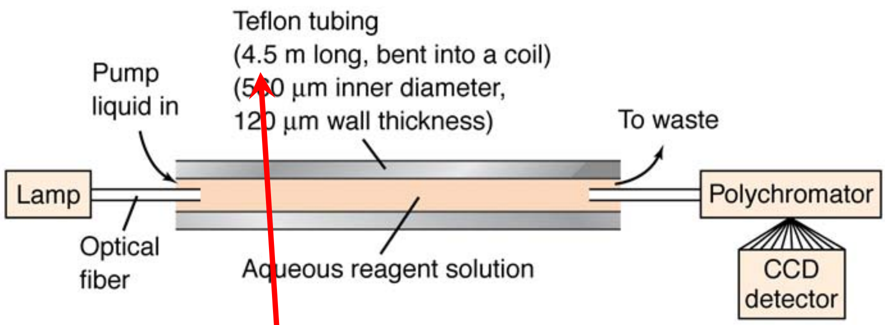


$$T = I / I_0 = e^{-Const \cdot Concentration}$$

The Beer-Bouguer-Lambert Law



$$A = -\log T = -\log\left(I / I_0\right) = \log\left(I_0 / I\right) = \varepsilon \cdot b \cdot c$$



Stanovení nanomolárních koncentrací dusičnanů a dusitanů pomocí „**Long Path Length** Absorbance“ spektroskopie

Use the newer recommended nomenclature.

Table 16.4

Spectrometry Nomenclature

Recommended Name	Older Names or Symbols
Absorbance (*A*)	Optical density (OD), extinction, absorbancy
Absorptivity (*a*)	Extinction coefficient, absorbancy index, absorbing index
Pathlength (*b*)	*l* or *d*
Transmittance (*T*)	Transmittancy, transmission
Wavelength (nm)	mμ (millicron)

This empirical ratio method is used because of deviations from Beer's law, scattered radiation, etc.

Log P_0/P is plotted against C.

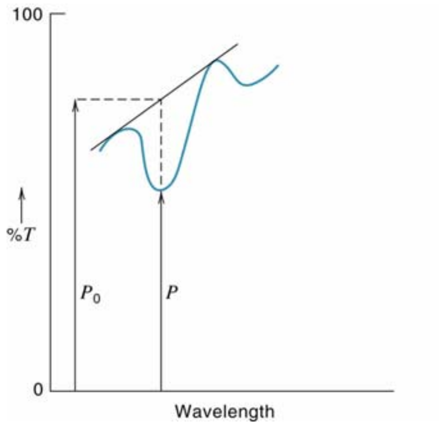


Fig. 16.11. Baseline method for quantitative determination in infrared region of spectrum.

The nominal wavelength is that set on the instrument.

The slit passes a band of wavelengths.

The bandwidth varies with wavelength with a prism, but is constant with a grating.

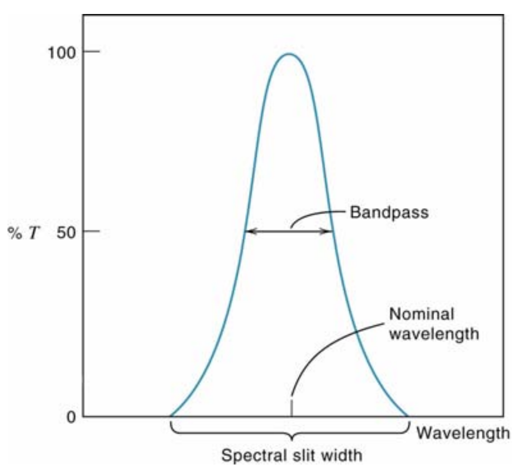
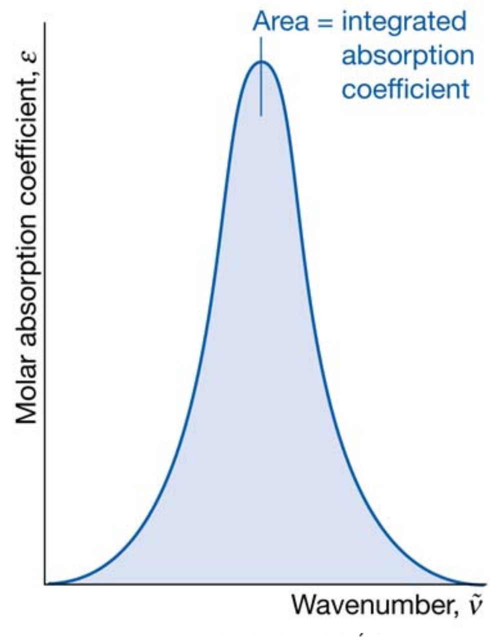


Fig. 16.21. Distribution of wavelengths leaving the slit of monochromator.



UV/VIS spektroskopie
Gaussovský pás

IR, NMR spektroskopie
Lorenzovský pás

Ideální případ !!!

Většinou kombinace obou funkcí

Šířka čáry

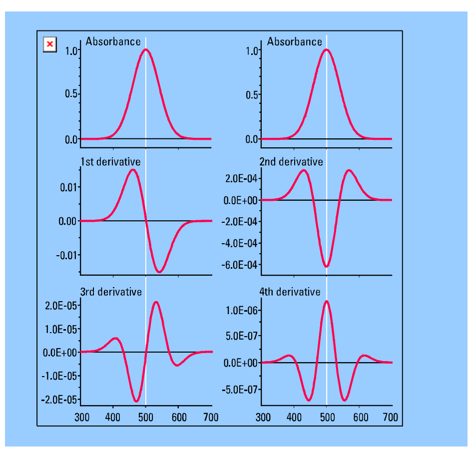
- **Dopplerovo rozšíření –** *detektor vs. zdroj*
- **„lifetime“ rozšíření -** *princip neurčitosti*

Derivative Spectra of a Gaussian Absorbance Band

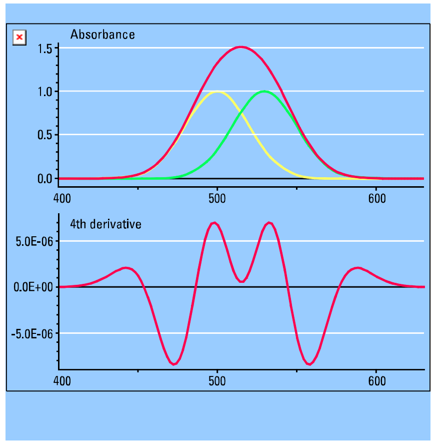
Absorbance: $A = f(\lambda)$

1st Derivative: $\dfrac{dA}{d\lambda} = f^{'}(\lambda)$

2nd Derivative: $\dfrac{d^2A}{d\lambda^2} = f^{''}(\lambda)$

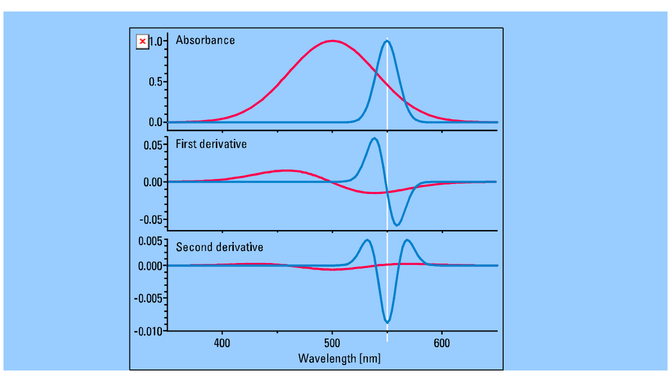


Resolution Enhancement



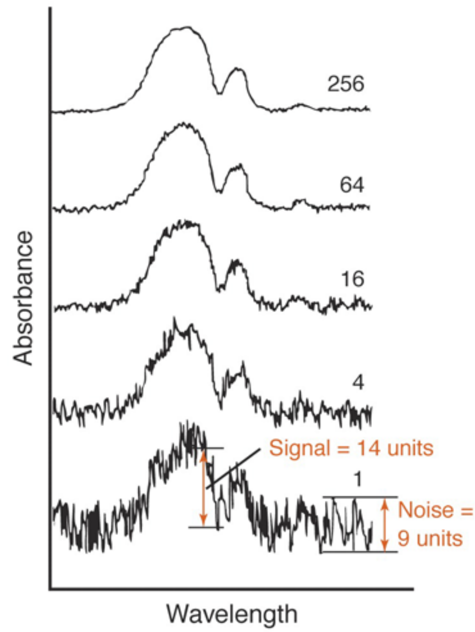
- Overlay of 2 Gaussian bands with a NBW of 40 nm separated by 30 nm
- Separated by 4th derivative

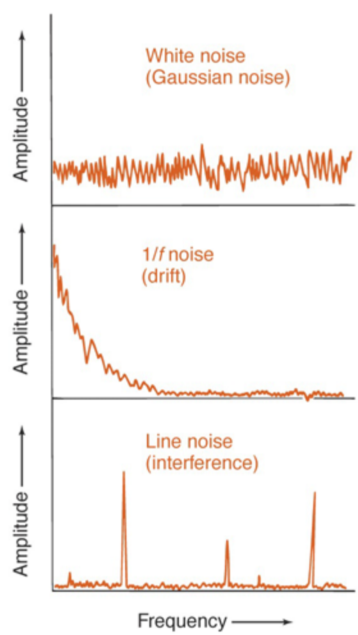
Discrimination of Broad Bands

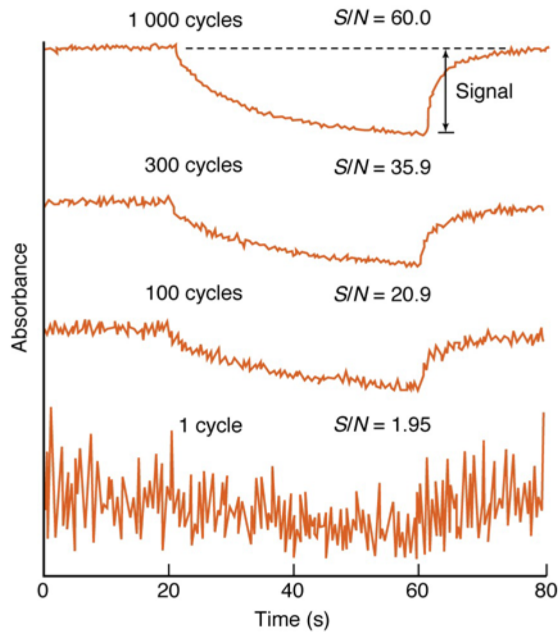


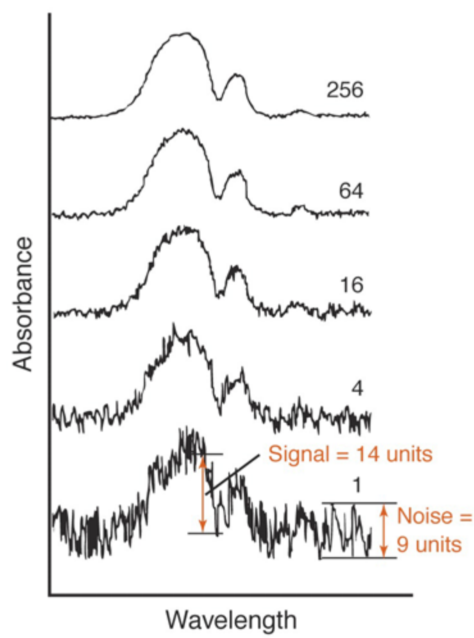
- Derivatives can eliminate background absorption
- Derivatives discriminate against broad absorbance bands

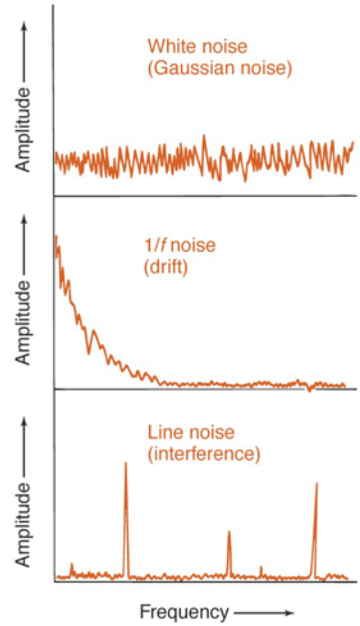
Akumulace signálu



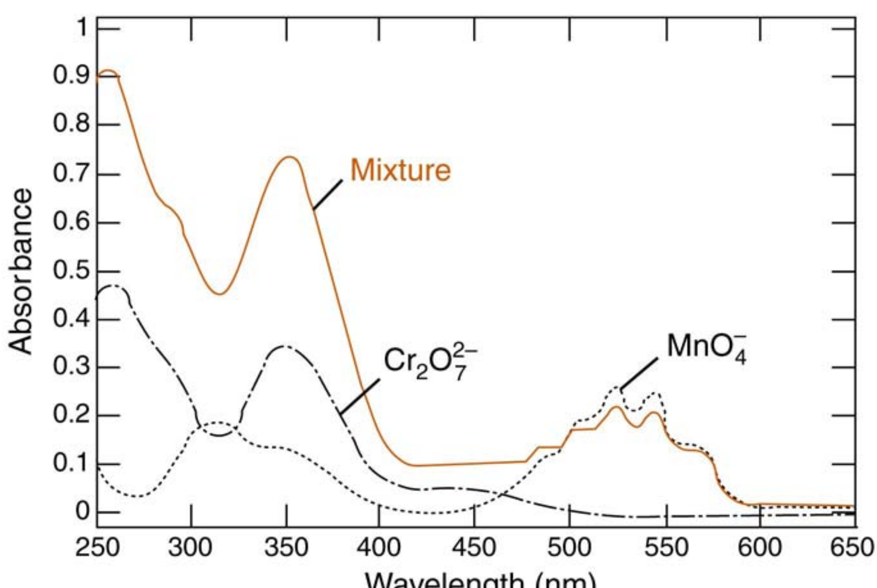


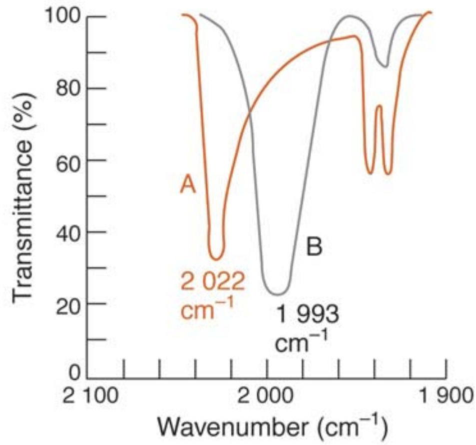

1 000 cycles
S/N = 60.0
Signal
300 cycles
S/N = 35.9
100 cycles
S/N = 20.9
1 cycle
S/N = 1.95
Absorbance
0
20
40
60
80
Time (s)


256
64
16
4
1
Signal = 14 units
Noise = 9 units
Absorbance
Wavelength



Vícesložková analýza



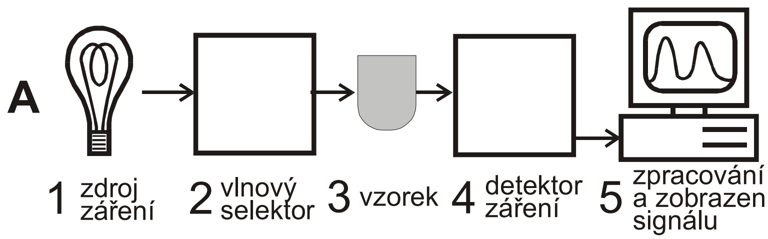


Wavenumber	Pure A	Pure B
2 022 cm^{-1}	31.0% *T*	97.4% *T*
1 993 cm^{-1}	79.7% *T*	20.0% *T*

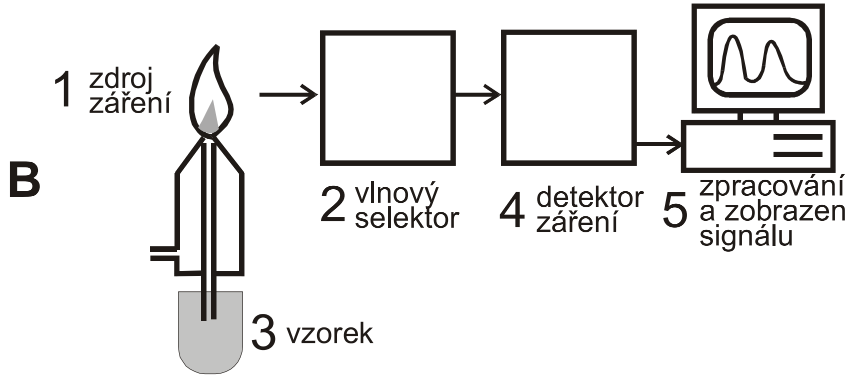
Instrumentace
Základní části spektrálních přístrojů

1. **zdroj záření**
2. **selektor vlnových délek**
3. **vzorek**
4. **detektor záření**
5. **vyhodnocovací zařízení a displej**

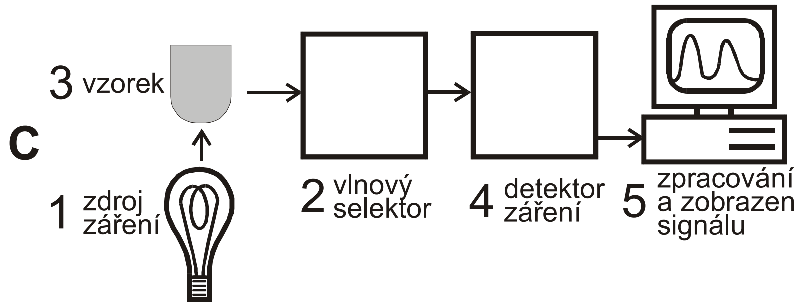
Základní uspořádání pro absorpční spektrometrii:



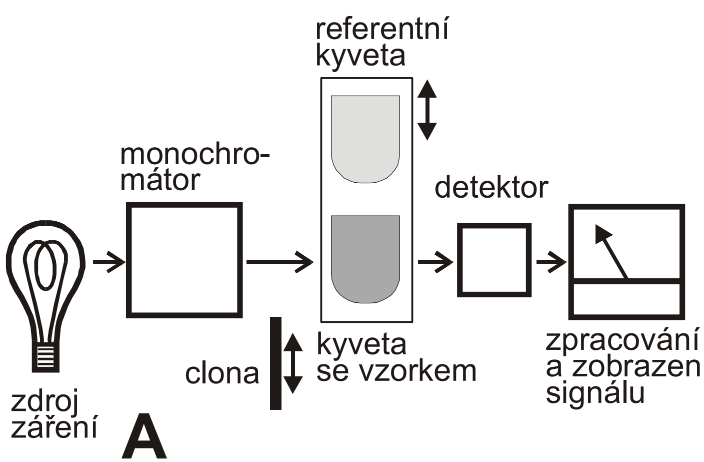
Základní uspořádání pro emisní spektrometrii:



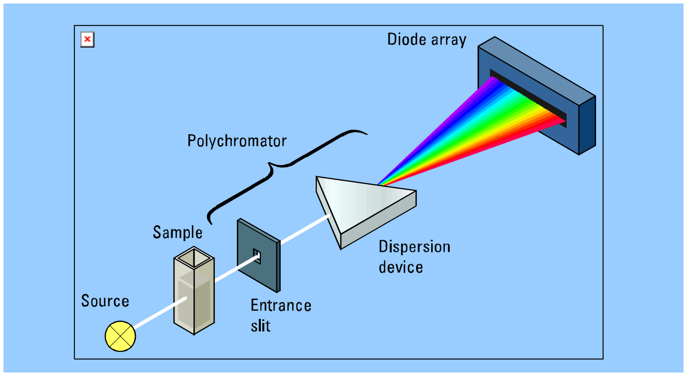
Základní uspořádání pro fluorescenční spektrometrii:



Jednopaprskové diferenční uspořádání spektrometru:

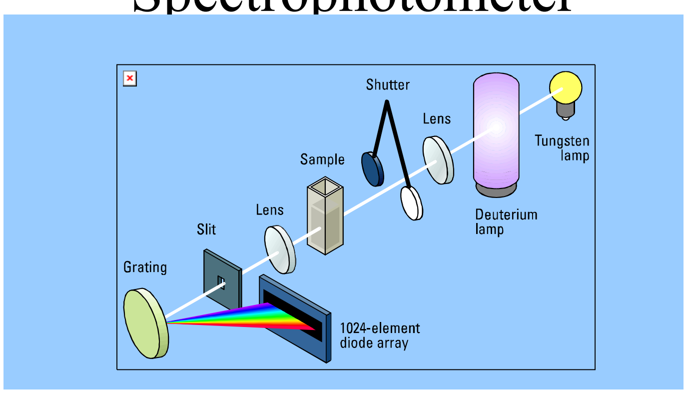


Diode-Array Spectrophotometer



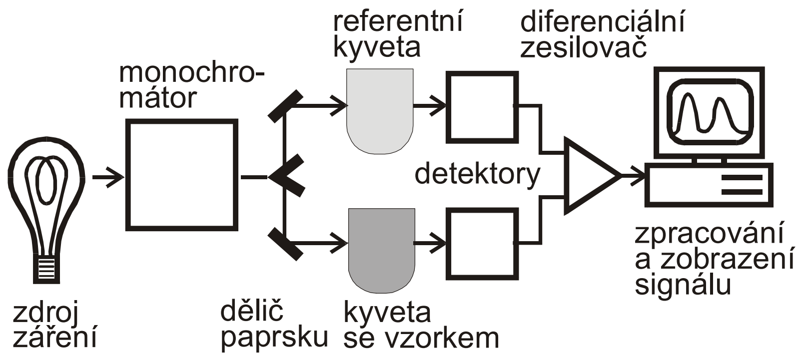
Schematic of a diode-array spectrophotometer

Diode-Array Spectrophotometer

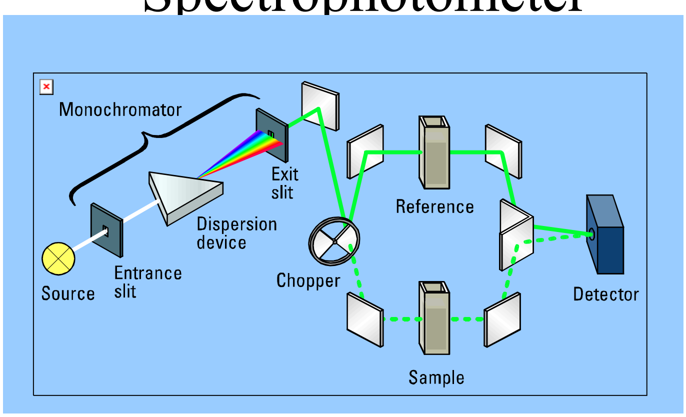


Optical diagram of the HP 8453 diode-array spectrophotometer

Dvoupaprskové uspořádání spektrometru:

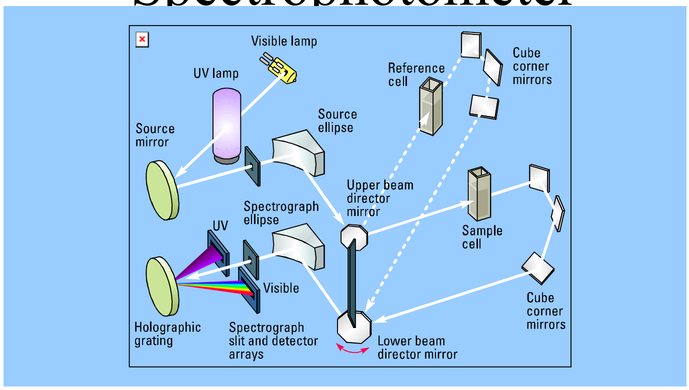


Conventional Spectrophotometer



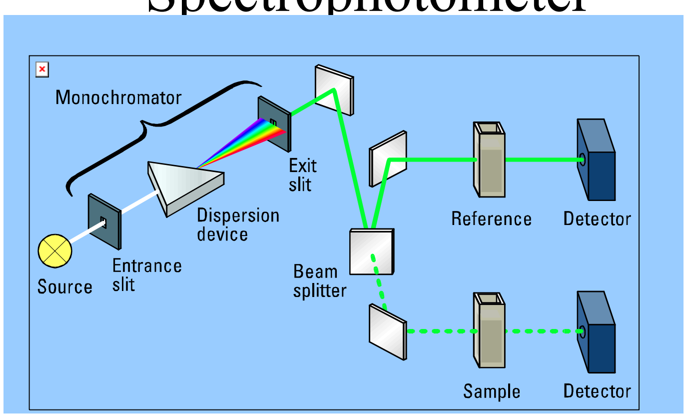
Optical system of a double-beam spectrophotometer

Diode-Array Spectrophotometer

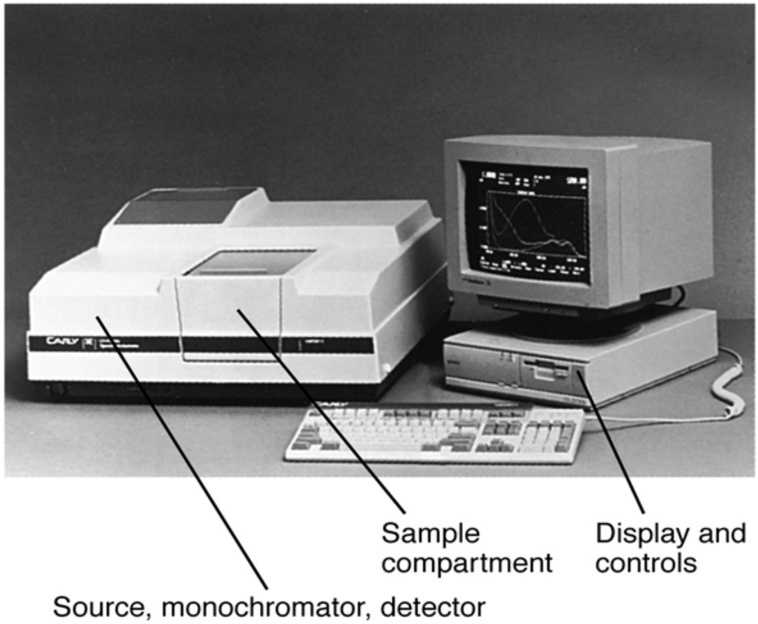


Optical system of the HP 8450A diode-array spectrophotometer

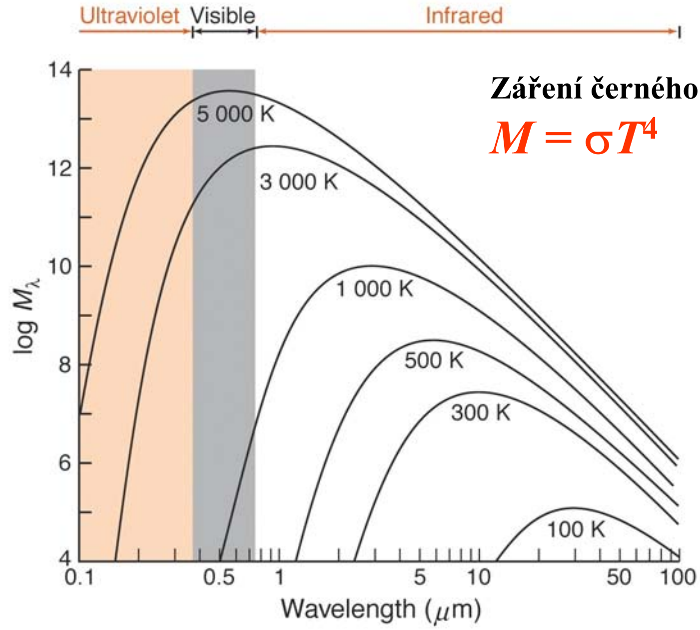
Conventional Spectrophotometer



Optical system of a split-beam spectrophotometer



1. zdroj	použití
a) zdroje spojitého záření	
xenonová lampa	molek. fluoresc. spektr.
H_2, D_2 výbojka	molek. abs. spektr., UV
W, W-X žárovka	molek. abs. spektr., UV, VIS, IČ
globar (SiC, 1500°C)	molek. abs. spektr., IČ
b) zdroje čárového spektra	
laser	molek. abs. spektr., UV, VIS, IČ; molek. fluoresc. spektr.
výbojka s dutou katodou	atom. abs. spektr., UV, VIS; atom. fluoresc. spektr.
Hg výbojka	atom. abs. spektr., UV, VIS; atom. fluoresc. spektr.
c) zdroj spojitého i čárového záření	
RTG lampa	RTG spektrometrie
d) v emisních spektrálních metodách je zdrojem záření excitovaný vzorek	



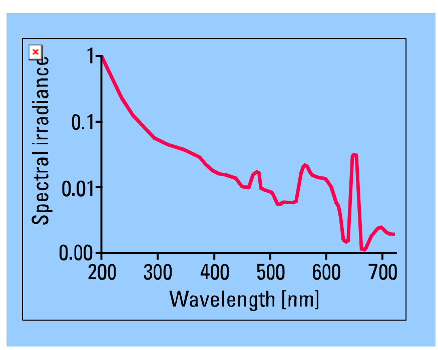
Záření černého tělesa

$$M = \sigma T^4$$

astronomie

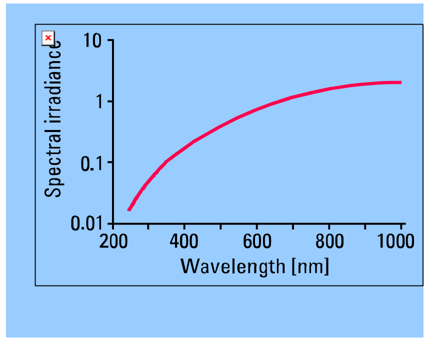
Intensity Spectrum of the Deuterium Arc Lamp

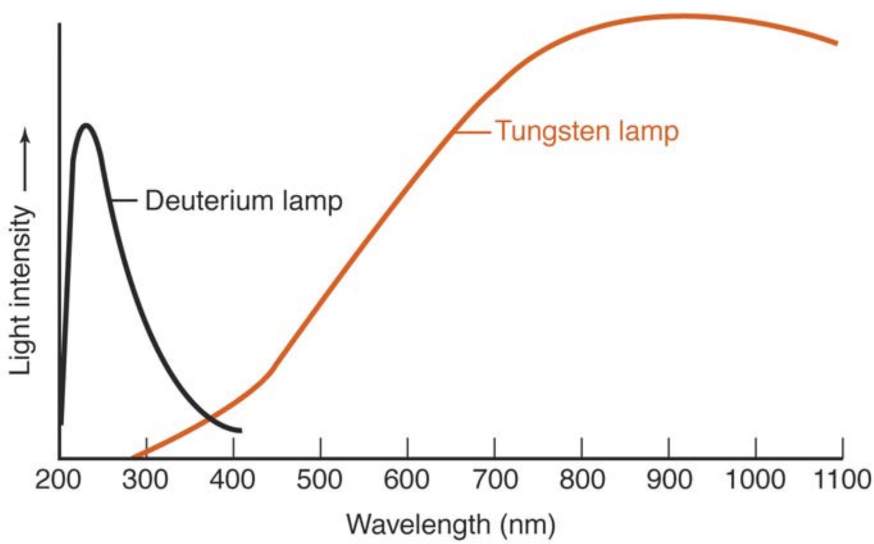
- Good intensity in UV range
- Useful intensity in visible range
- Low noise
- Intensity decreases over lifetime



Intensity Spectrum of the Tungsten-Halogen Lamp

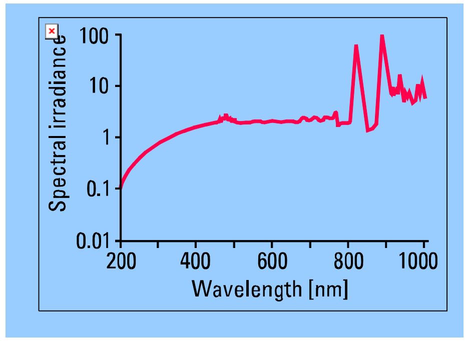
- Weak intensity in UV range
- Good intensity in visible range
- Very low noise
- Low drift



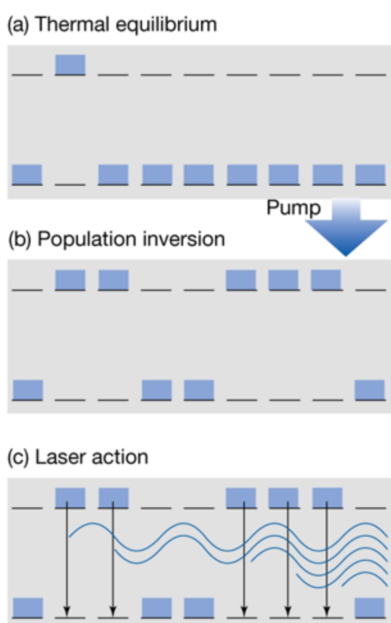


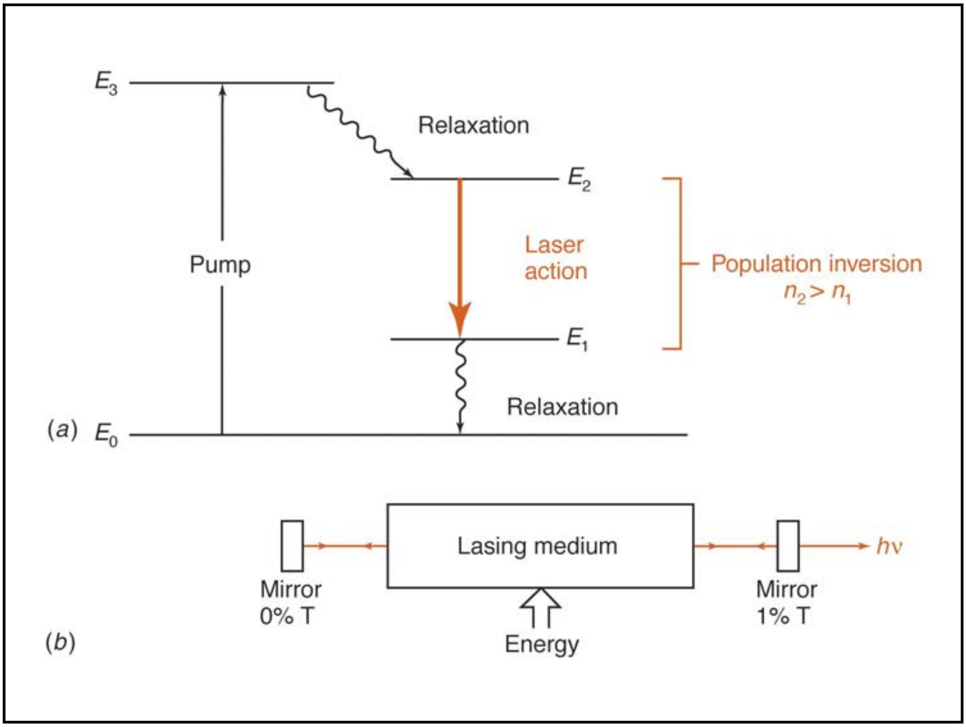
Intensity Spectrum of the Xenon Lamp

- High intensity in UV range
- High intensity in visible range
- Medium noise

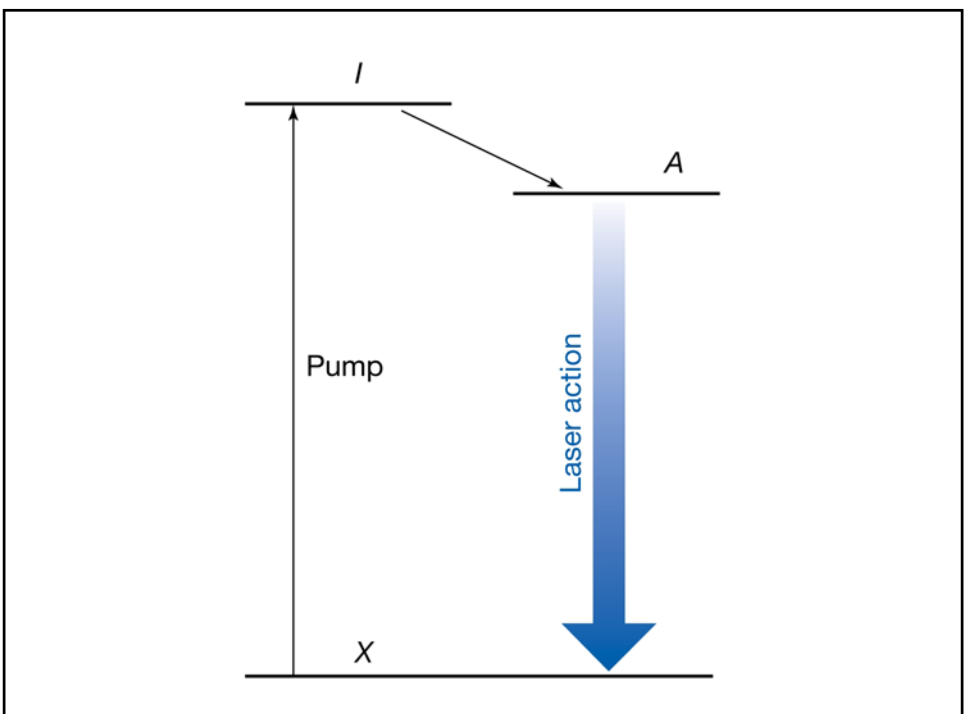


Lasery

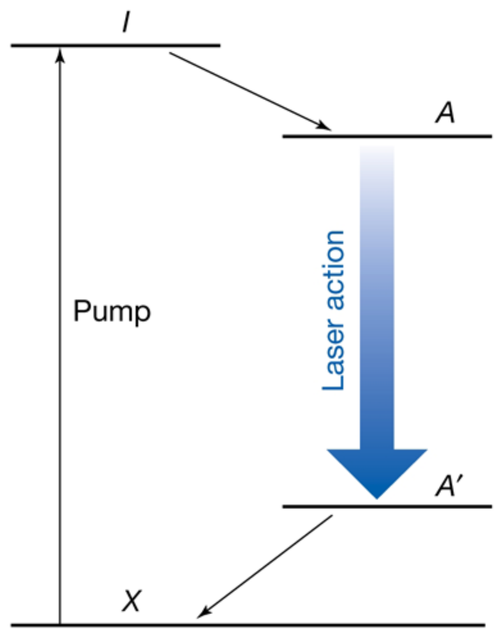




E_3
Relaxation
E_2
Laser action
Population inversion
$n_2 > n_1$
Pump
E_1
Relaxation
(a) E_0
Lasing medium
$h\nu$
Mirror 0% T
Mirror 1% T
Energy
(b)



I
A
Pump
Laser action
X



Vlastnosti laserů

- monochromatické záření
- extrémně úzké – vysoký výkon při jedné λ
- kolimované
- polarizované
- koherentní

Nevýhody
- drahé
- náročné údržba
- omezený počet l pro použití

Lasers are intense monochromatic sources, good as fluorescence sources, since F α Intensity.

Table 16.5
Characteristics of Common Lasers

Laser	Wavelength (nm)	Power (W)
Ionic crystal		
Ruby[a]	694.3	1–10 MW
Nd: YAG[a]	1064.0	25 MW (8–9 ns)
Gas		
He–Ne	632.8	0.001–0.05
He–Cd	441.6	0.05
	325.0	0.01
Ar^+	514.5	7.5
	496.6	2.5
	488.0	6.0
	476.5	2.5
	465.8	7.0
	457.9	1.3
	333.6–363.8 (4 lines)	3.0
Kr^+	752.5	1.2
	647.1	3.5
	530.9	1.5
	482.5	0.4
	468.0	0.5
	413.1	1.8
	406.7	0.9
	337.5–356.4 (3 lines)	2.0
Nitrogen[a]	337.1	200 kW

[a]Operated in pulsed mode; values given are peak power (pulse width).
From G. D. Christian and J. E. O'Reilly, *Instrumental Analysis*, 2nd ed. Boston: Allyn and Bacon, Inc., 1986.
Reproduced by permission of Allyn and Bacon, Inc.

2. Vlnové selektory

Filtry

•absorpční

•interferenční

Monochromátory

• hranolové

• mřížkové

3. Vzorek

Molekulové spektrální. metody –

• kyvety, (hranaté, válcové) z vhodného materiálu;

Atomové spektrální metody

• oblak atomů.

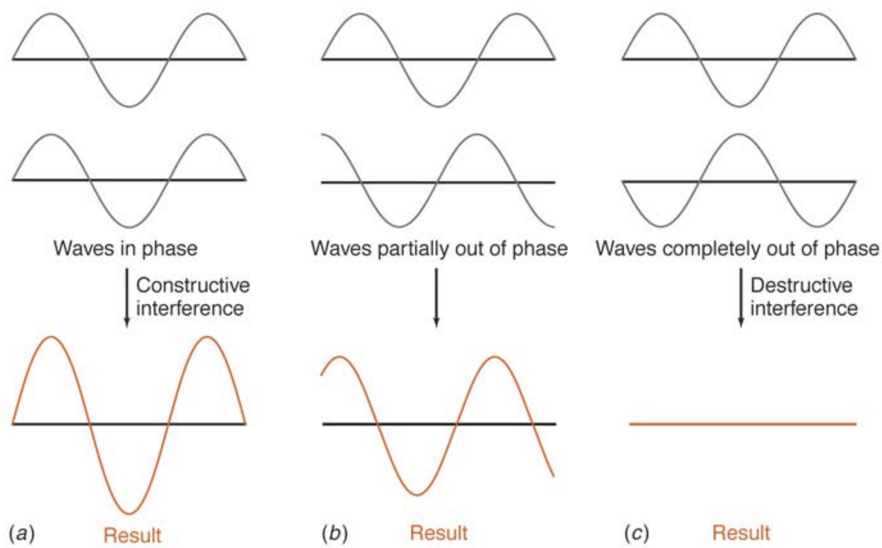
4. Detektory

•fotonky, fotonásobiče

•polovodičové detektory

•detektory s diodovým polem

•termočlánky



Modern IR spectrometers are Fourier transform spectrometers, rather than dispersive.

The beam is spit into two paths.

When reflected, they are out of phase due to the moving mirror.

They recombine to give an interference pattern of all wavelengths (pattern changes with time).

A time-domain spectrum is recorded (interferogram – see next slide).

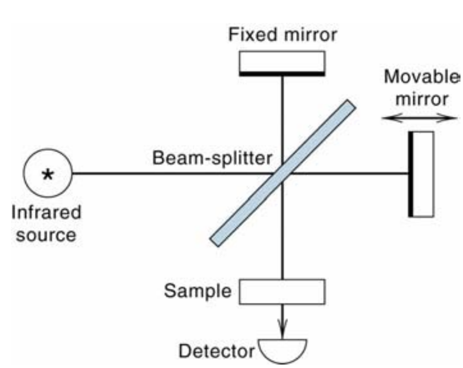
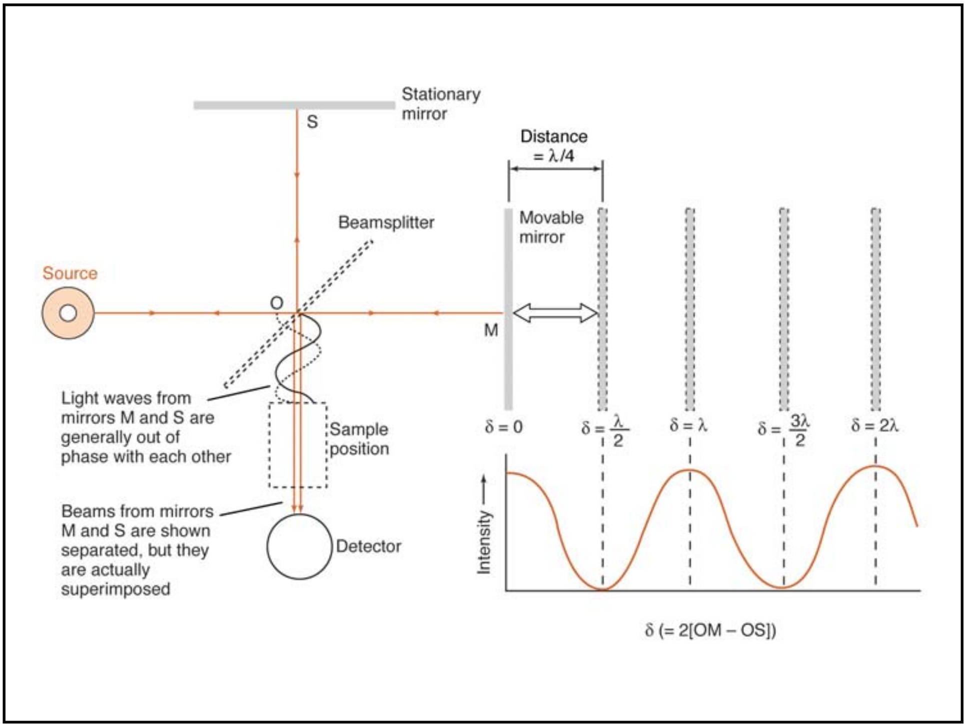
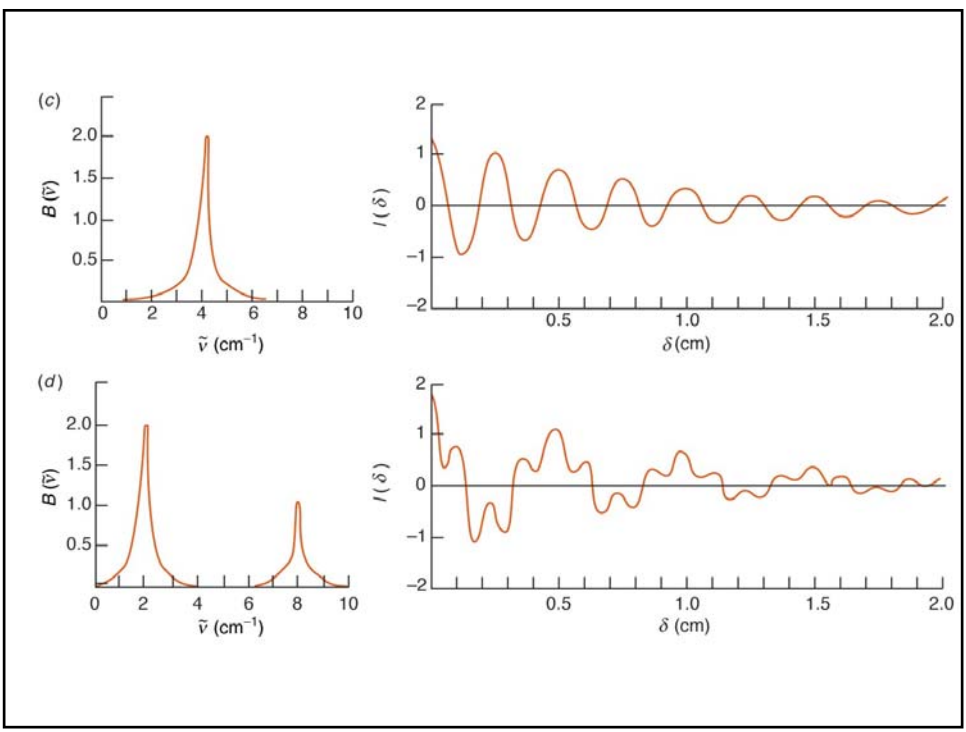


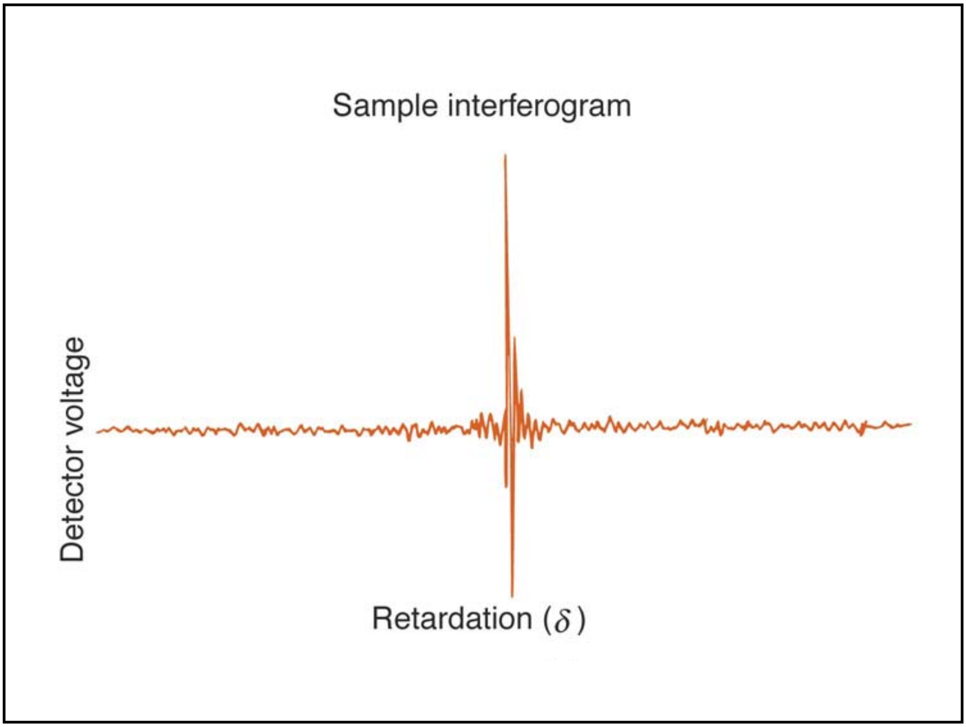
Fig. 16.25. Schematic of interferometer for FTIR spectroscopy.

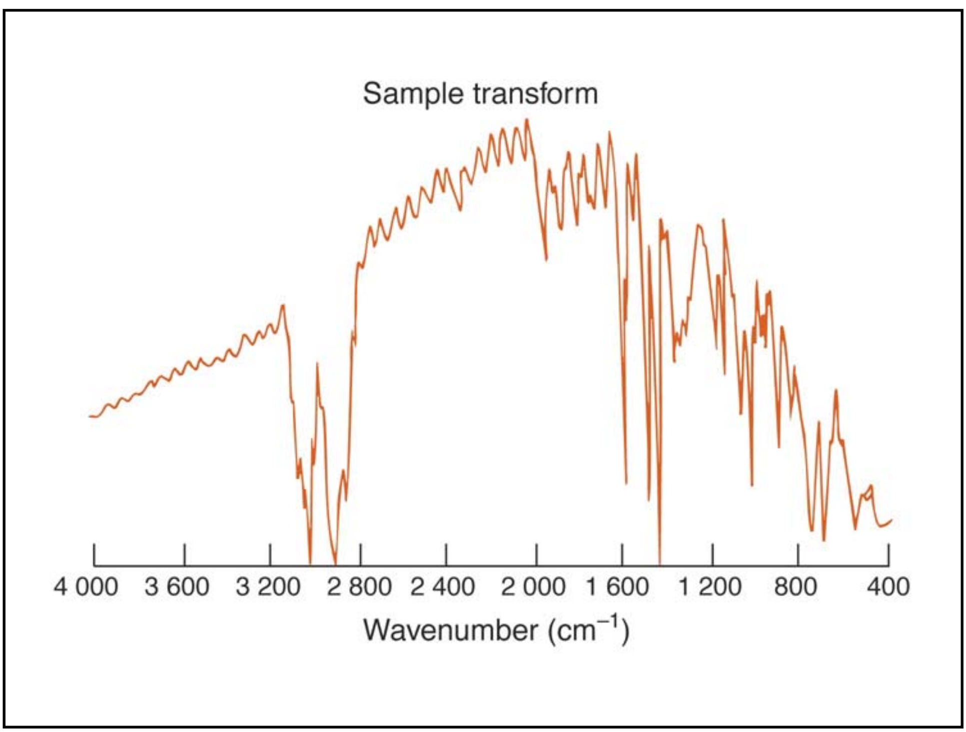


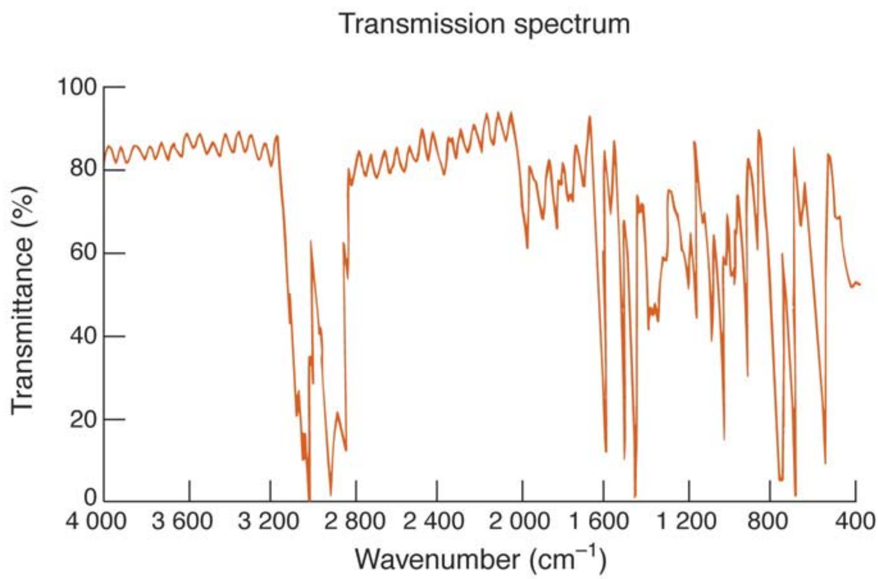
Stationary mirror
S
Distance = λ/4
Beamsplitter
Movable mirror
Source
O
M
Light waves from mirrors M and S are generally out of phase with each other
Sample position
Beams from mirrors M and S are shown separated, but they are actually superimposed
Detector
δ = 0
δ = λ/2
δ = λ
δ = 3λ/2
δ = 2λ
Intensity
δ (= 2[OM – OS])



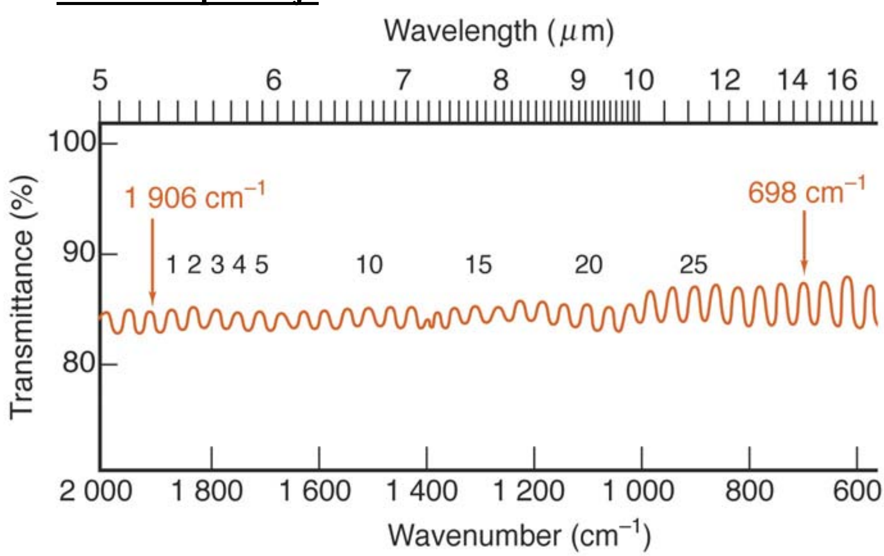
(c)
B (ν̃)
ν̃ (cm⁻¹)
I (δ)
δ (cm)
(d)
B (ν̃)
ν̃ (cm⁻¹)
I (δ)
δ (cm)


Sample interferogram
Detector voltage
Retardation (δ)


Sample transform
4 000
3 600
3 200
2 800
2 400
2 000
1 600
1 200
800
400
Wavenumber (cm⁻¹)



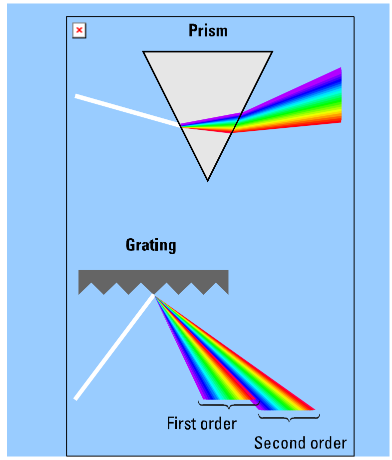
Kalibrace přístroje



Dispersion Devices

- Non-linear dispersion
- Temperature sensitive

- Linear Dispersion
- Different orders



Dispersion by prisms is good at short wavelengths, poor at long wavelengths (IR).

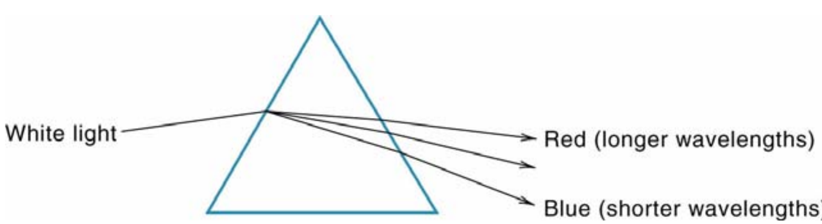
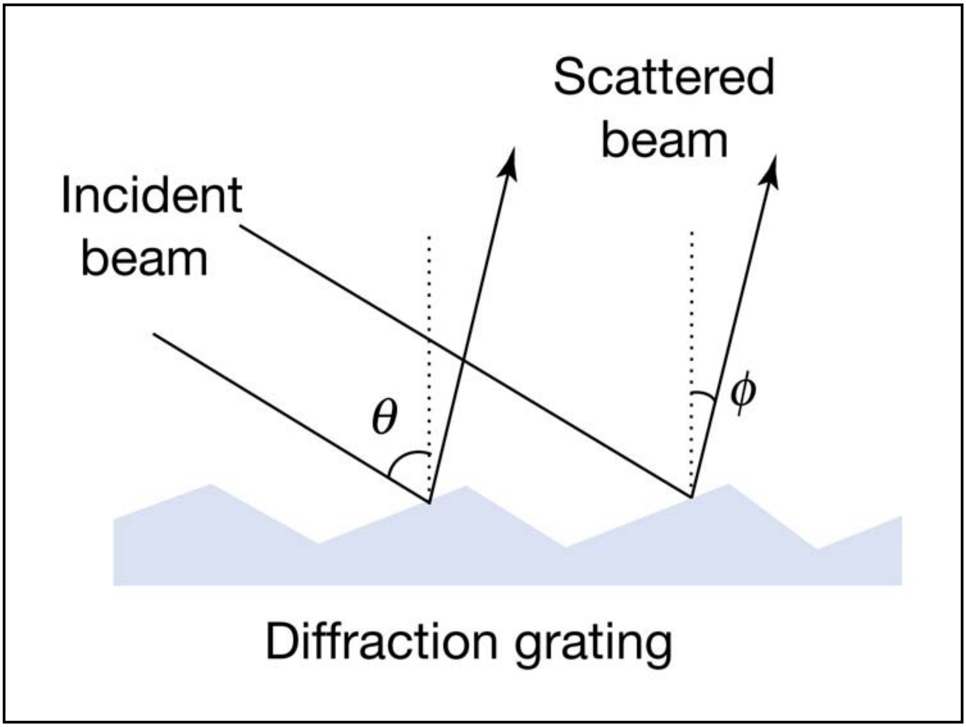
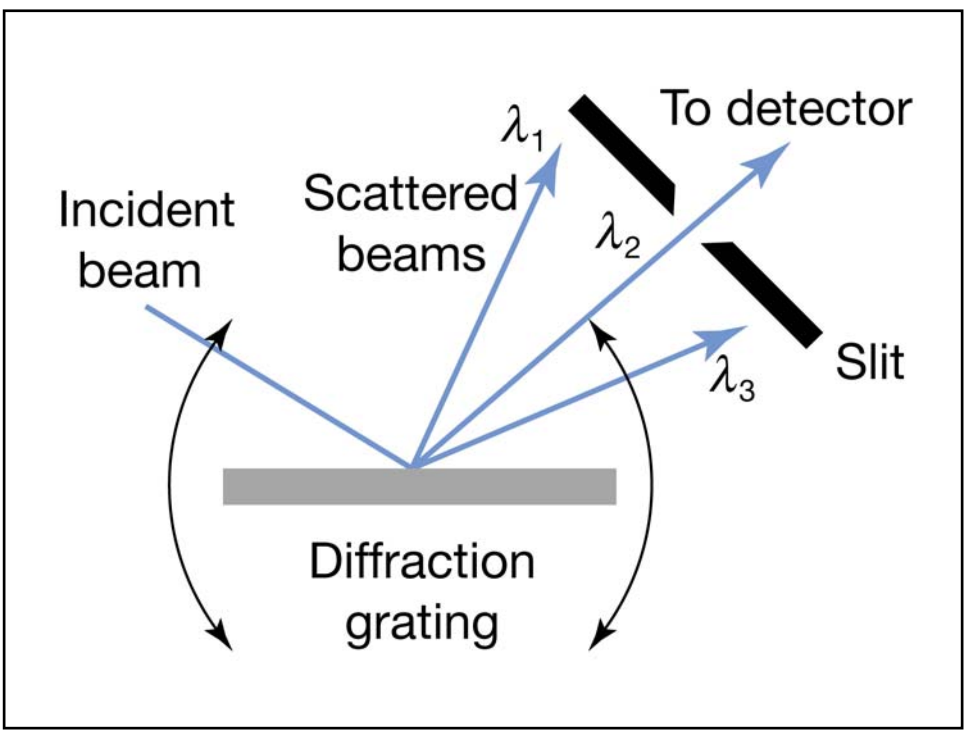


Fig. 16.14. Dispersion of polychromatic light by prism.


Scattered beam
Incident beam
θ
ϕ
Diffraction grating


To detector
λ1
Scattered beams
Incident beam
λ2
Slit
λ3
Diffraction grating

Dispersion by a grating is independent of wavelength, but intensity varies with wavelength.

Gratings are blazed for certain wavelength regions.

Higher order radiation is produced (multiples of the primary, 1st order, radiation).

Radiation at wavelengths shorter than the spectral region must be filtered out to prevent its 2nd order radiation from overlapping the spectral region.

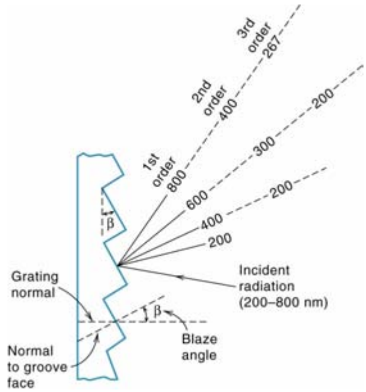
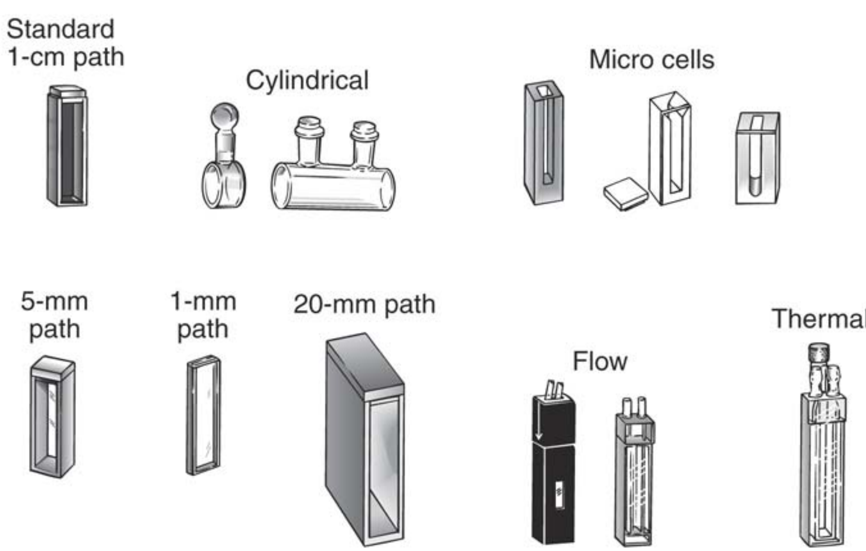


Fig. 16.15. Diffraction radiation from grating.



The standard cell is 1 x 1 cm.

Quartz is used for UV and visible.

Glass and clear plastic are used for visible.

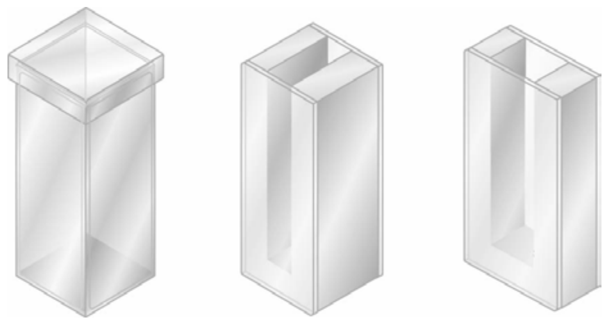
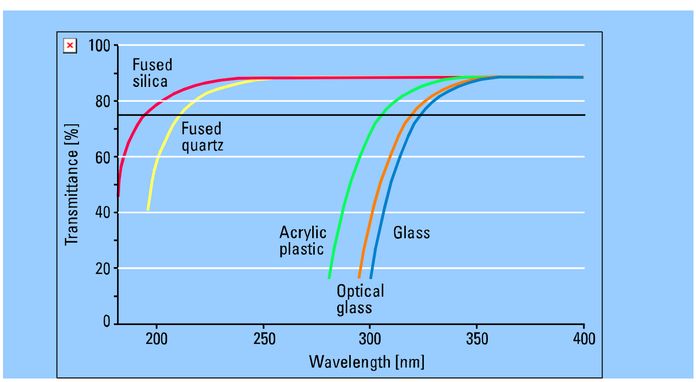


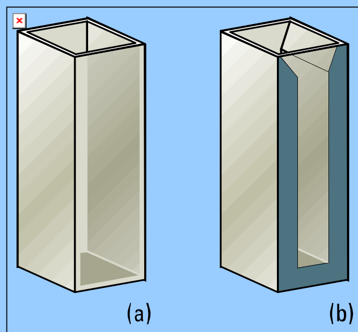
Fig. 16.16 Some typical UV and visible absorption cells.

Transmission Characteristics of Cell Materials



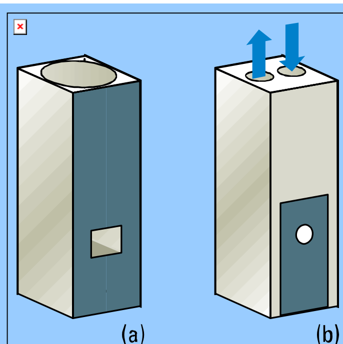
Note that all materials exhibit at least approximately 10% loss in transmittance at all wavelengths

Cell Types I



Open-topped rectangular standard cell (a)
and apertured cell (b) for limited sample volume

Cell Types II



Micro cell (a) for very small volumes
and flow-through cell (b) for automated applications

Salt crystals are used as cell material for the IR region.

NaCl must be protected from moisture, and is polished to remove "fogging".

AgCl can be used for wet samples.

Table 16.6

Properties of Infrared Materials

Material	Useful Range (cm^{-1})	General Properties
NaCl	40,000–625	Hygroscopic, water soluble, low cost, most commonly used material.
KCl	40,000–500	Hygroscopic, water soluble.
KBr	40,000–400	Hygroscopic, water soluble, slightly higher in cost than NaCl and more hygroscopic.
CsBr	40,000–250	Hygroscopic, water soluble.
CsI	40,000–200	Very hygroscopic, water soluble, good for lower wavenumber studies.
LiF	83,333–1425	Slightly soluble in water, good UV material.
CaF_2	77,000–1110	Insoluble in water, resists most acids and alkalis.
BaF_2	67,000–870	Insoluble in water, brittle, soluble in acids and NH_4Cl.
AgCl	10,000–400	Insoluble in water, corrosive to metals. Darkens upon exposure to short-wavelength visible light. Store in dark.
AgBr	22,000–333	Insoluble in water, corrosive to metals. Darkens upon exposure to short-wavelength visible light. Store in dark.
KRS-5	16,600–285	Insoluble in water, highly toxic, soluble in bases, soft, good for ATR work.
ZnS	50,000–760	Insoluble in water, normal acids and bases, brittle.
ZnSe	20,000–500	Insoluble in water, normal acids and bases, brittle.
Ge	5000–560	Brittle, high index of refraction.
Si	83,333–1430 400–30	Insoluble in most acids and bases.
UV quartz	56,800–3700	Unaffected by water and most solvents.
IR quartz	40,000–3000	Unaffected by water and most solvents.
Polyethylene	625–10	Low-cost material for far-IR work.

Adapted from McCarthy Scientific Co. Catalogue 489, with permission.

A short path cell is used with pure substances for qualitative measurements (e.g., 0.01-0.05 mm).

High concentration solutions are usually used since most solvent absorb some in the IR (ca. 0.1 mm pathlength).

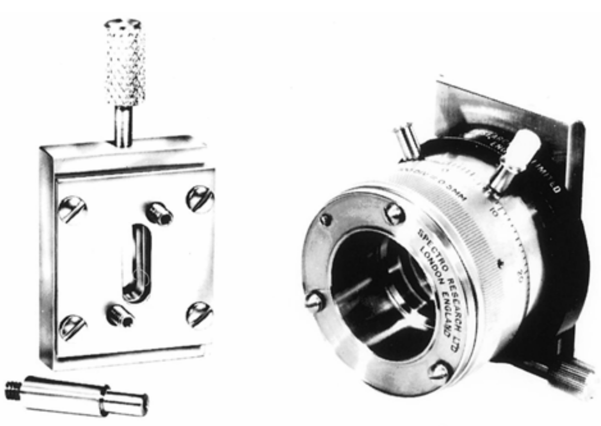
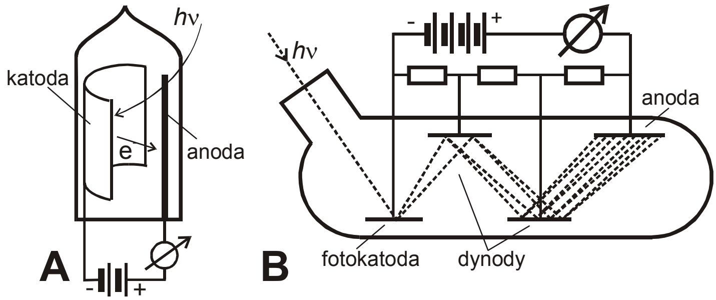
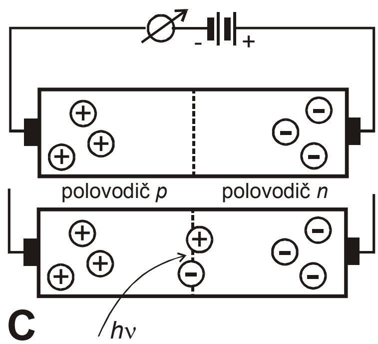
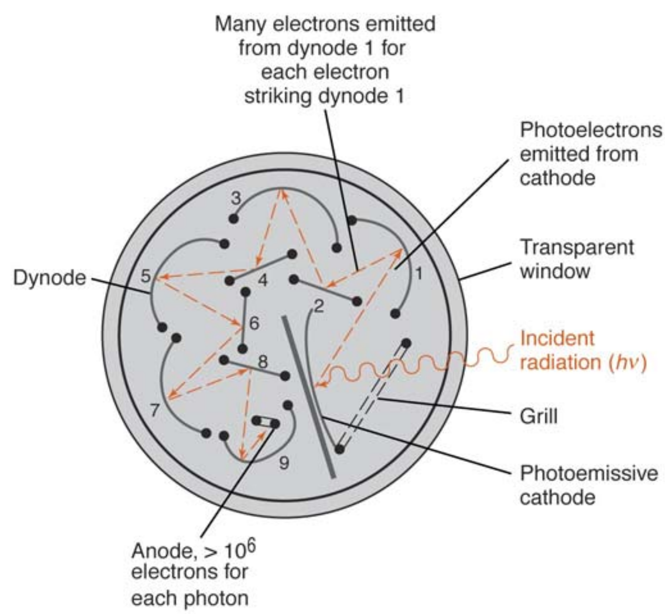


Fig. 16.17. Typical infrared cells. (a) Fixed-path cell. (b) Variable length cell.



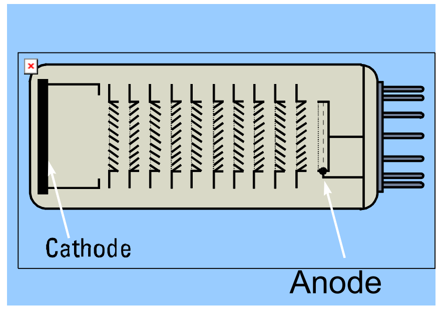


A. Fotonka
B. Fotonásobič
C. Polovodičový detektor



Photomultiplier Tube Detector

- High sensitivity at low light levels
- Cathode material determines spectral sensitivity
- Good signal/noise
- Shock sensitive



PM tubes are sensitive, but different photoemissive surfaces are responsive to different wavelengths.

Einstein received the 1921 Nobel Prize in Physics for explaining the photoelectric effect in 1905.

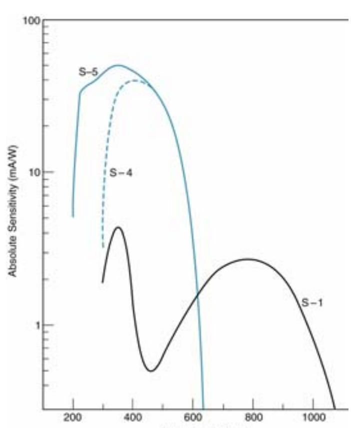
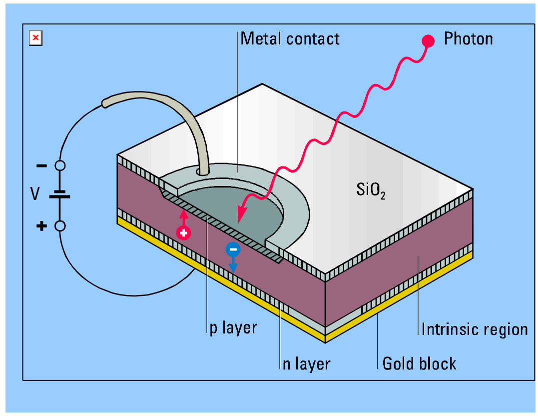


Fig. 16.18. Some spectral responses of photomultipliers. S-5 = RCA 1P28; S-4 = RCA 1P21; S-1 = RCA 7102.

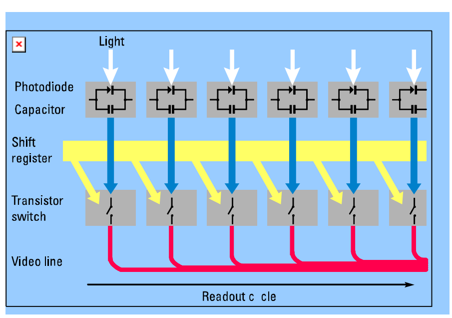
The Photodiode Detector

- Wide dynamic range
- Very good signal/noise at high light levels
- Solid-state device



Schematic Diagram of a Photodiode Array

- Same characteristics as photodiodes
- Solid-state device
- Fast read-out cycles



These detectors allow recording of an entire spectrum at once.

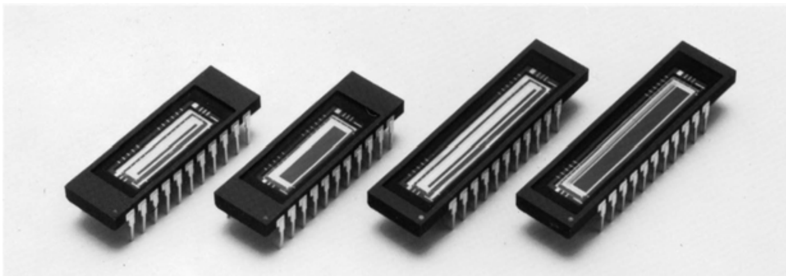


Fig. 16.19. Photo of 1024-element diode arrays.

Diode arrays are useful for UV to IR radiation.

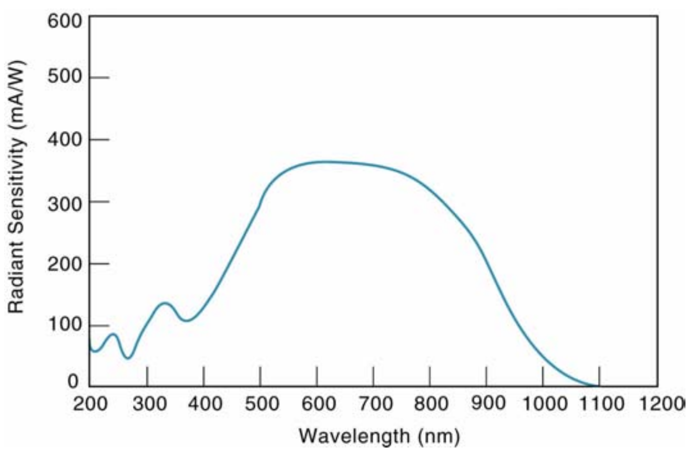
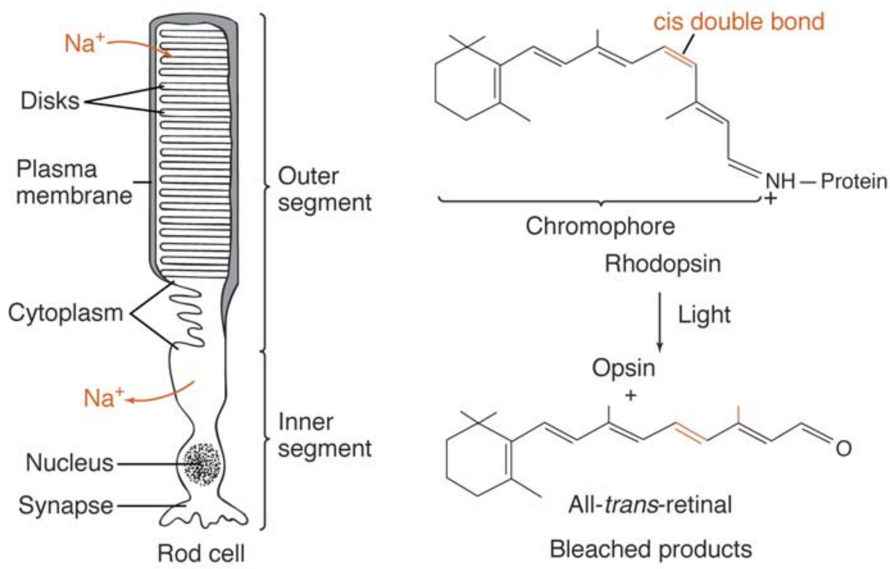
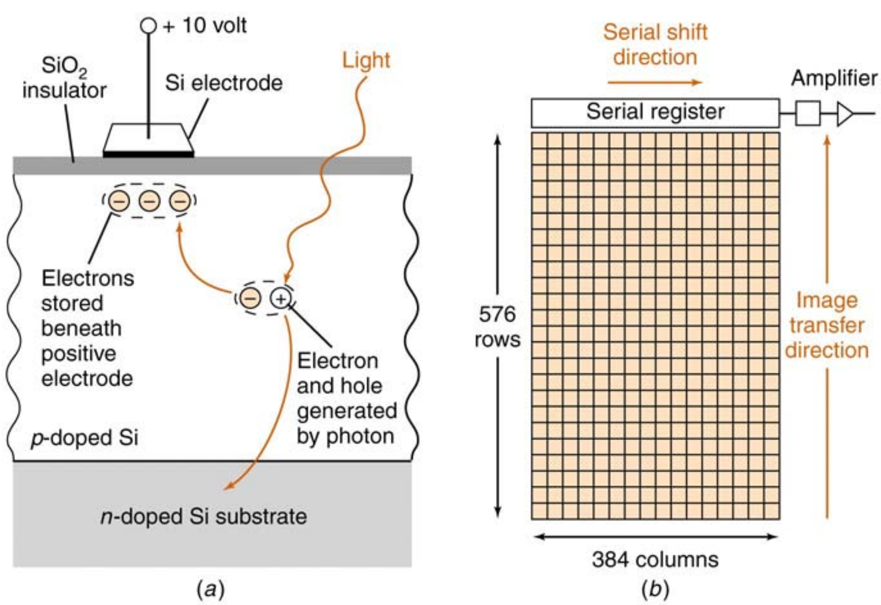


Fig. 16.20. Typical spectral response of diode array.



Charged Coupled Device



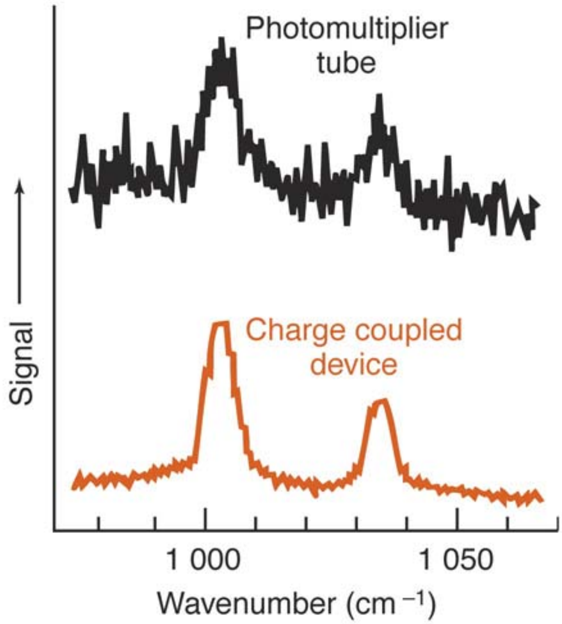
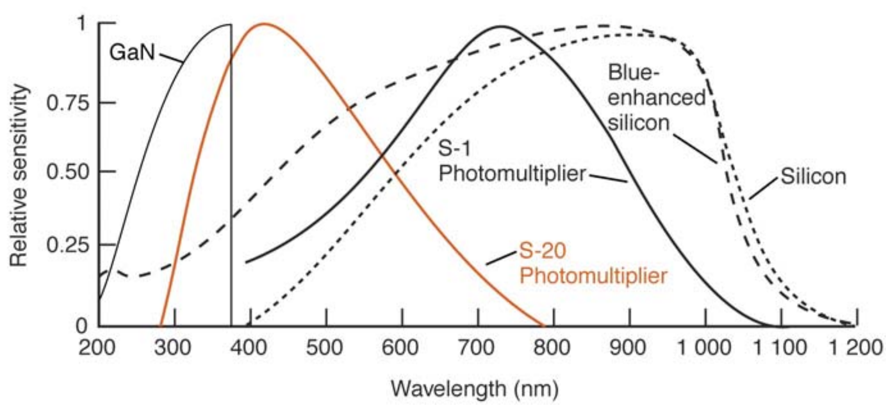
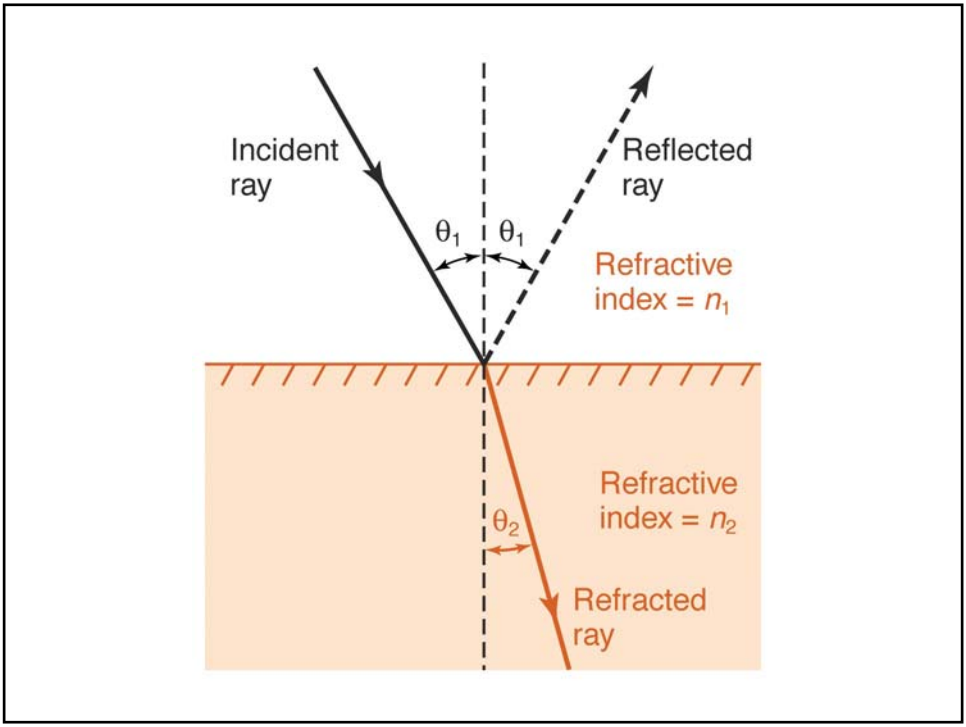


Table 20-2 **Minimum detectable signal (photons/s/detector element) of ultraviolet/visible detectors**

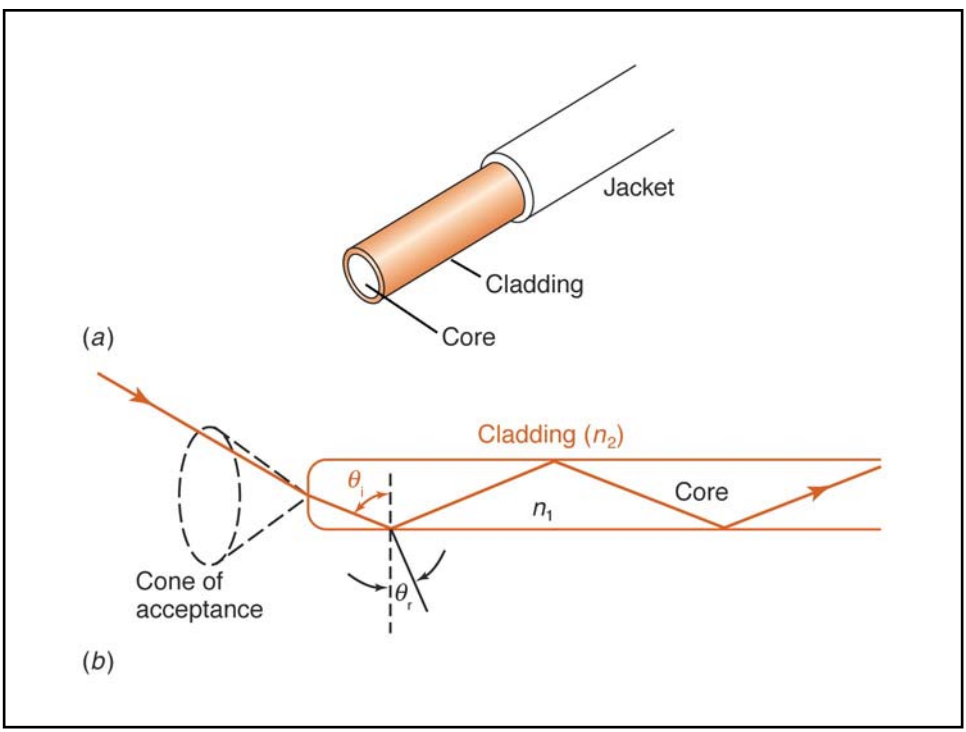
Signal acquisition time (s)	Photodiode array		Photomultiplier tube		Charge coupled device	
	Ultraviolet	Visible	Ultraviolet	Visible	Ultraviolet	Visible
1	6 000	3 300	30	122	31	17
10	671	363	6.3	26	3.1	1.7
100	112	62	1.8	7.3	0.3	0.2

SOURCE: R. B. Bilhorn, J. V. Sweedler, P. M. Epperson, and M. B. Denton, "Charge Transfer Device Detectors for Analytical Optical Spectroscopy," *Appl. Spectros.* **1987**, *41*, 1114.





Incident ray
Reflected ray
θ1
θ1
Refractive index = n1
θ2
Refractive index = n2
Refracted ray



Jacket
Cladding
Core
(a)
Cladding (n2)
θi
n1
Core
θr
Cone of acceptance
(b)

The cladding has a higher refractive index than the core.

The buffer layer is a protective layer.

Light entering at no greater angle than θ_a will be internally reflected and transmitted.

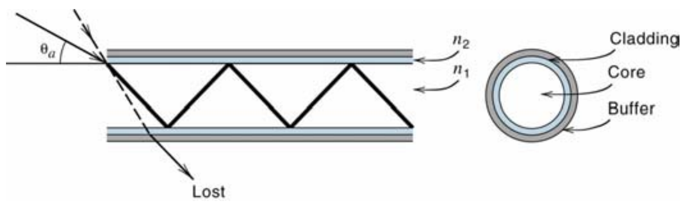
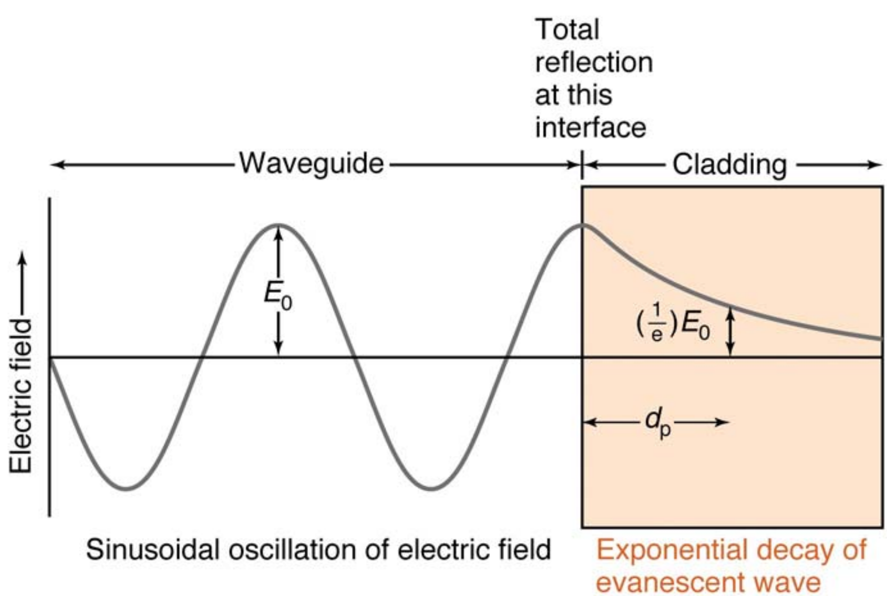
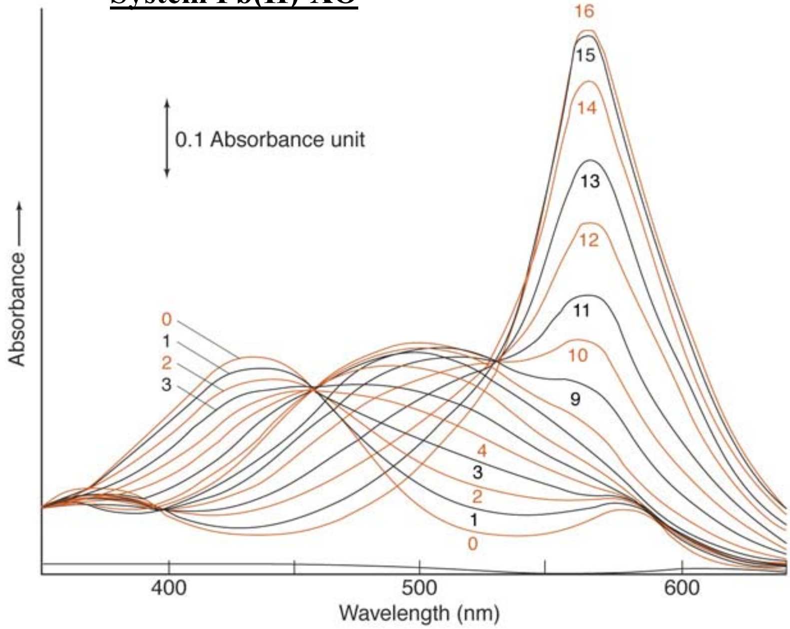
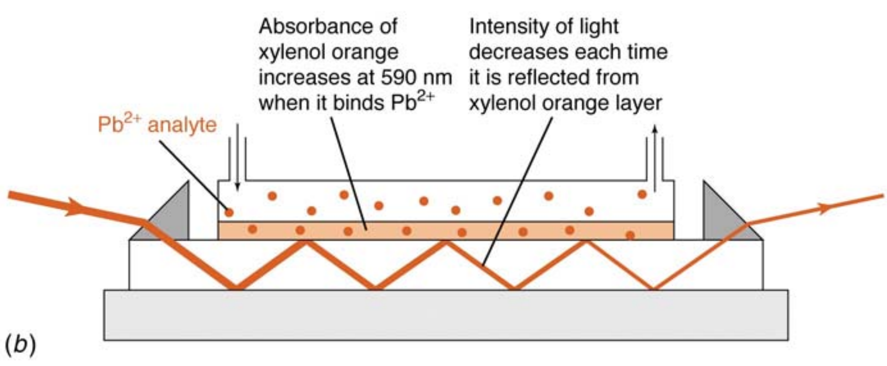


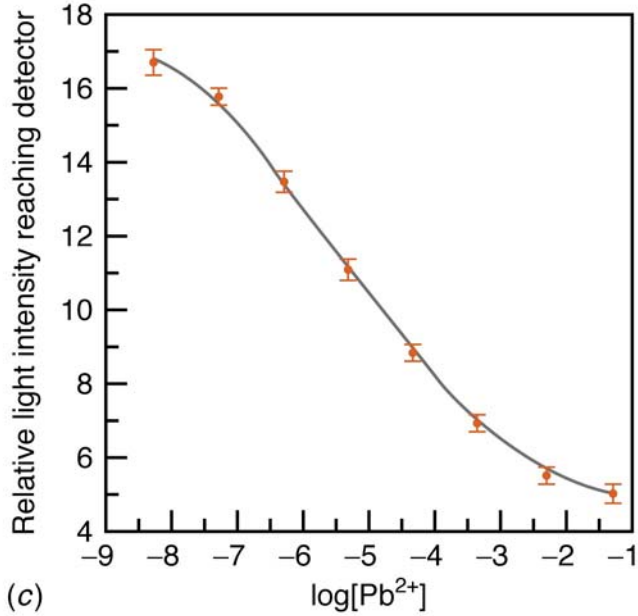
Fig. 16.32. Fiber-optic structure.

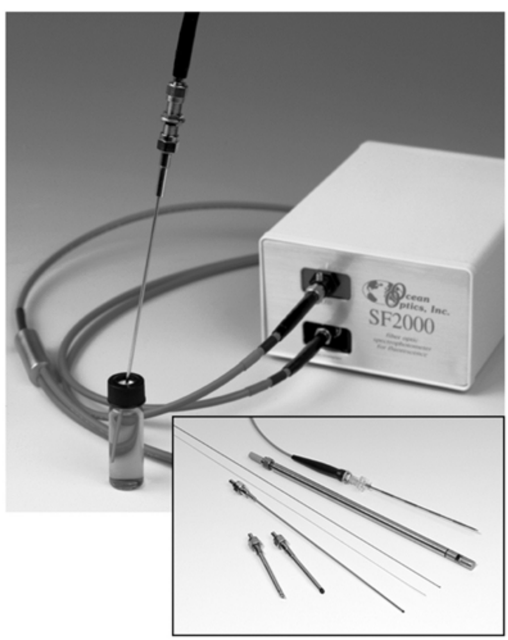


System Pb(II)-XO






Relative light intensity reaching detector
18
16
14
12
10
8
6
4
−9 −8 −7 −6 −5 −4 −3 −2 −1
log[Pb2+]
(c)


Ocean Optics, Inc.
SF2000

The detector is a 2048-element charge-couple device (CCD).

The light from the fiber optic cable is dispersed across the array via a fixed grating.

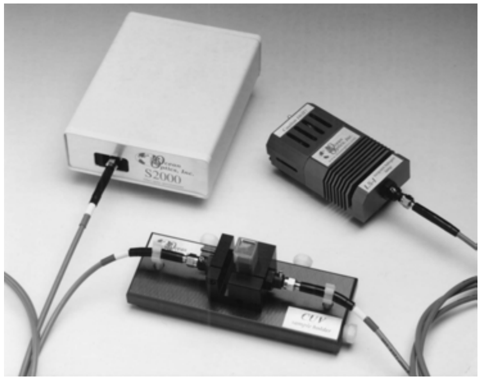
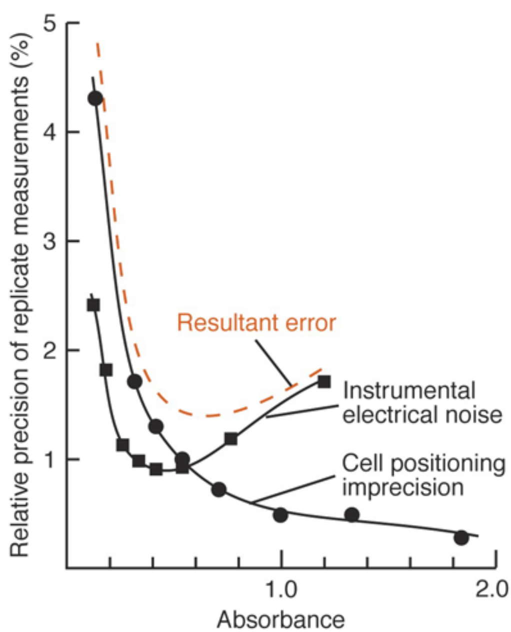


Fig. 16.34. Miniature fiber spectrometer. Box is the spectrometer. Light source is to right, and fiber-optic cable guides light to cuvet. Second cable takes transmitted light to spectrometer.



It is difficult to precisely measure either very small or very large decreases in absorbance.

For thermal-noise limited detectors (as used in IR), the error is minimum for A = 0.434; working range 0.1-1 A.

For shot-noise limited phototube and photomultiplier detectors, the error is minimum at A = 0.87; working range is 0.1-1.5 A.

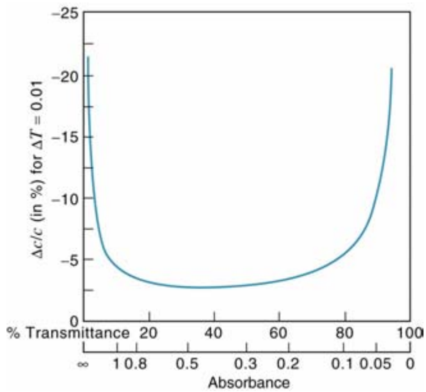


Fig. 16.27. relative concentration error as function of transmittance for 1% uncertainy in %T.

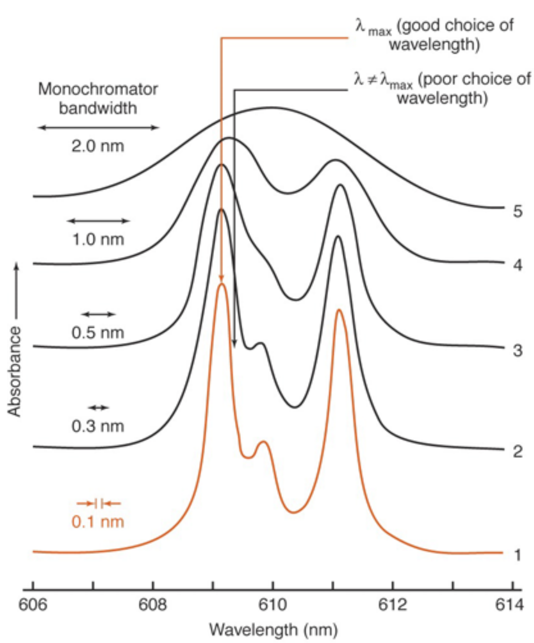
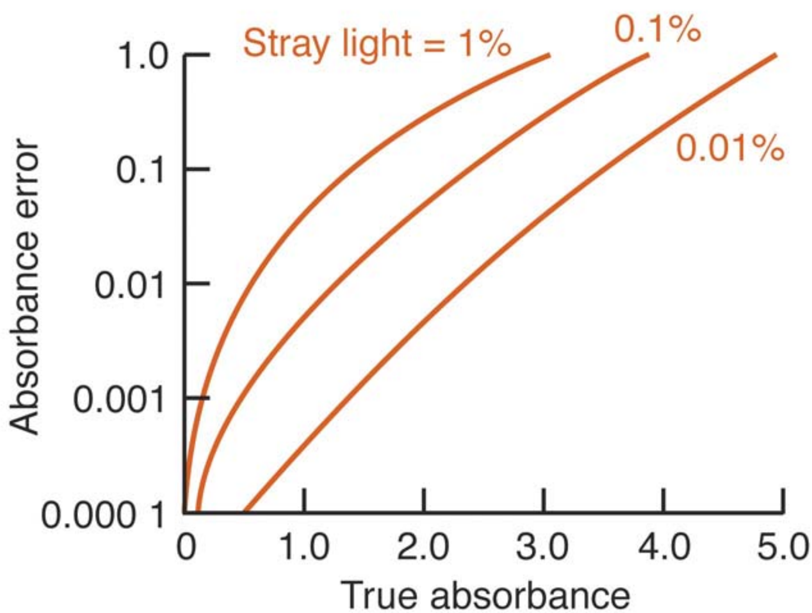


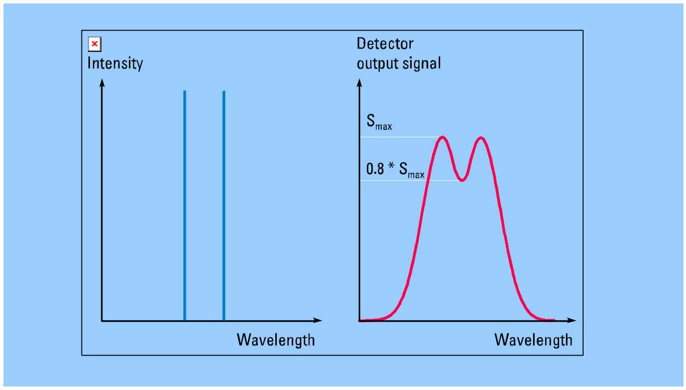
Table 20-1 **Calibration standard for ultraviolet absorbance**

H_2SO_4 Wavelength (nm)	Absorbance of $K_2Cr_2O_7$ (60.06 mg/L) in 5.0 mM in 1-cm cell
235	0.748 ± 0.010
257	0.865 ± 0.010
313	0.292 ± 0.010
350	0.640 ± 0.010

SOURCE: S. Ebel, "Validation of Analysis Methods," *Fresenius J. Anal. Chem.*

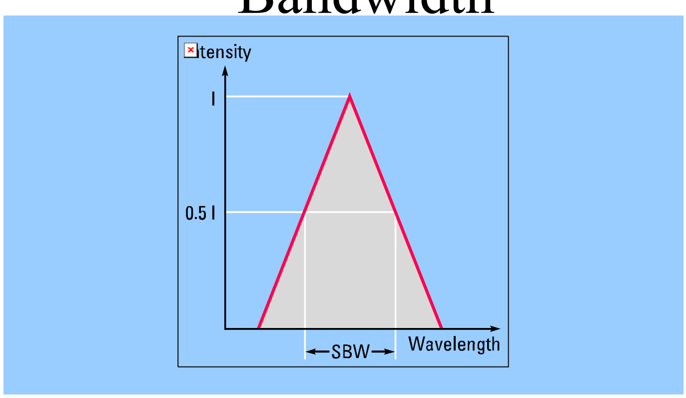


Definition of Resolution



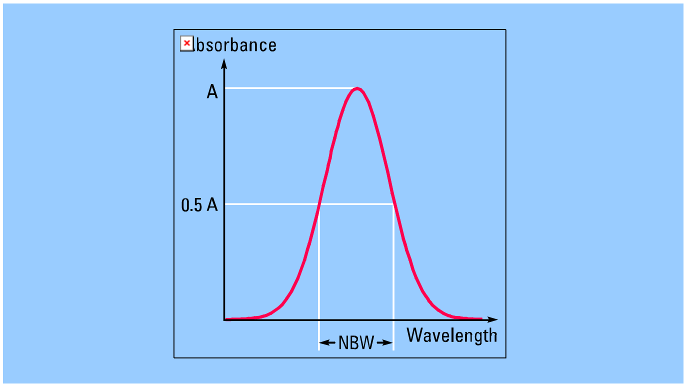
Spectral resolution is a measure of the ability of an instrument to differentiate between two adjacent wavelengths

Instrumental Spectral Bandwidth



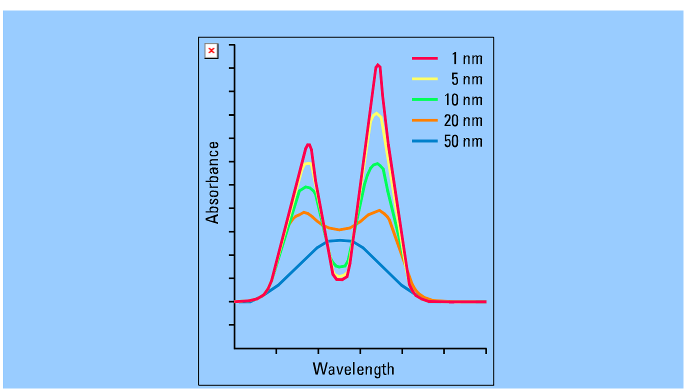
The SBW is defined as the width, at half the maximum intensity, of the band of light leaving the monochromator

Natural Spectral Bandwidth



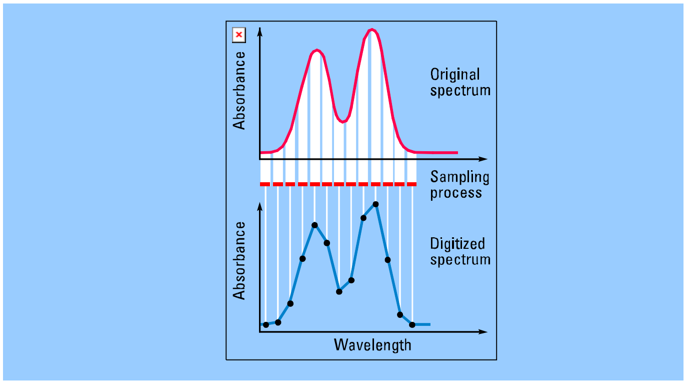
The NBW is the width of the sample absorption band at half the absorption maximum

Effect of SBW on Band Shape



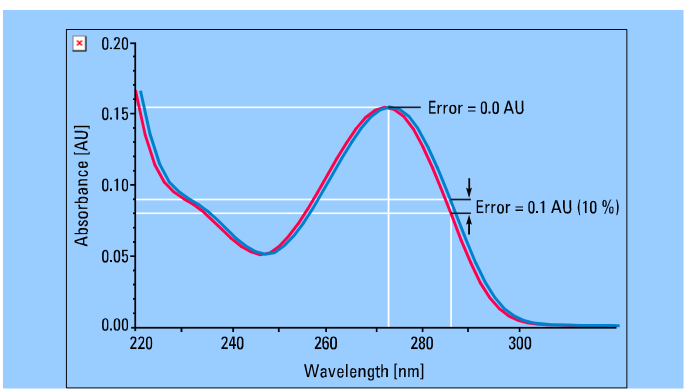
The SBW/NBW ratio should be 0.1 or better to yield an absorbance measurement with an accuracy of 99.5% or better

Effect of Digital Sampling



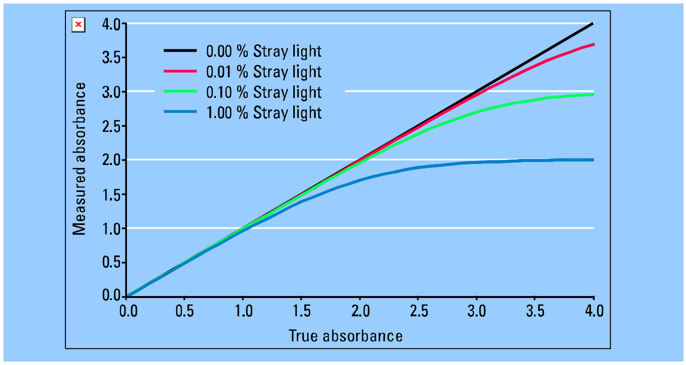
The sampling interval used to digitize the spectrum for computer evaluation and storage also effects resolution

Wavelength Resettability



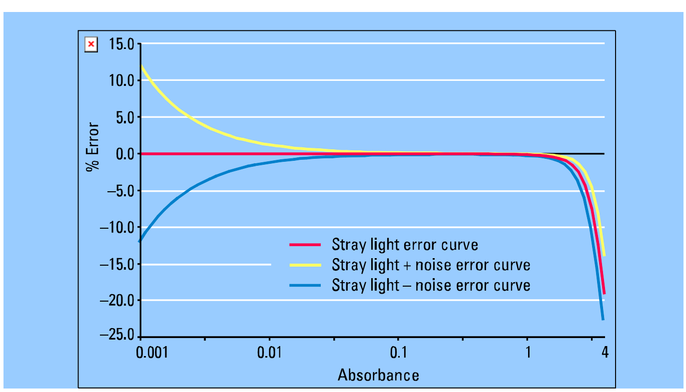
Influence of wavelength resettability on measurements at the maximum and slope of an absorption band

Effect of Stray Light



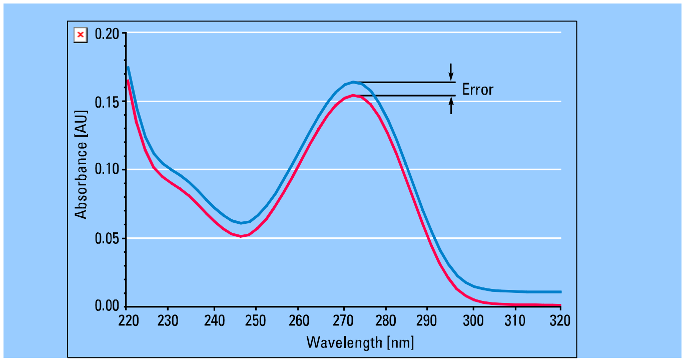
Effect of various levels of stray light on measured absorbance compared with actual absorbance

Theoretical Absorbance Error



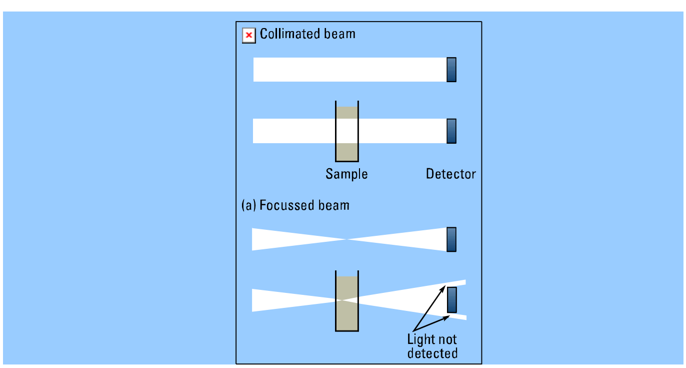
The total error at any absorbance is the sum of the errors due to stray light and noise (photon noise and electronic noise)

Effect of Drift



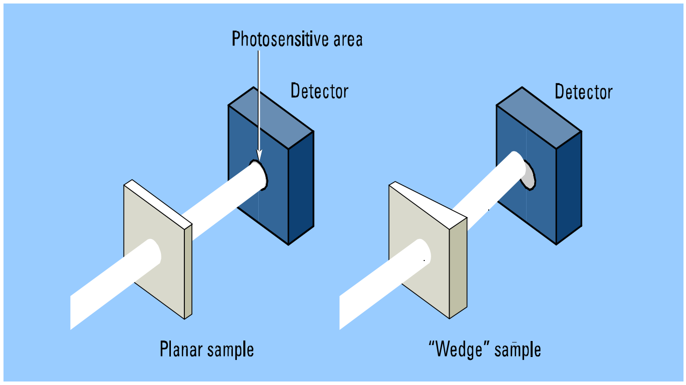
Drift is a potential cause of photometric error and results from variations between the measurement of I_0 and I

Effect of Refractive Index



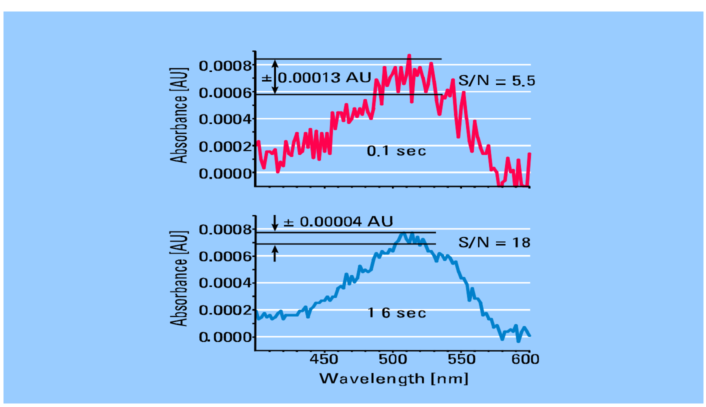
Changes in the refractive index of reference and sample measurement can cause wrong absorbance measurements

Non-planar Sample Geometry



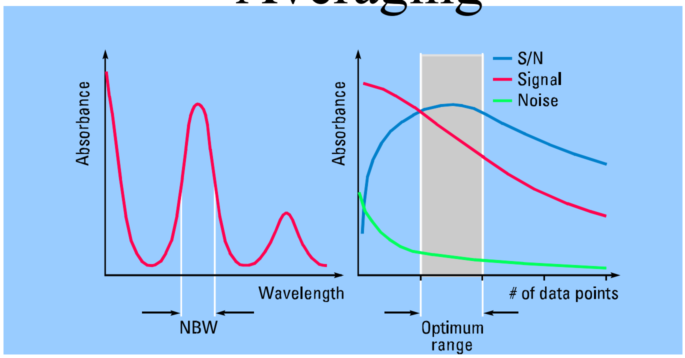
Some sample can act as an active optical component in the system and deviate or defocus the light beam

Effect of Integration Time



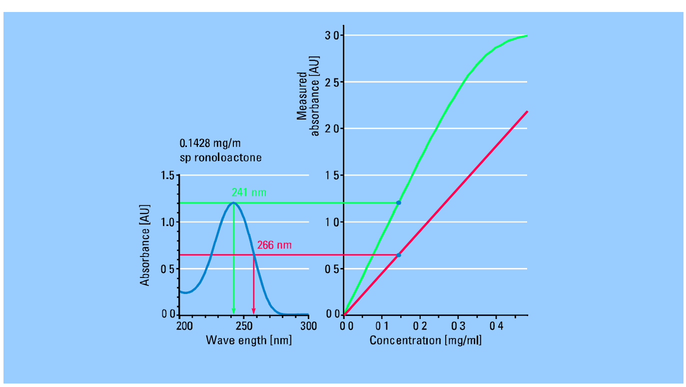
Averaging of data points reduces noise by the square root of the number of points averaged

Effect of Wavelength Averaging



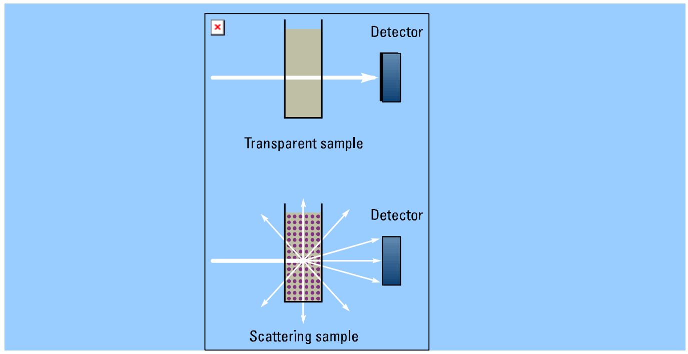
• Wavelength averaging reduces also the noise (square root of data points)
• Amplitude of the signal is affected

Increasing Dynamic Range



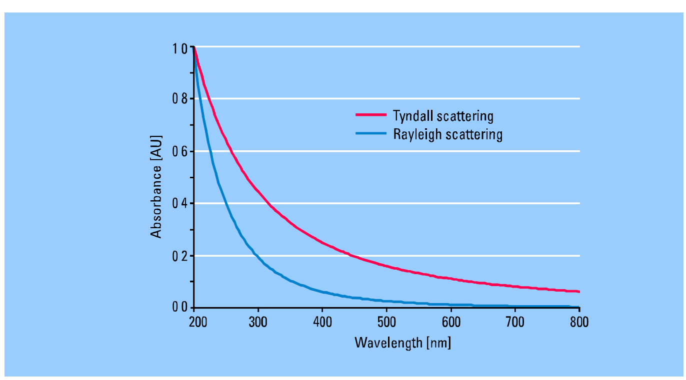
Selection of a wavelength in the slope of a absorption band can increase the dynamic range and avoid sample preparation like dilution

Scattering



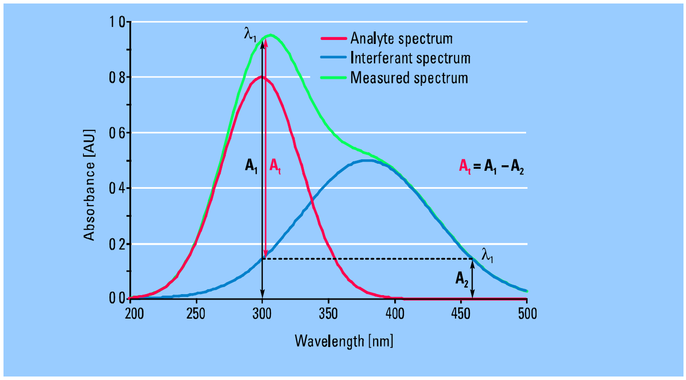
Scattering causes an apparent absorbance because less light reaches the detector

Scatter Spectra



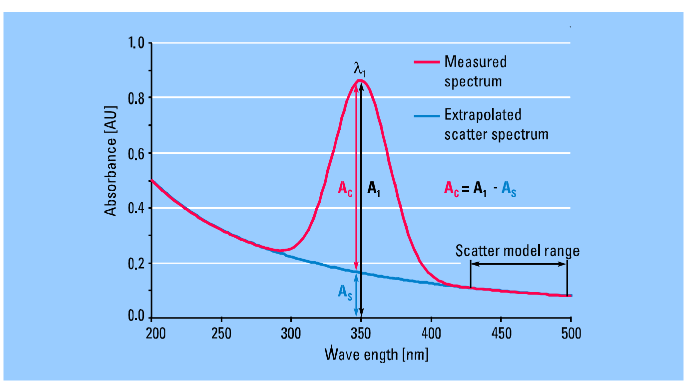
- Rayleigh scattering: Particles small relative to wavelength
- Tyndall scattering: Particles large relative to wavelength

Isoabsorbance Corrections



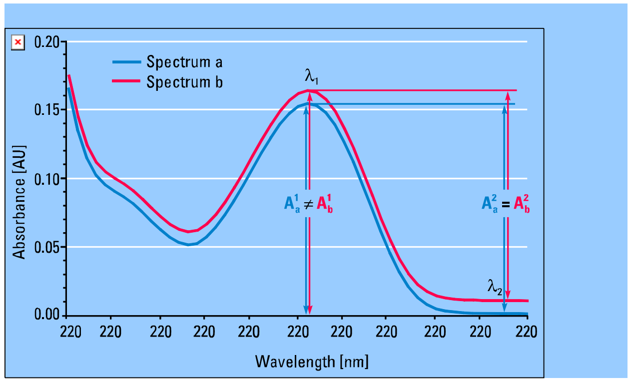
Absorbance at the reference wavelength must be equivalent to the interference at the analytical wavelength

Background Modeling



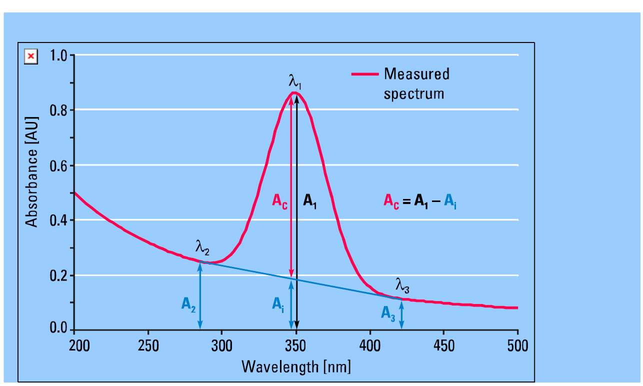
Background modeling can be done if the interference is due to a physical process

Internal Referencing



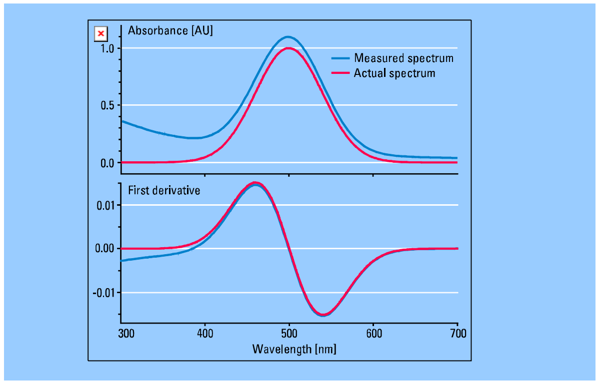
Corrects for constant background absorbance over a range

Three-Point Correction



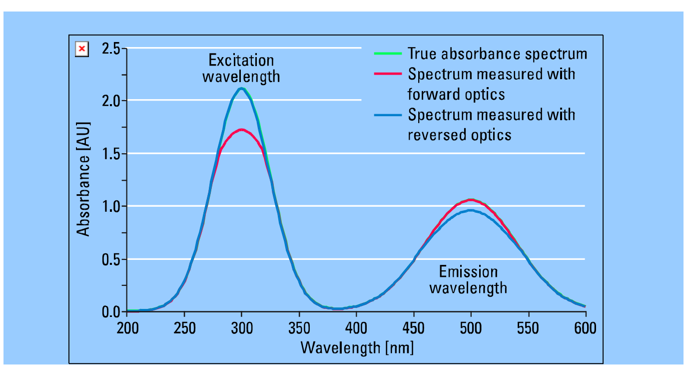
- Uses two reference wavelengths
- Corrects for sloped linear background absorbance

Scatter Correction by Derivative Spectroscopy



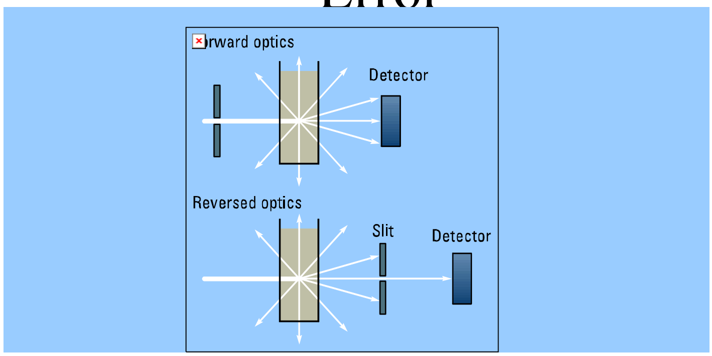
Scatter is discriminated like a broad-band absorbance band

Effect of Fluorescence



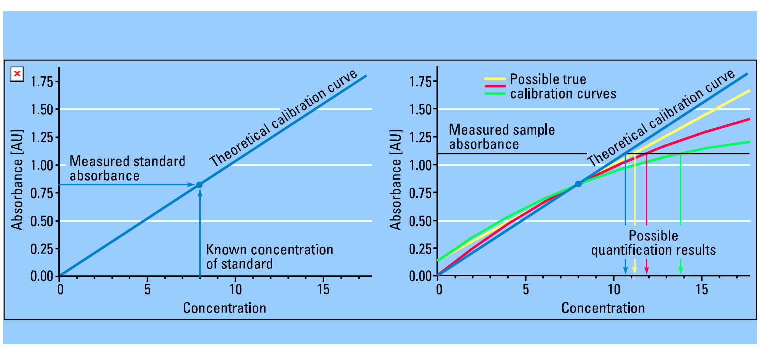
The emitted light of a fluorescing sample causes an error in the absorbance measurement

Acceptance Angles and Magnitude of Fluorescence Error



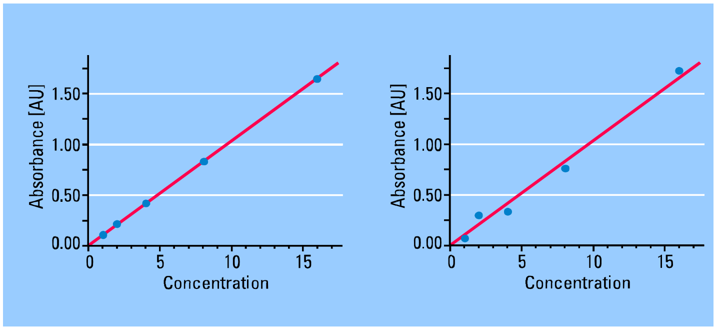
- Forward optics: Absorbance at the excitation wavelengths are too low
- Reversed optics: Absorbance at the emission wavelengths are too low

Inadequate Calibration



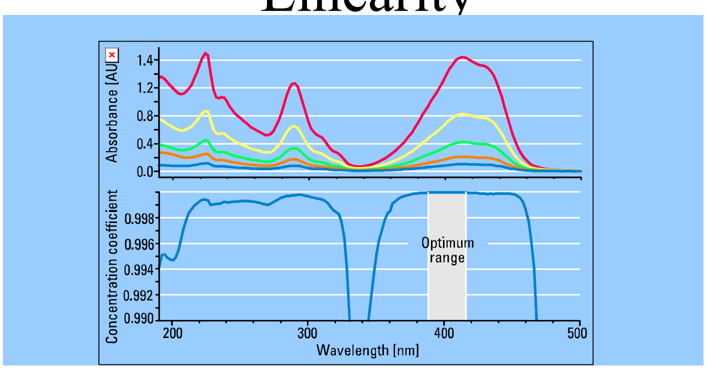
- Theoretically only one standard is required to calibrate
- In practice, deviations from Beer's law can cause wrong results

Calibration Data Sets



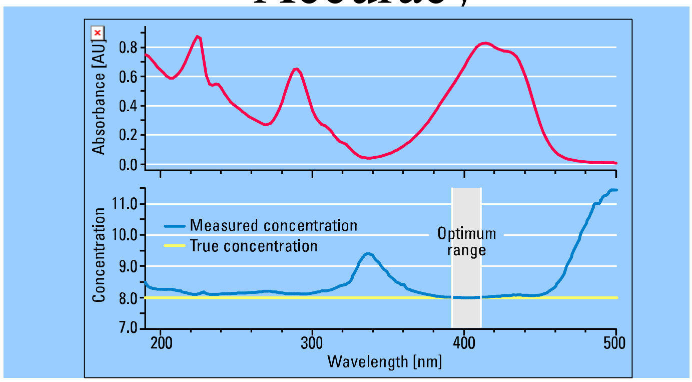
- Forward optics: Absorbance at the excitation wavelengths are too low
- Reversed optics: Absorbance at the emission wavelengths are too low

Wavelength(s) for Best Linearity



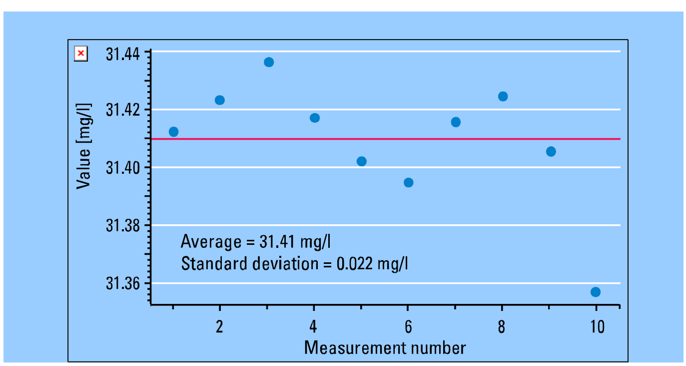
- A linear calibration curve is calculated at each wavelength
- The correlation coefficient gives an estimate on the linearity

Wavelength(s) for Best Accuracy



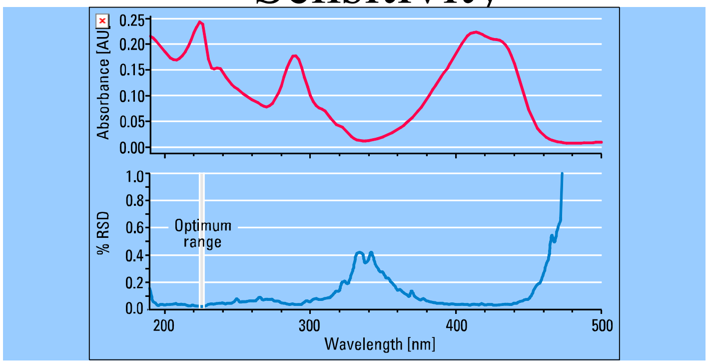
- The quantification results are calculated at each wavelength
- The calculated concentration are giving an estimate of the accuracy

Precision of an Analysis



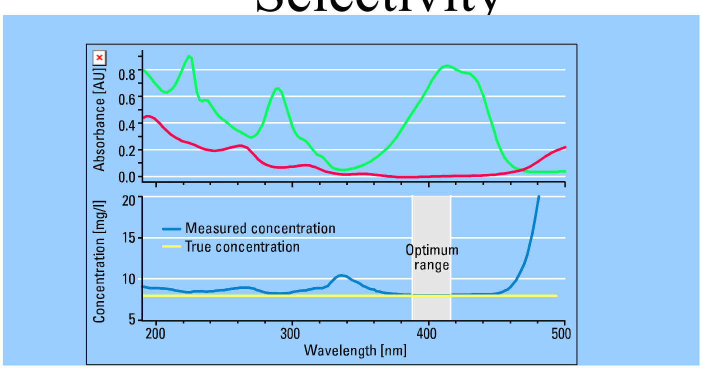
Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings

Wavelength(s) for Best Sensitivity



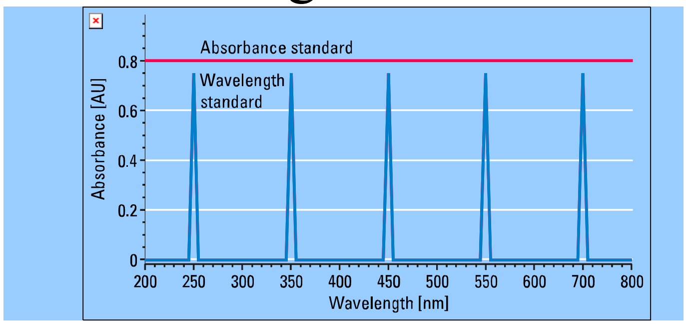
- Calculation of relative standard deviation of the measured values at each wavelength
- The wavelength with lowest %RSD likely will yield the best sensitivity

Wavelength(s) for Best Selectivity



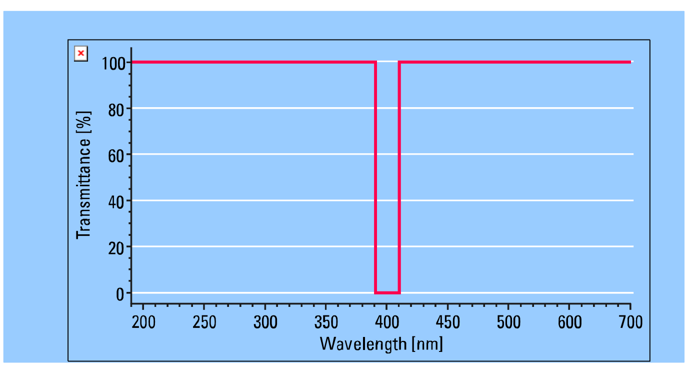
Selectivity is the ability of a method to quantify accurately and specifically the analyte or analytes in the presence of other compounds

Ideal Absorbance and Wavelength Standards



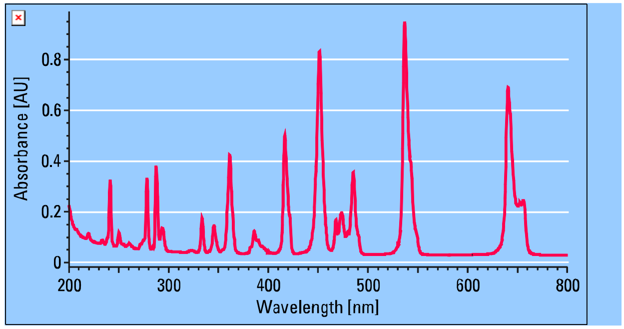
- An ideal absorbance standard would have a constant absorbance at all wavelengths
- An ideal wavelength standard would have very narrow, well-defined peaks

Ideal Stray Light Filter



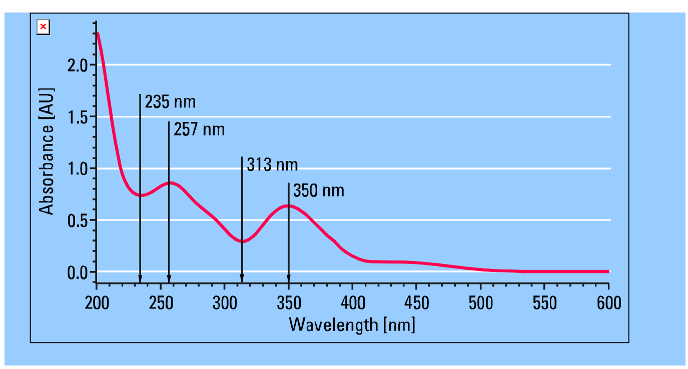
An ideal stray light filter would transmit all wavelengths except the wavelength used to measure the stray light

Holmium Perchlorate Solution



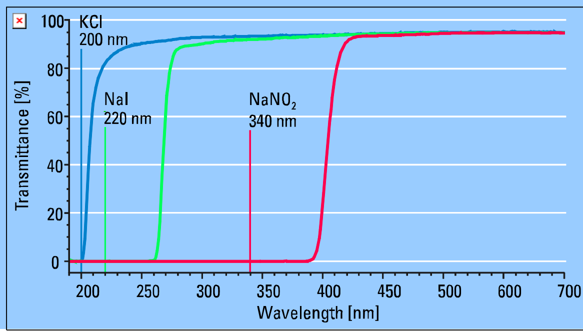
The most common wavelength accuracy standard is a holmium perchlorate solution

Potassium Dichromate Solution



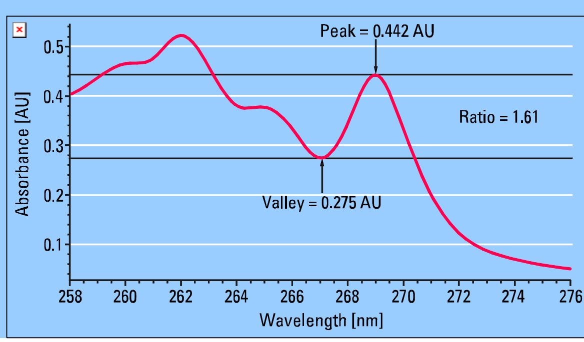
The photometric accuracy standard required by several pharmacopoeias is a potassium dichromate solution

Stray Light Standard Solutions



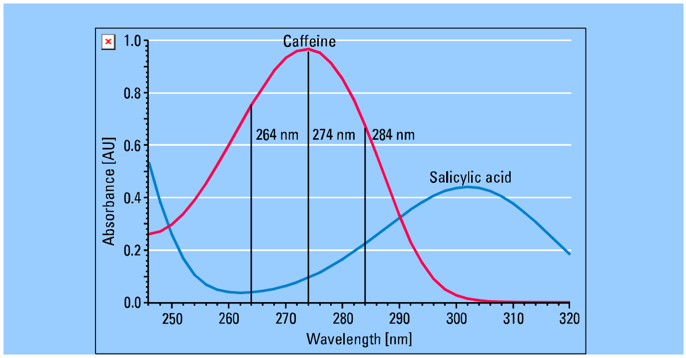
The most common stray light standard and the respectively used wavelengths

Toluene in Hexane (0.02% v/v)



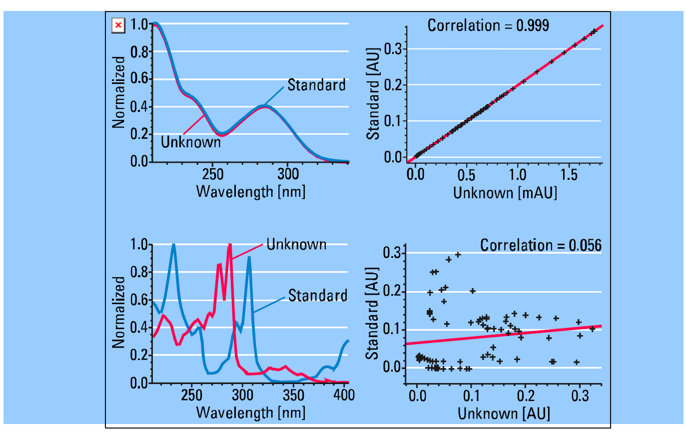
The resolution is estimated by taking the ratio of the absorbance of the maximum near 269 nm and minimum near 266 nm

Confirmation Analysis



In confirmation analysis, the absorbance at one or more additional wavelengths are used to quantify a sample

Spectral Similarity



Comparative plots of similar and dissimilar spectra