The folowing four “Rules-Of-Thumb” summarize the more important aspects of electronic absorption:

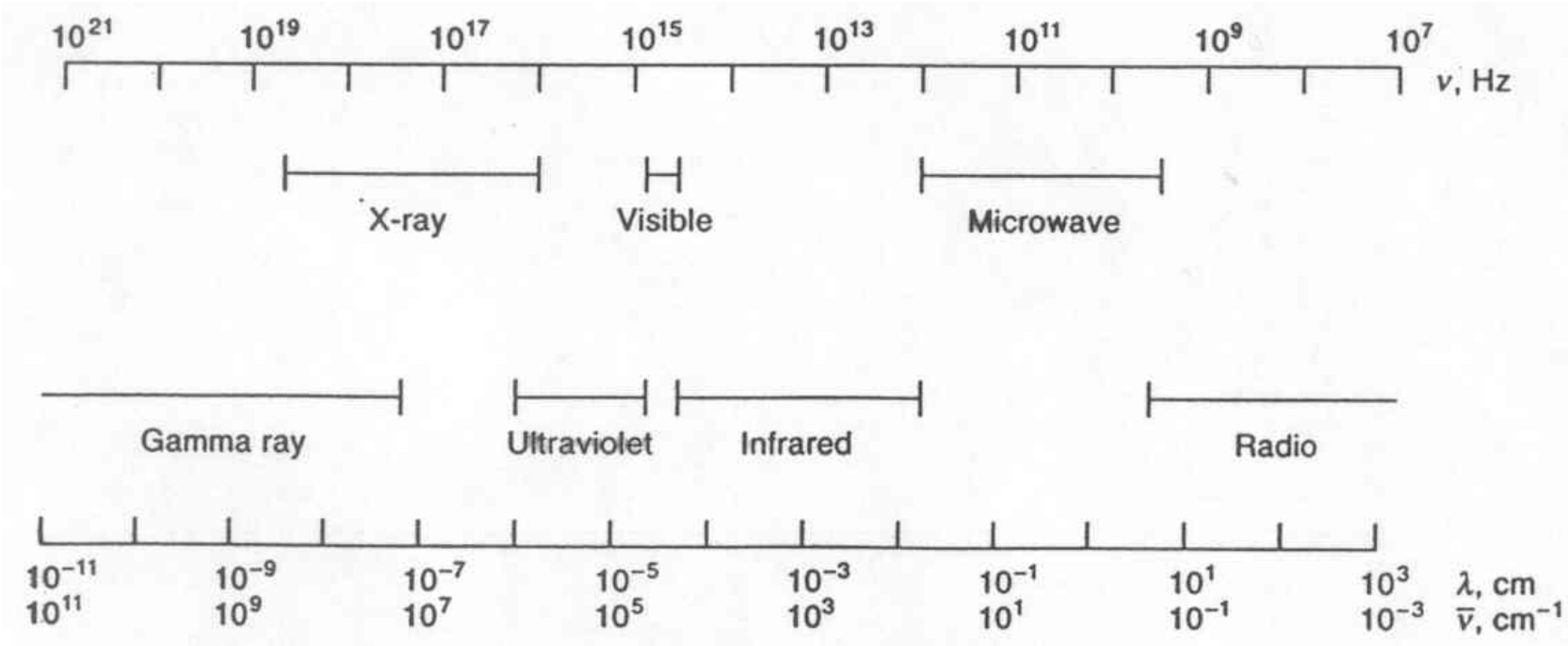
1. The amount of energy absorbed by an electron equals the quanta of energy of the photon absorbed.

2. Ground states and excited states are populated by rotational and vibrational substrates of the electron.

3. Rotational and vibrational absorbance by molecules occurs in the infrared and far infrared region of the spectrum.

4. Electronic absorbance that is useful to the spectroscopist occurs between 190 nm and 900 nm.

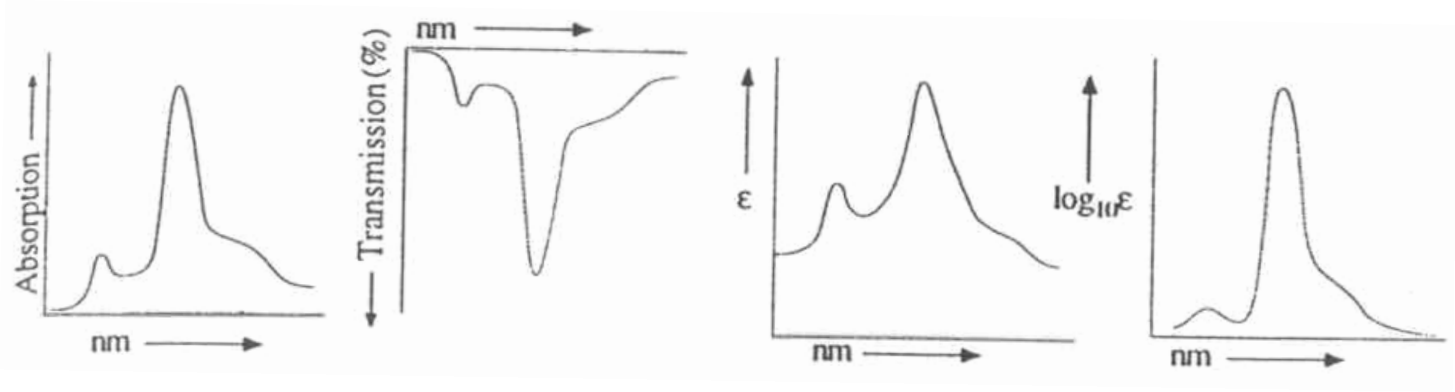
Figure 1: Regions of the electromagnetic spectrum



KVANTITATIVNA UV-VIS SPEKTROSKOPIJA

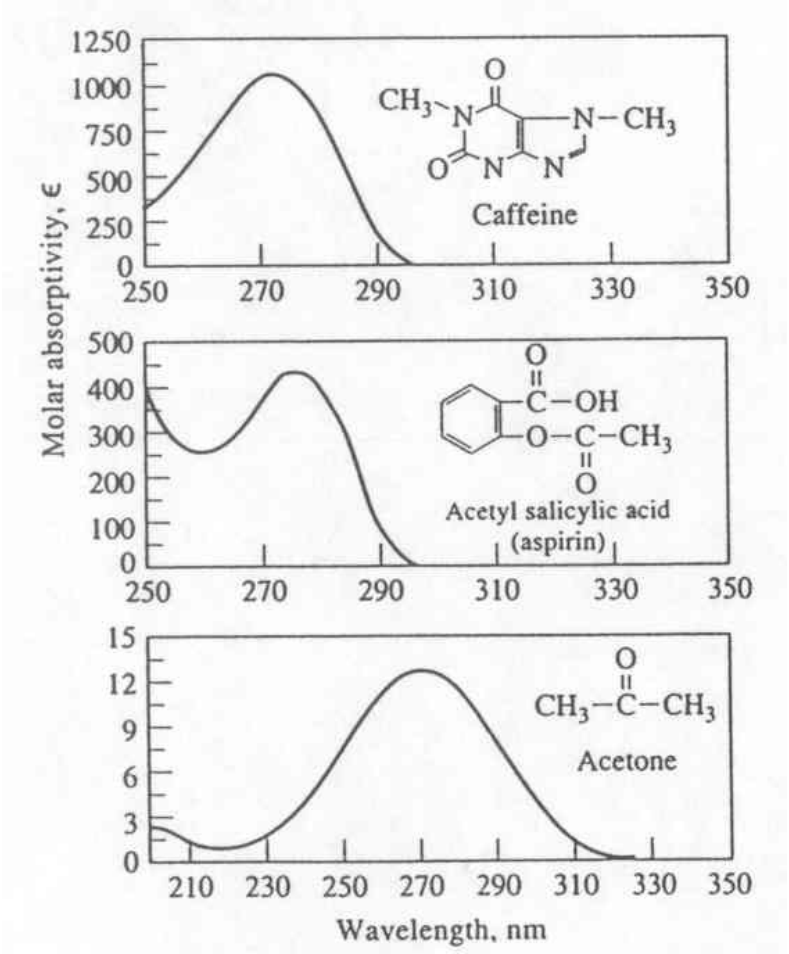
I. SPLOŠNO

- **Spektroskopija**
- **Splošne lastnosti e-m sevanja in parametri**
- **Elektromagnetni spekter**
- **Absorpcija svetlobe in absorpcijski spektri**



- **UV-VIS molekularna absorpcijska spektroskopija**

- **Kvalitativne aplikacije**



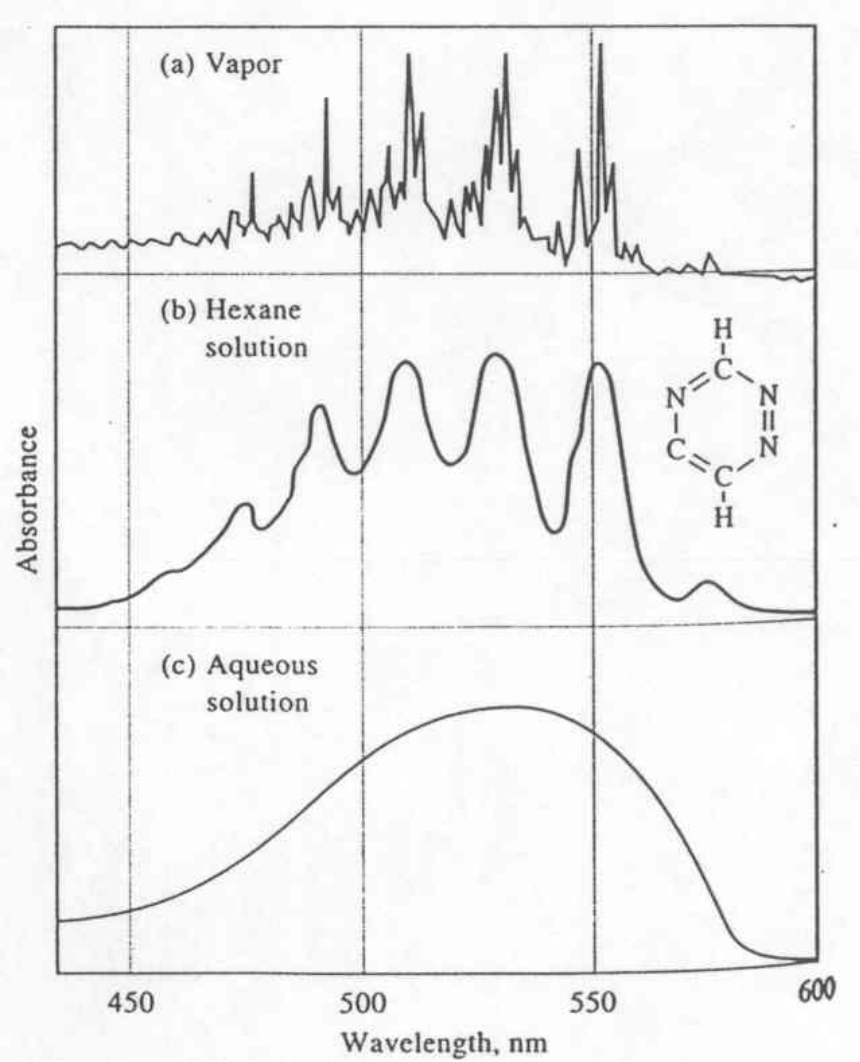
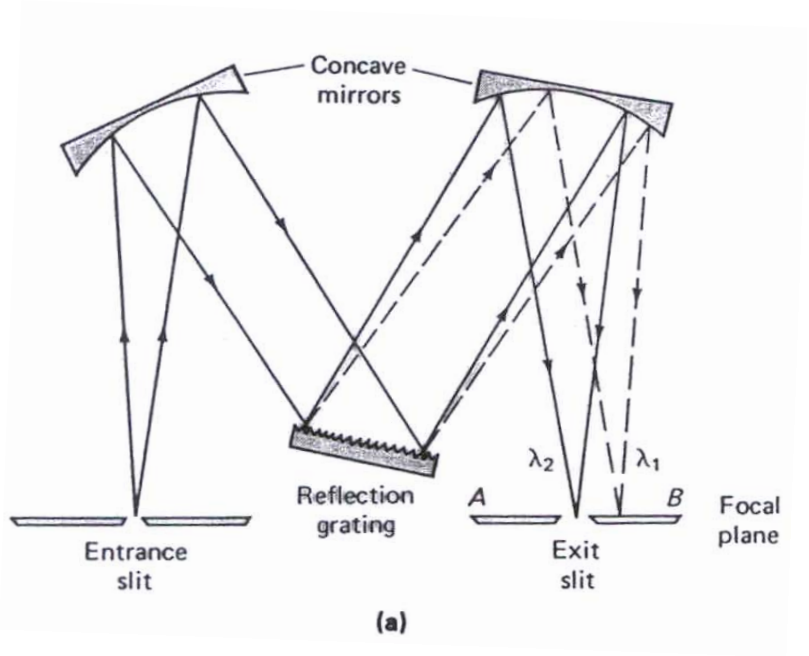
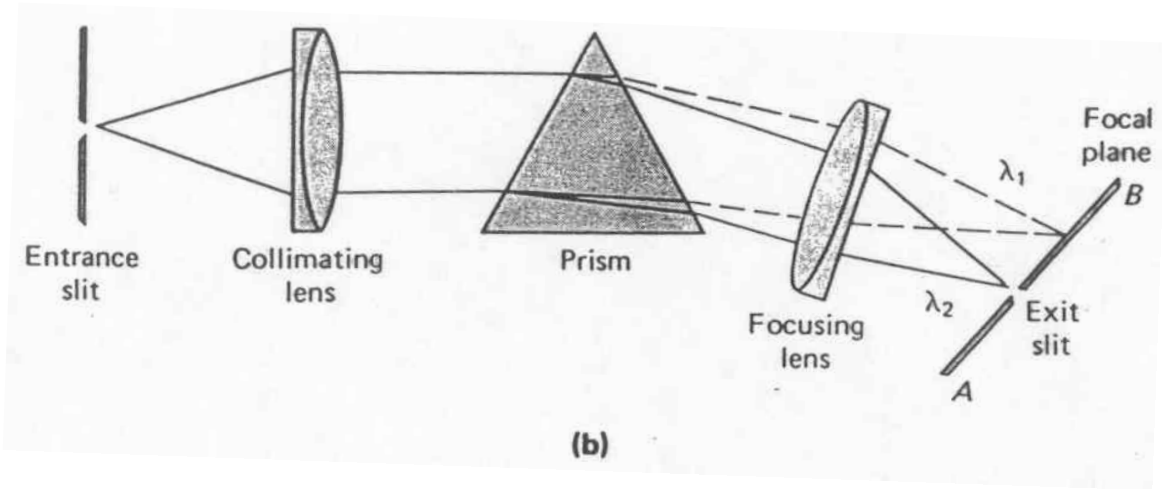


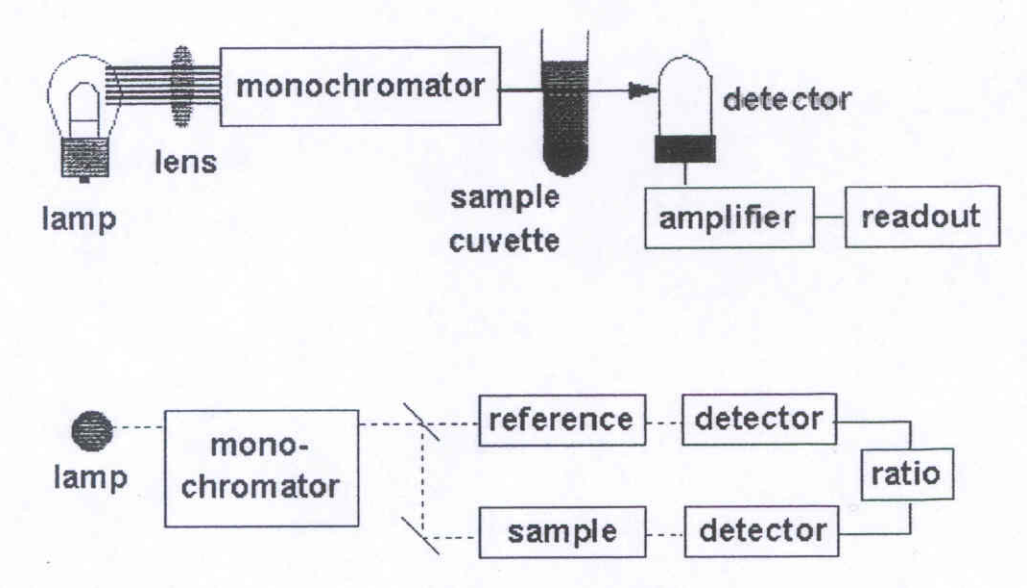
Figure 2: Two types of monochromators: (a) Czerney - Turner grating Monochromator; (b) Bunsen prism monochromator (in both instances, $\lambda_1 > \lambda_2$)

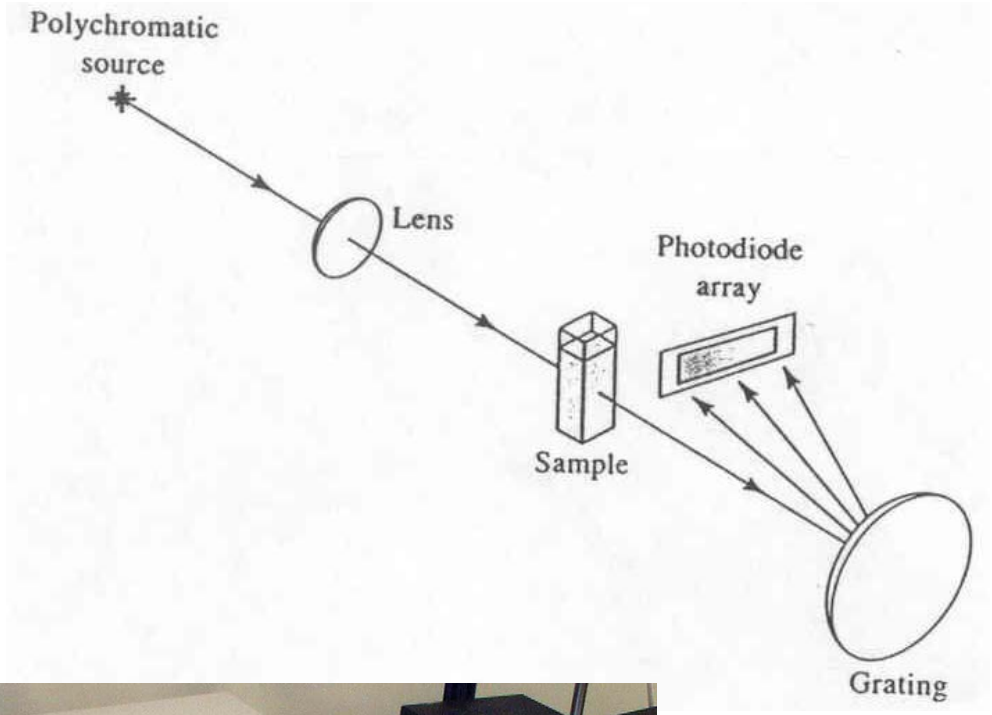




II. KVANTITATIVNE APLIKACIJE

1. **Značilnosti**
2. **Uporabnost**
3. **Tipični instrumenti**




Polychromatic
source
Lens
Photodiode
array
Sample
Grating

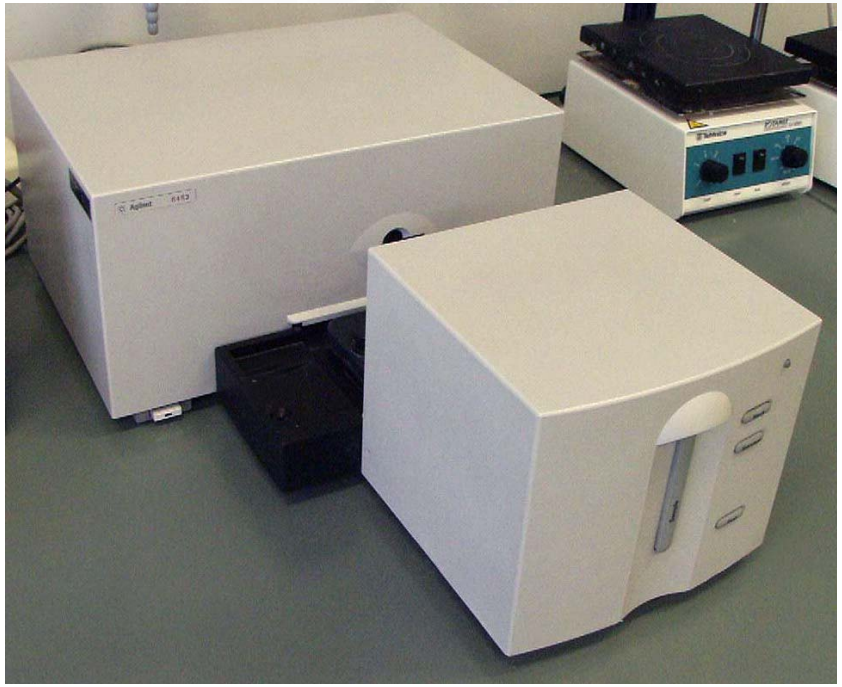
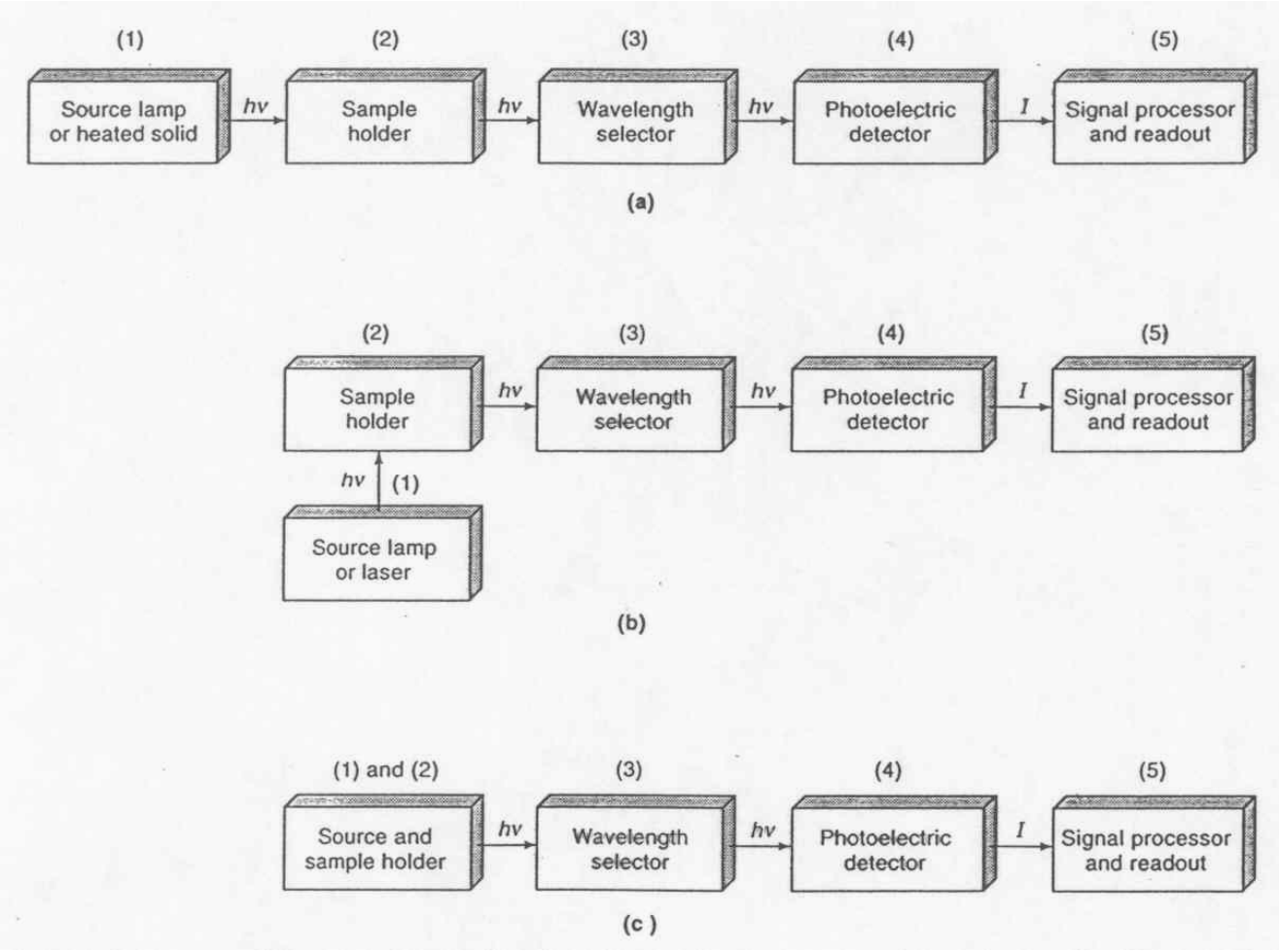


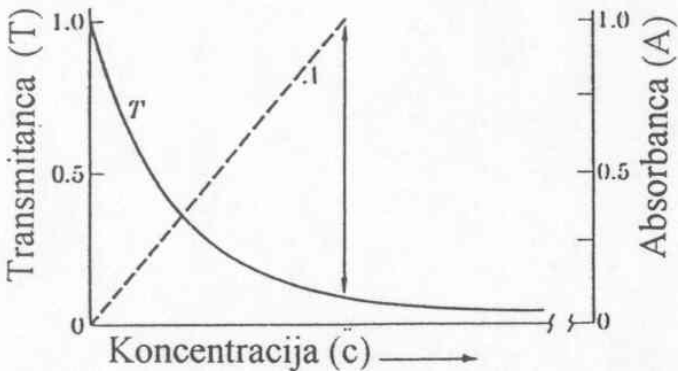
Figure 3: Components of various types of instruments for optical spectroscopy: (a) absorption; (b) fluorescence, phosphorescence, and scatering; (c) emission and chemiluminescence



4. Kvantitativne mere oslabitve svetlobnega žarka

Transmitanca: $T = P/P_0$

Absorbanca: $A = - \log T = \log P_0/P$



5. Beer – Lambert-ov zakon

$A = a \cdot b \cdot c$ a [L · g^{-1} · cm^{-1}] ..... absorptivnost

$A = \varepsilon \cdot b \cdot c$ ε [L · mol^{-1} · cm^{-1}]..... molarna absorptivnost

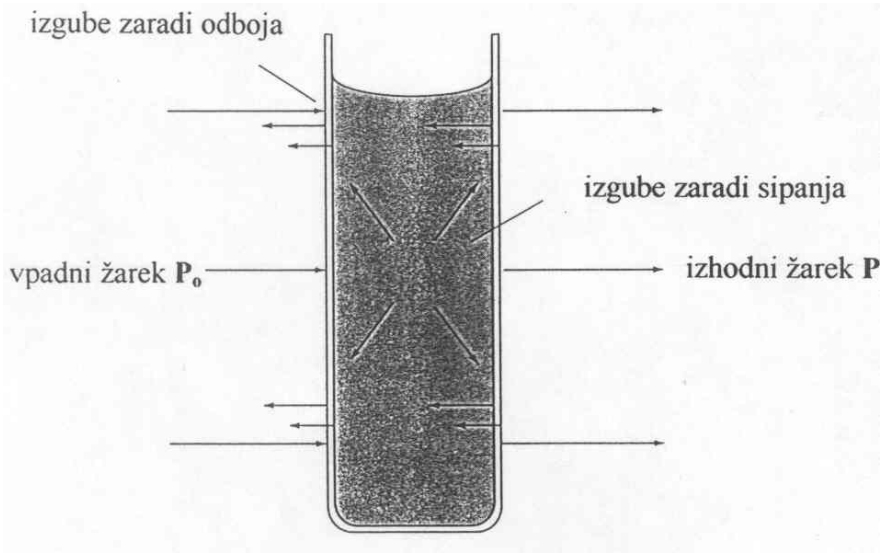
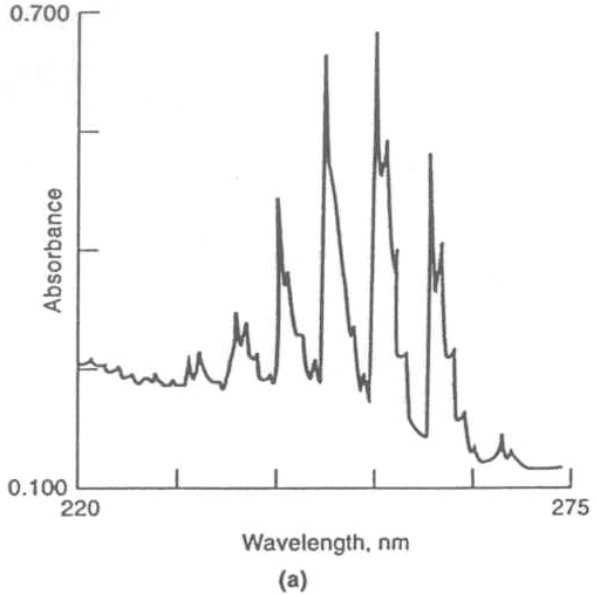
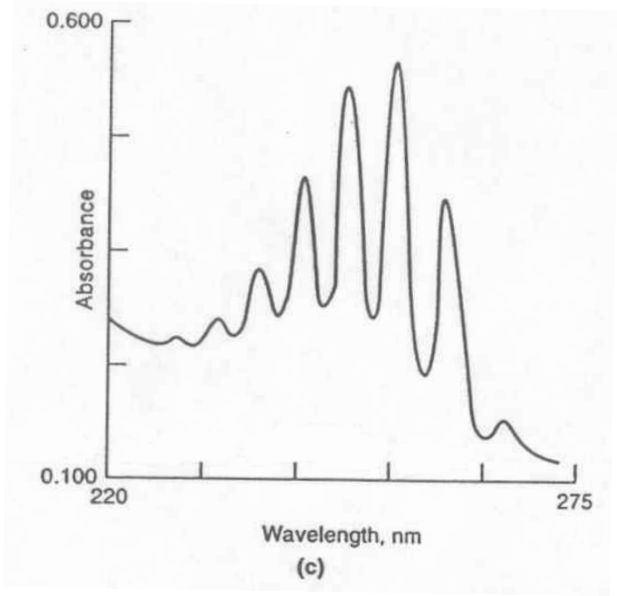


Figure 4: Effect of bandwidth on spectral detail. (a) 0.5 nm; (b) 1.0 nm; (c) 2.0 nm





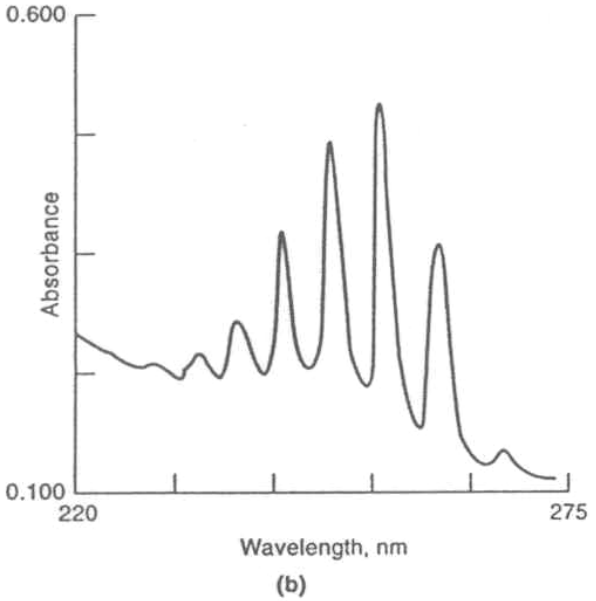


Figure 5:

Effect of bandwidth on spectral detail.

The sample was a didymium glass.

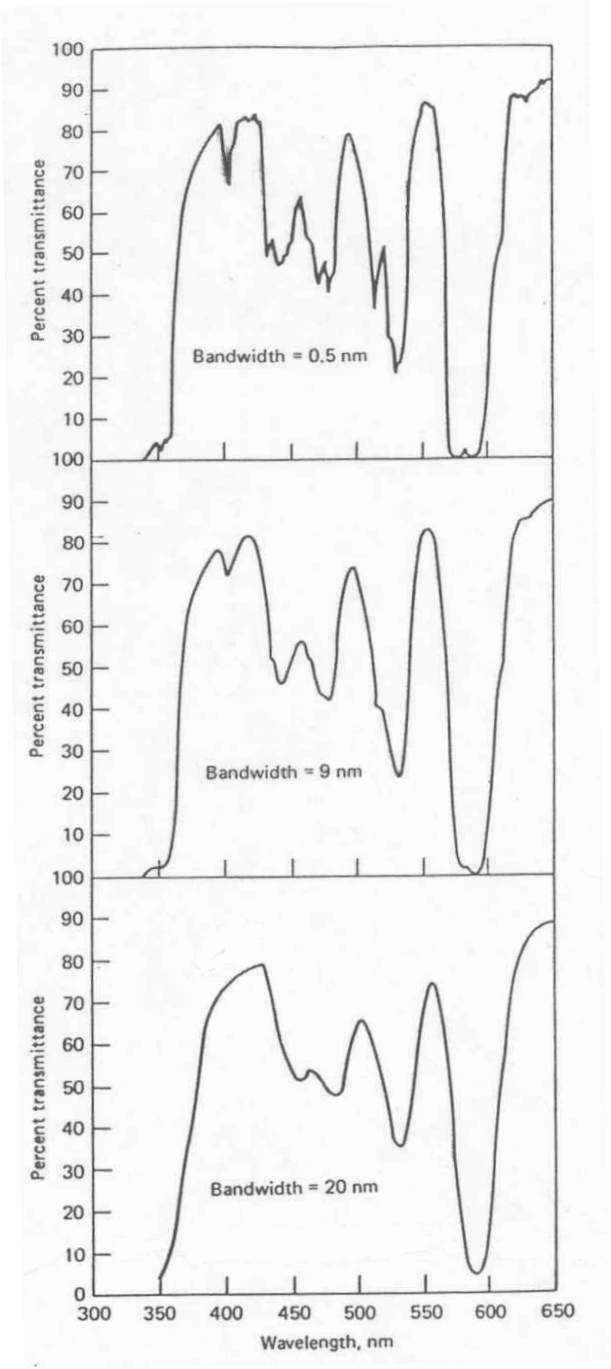


Figure 6: Spectra of cerium (IV) obtained with a spechtrometer having glass optics (A) and quartz optics (B). The false peak in A arises from transmission of stray radiation of longer wavelenghts.

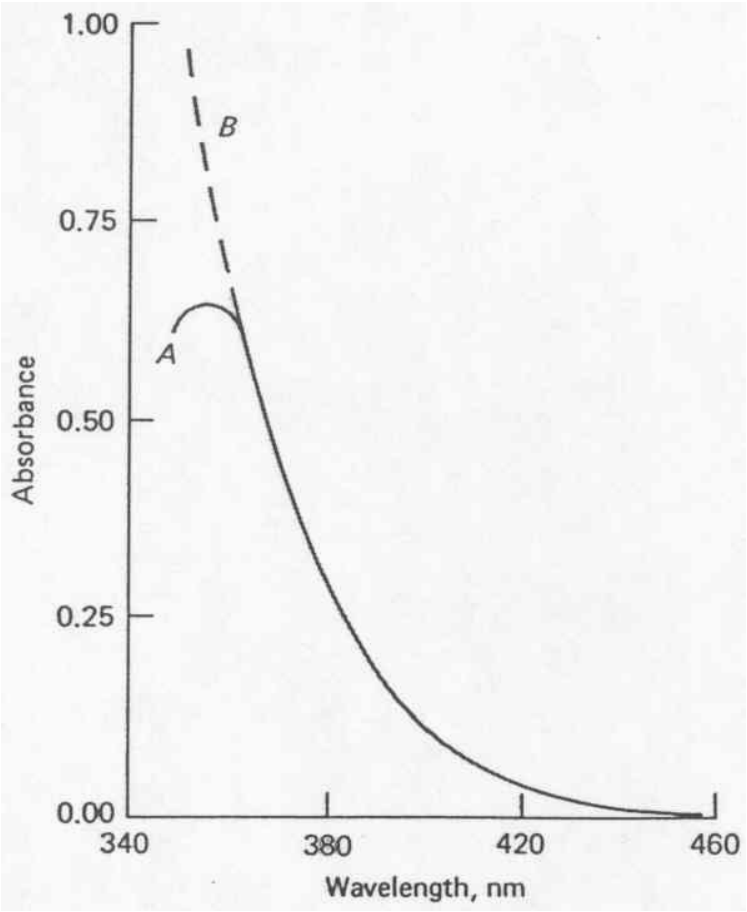
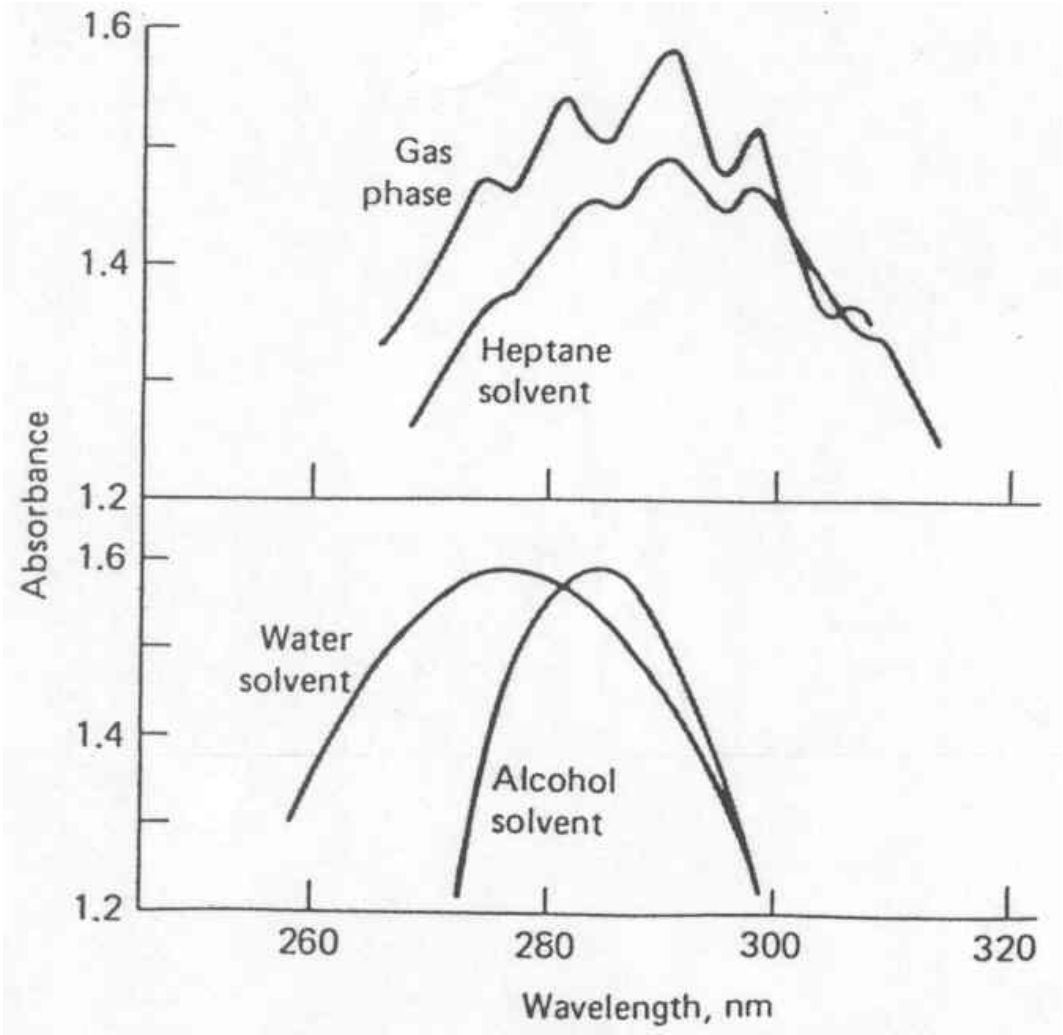


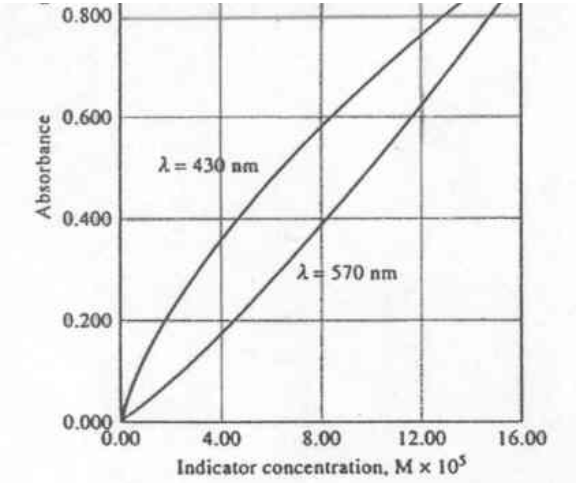
Figure 7: Effect of solvent on the absorption spectrum of acetaldehyde



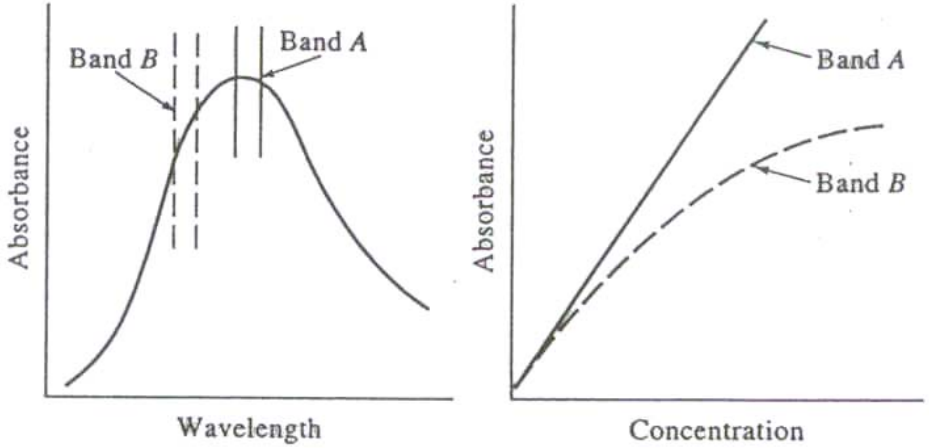
6. Omejitve Beer-ovega zakona

a) Resnične omejitve (visoke koncentracije, velike molekule)

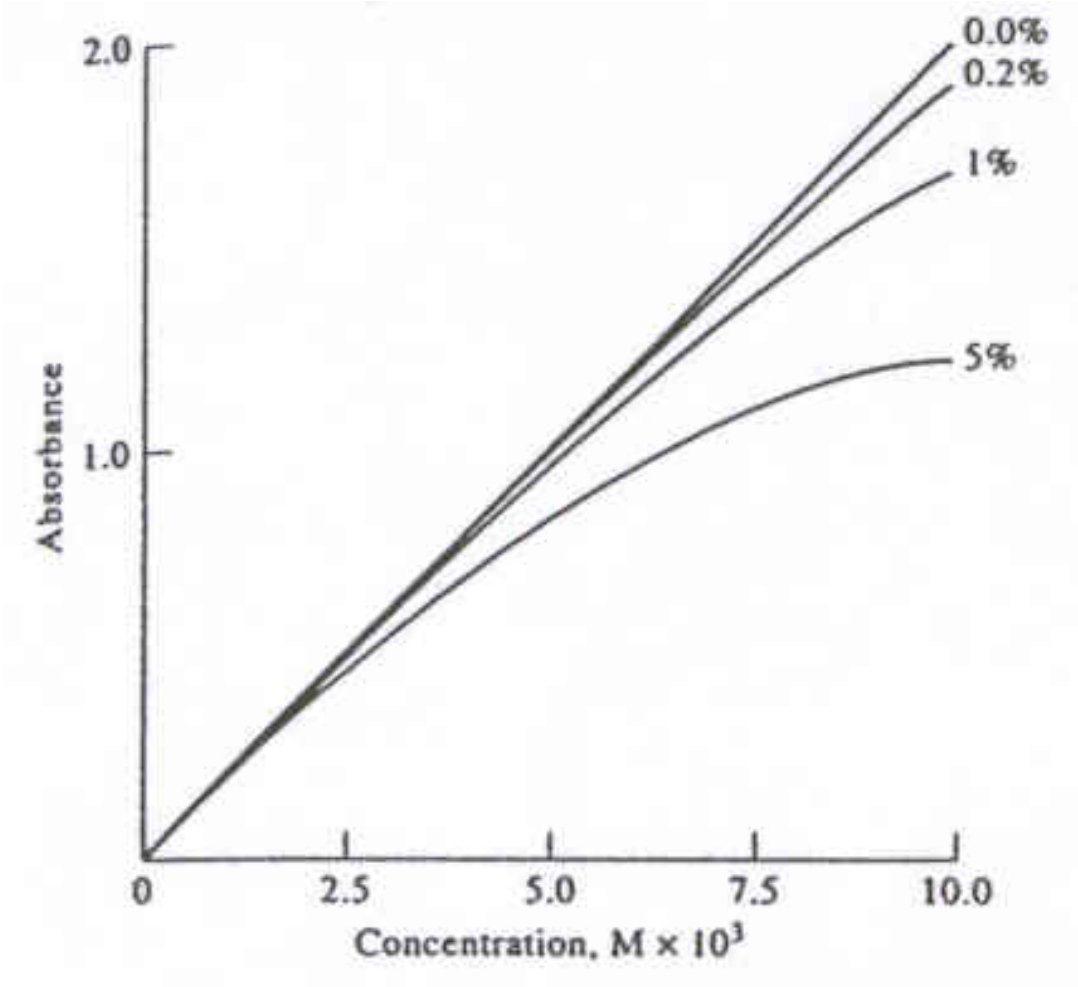
b) Navidezni kemični odkloni



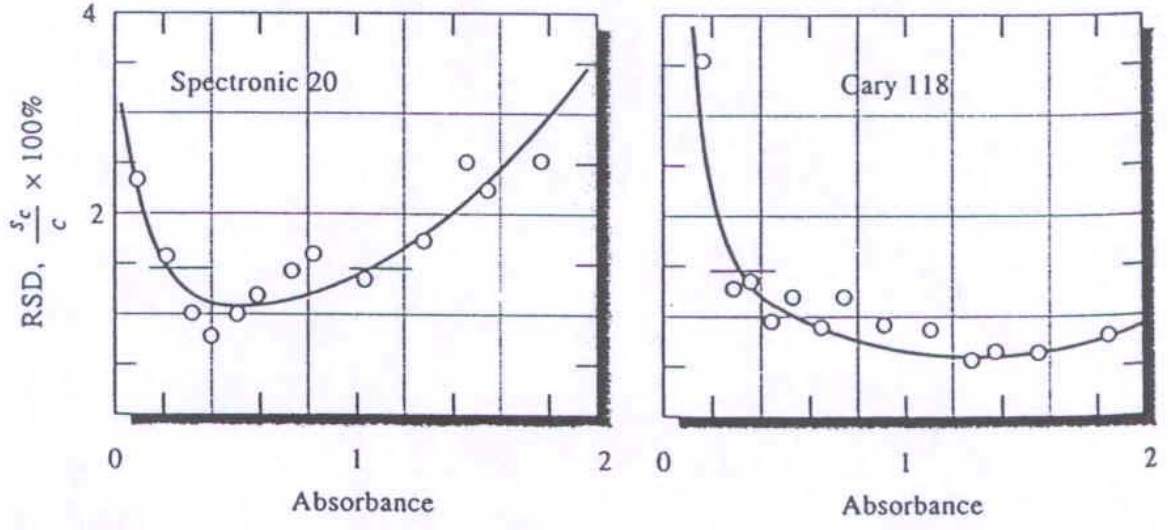
c) Navidezni instrumentalni odkloni



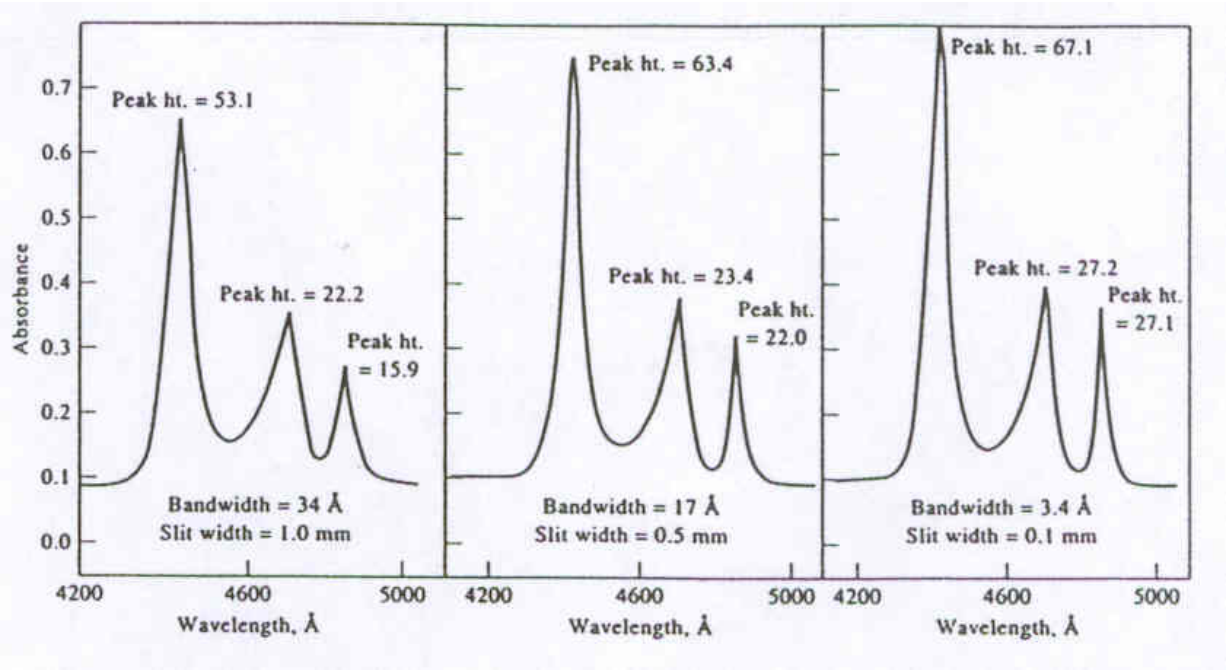
d) Instrumentalni odkloni zaradi svetlobe



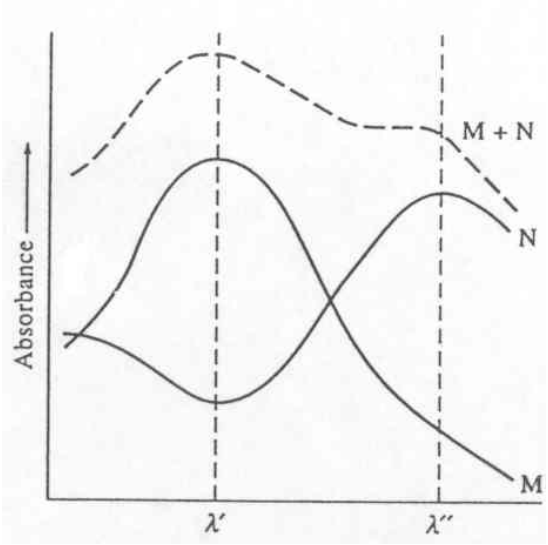
7. Vpliv instrumentalnega šuma na meritve



8. Vpliv širine reže na meritve

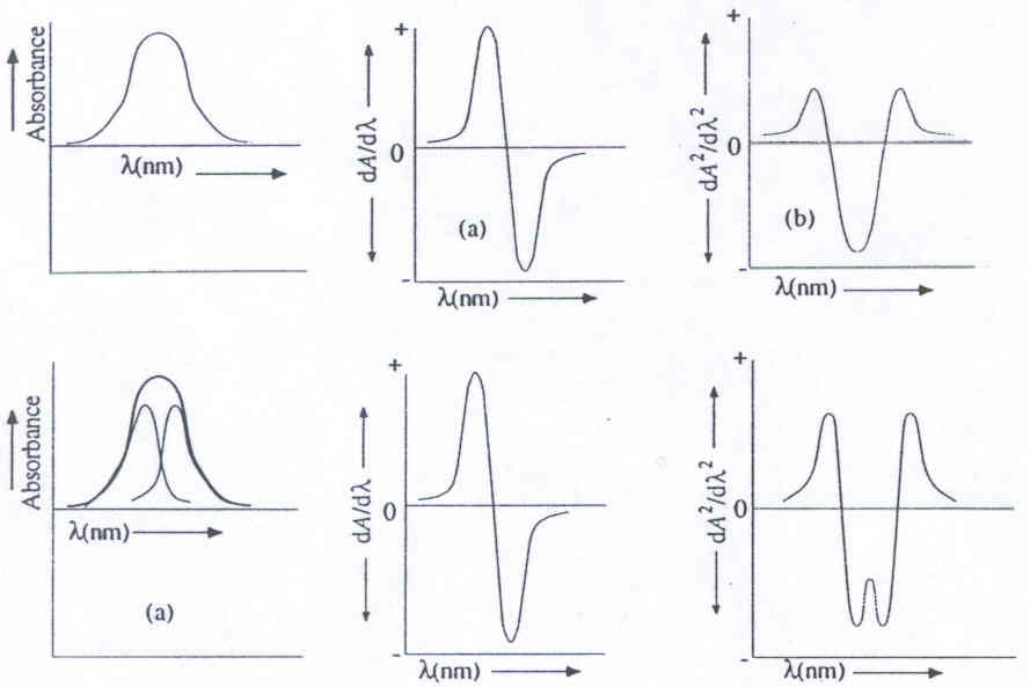


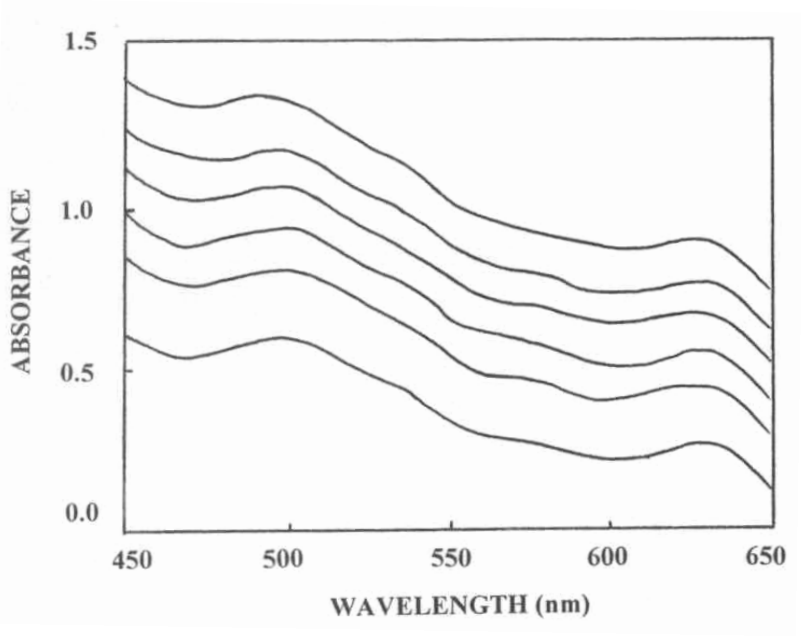
9. Analiza mešanic

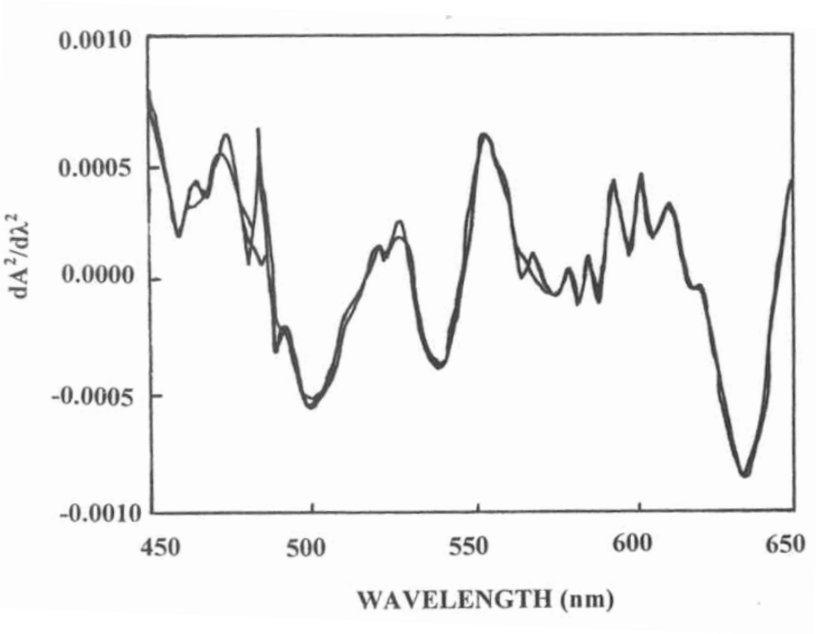


10. Dvovalovna spektrofotometrija

11. Derivacijska spektrofotometrija




1.5
1.0
0.5
0.0
ABSORBANCE
450
500
550
600
650
WAVELENGTH (nm)


0.0010
0.0005
0.0000
-0.0005
-0.0010
dA²/dλ²
450
500
550
600
650
WAVELENGTH (nm)

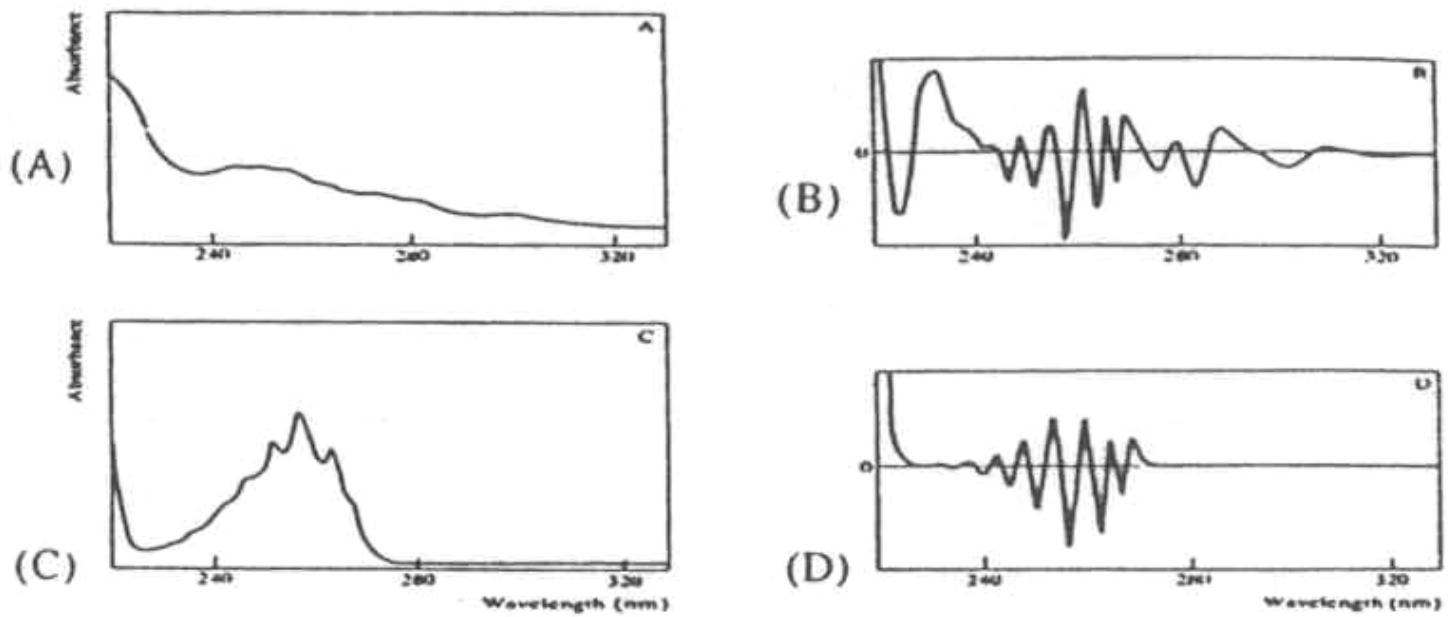

(A)
Absorbance
A
240
280
320
(B)
B
0
240
280
320
(C)
Absorbance
C
240
280
320
Wavelength (nm)
(D)
D
0
240
280
320
Wavelength (nm)

Figure 8: Comparison of a derivative spectrum (a) with a standard transmittance spectrum (b).

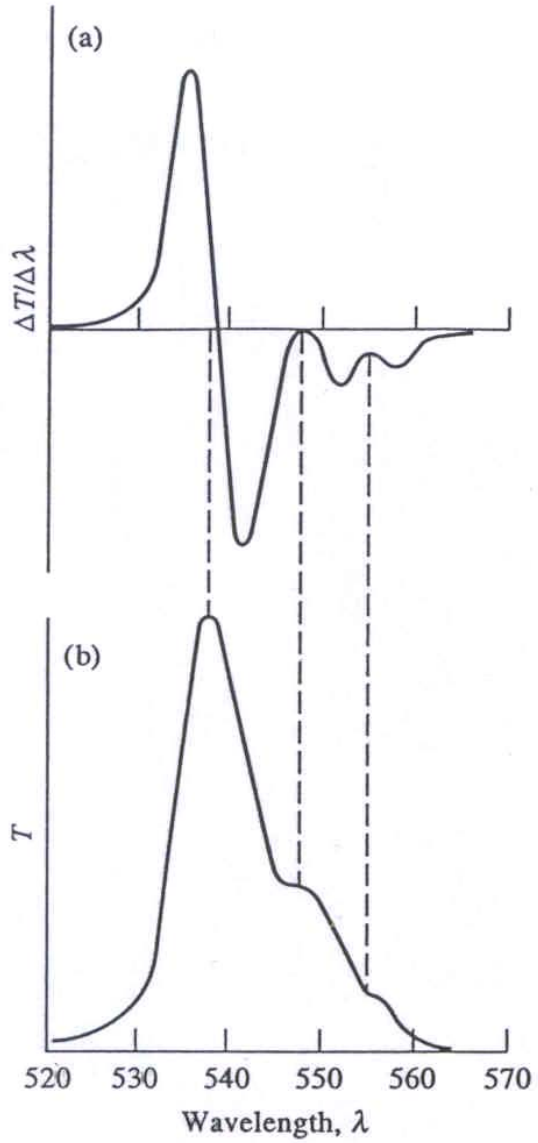
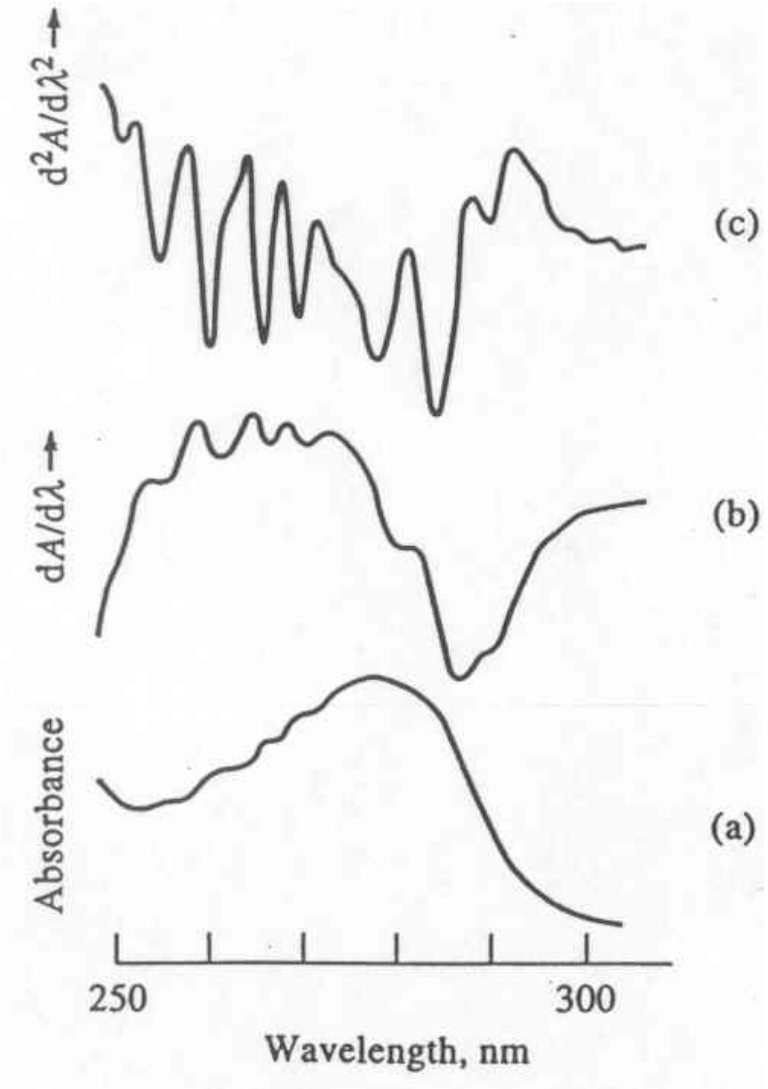
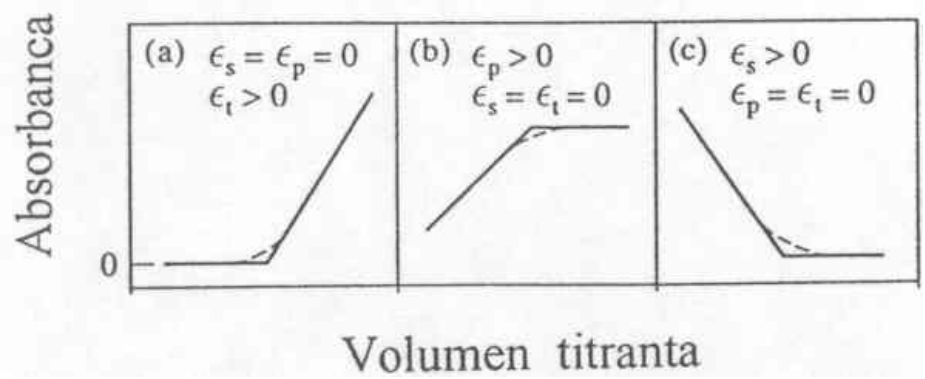


Figure 9: Absorption spectra of bovine albumin: (a) ordinary spectrum, (b) first derivative spectrum, (c) second derivative spectrum



12. Spektrofotometrične titracije



13. Avtomatizirane spektrofotometrične metode

III. KVANTITATIVNE MERITVE

1. Uporaba absorpcijskega koeficienta

2. Primerjalne metode

$c_x = (A_x/A_{st}) \cdot c_{st}$ umeritvena premica

3. Validacija metod

Q2A: Text on Validation of Analytical procedures, 1994

Q2B: Validation of Analytical procedures: Methodology, 1996

TIPI ANALIZNIH POSTOPKOV, KI NAJ SE VALIDIRAJO

- Identifikacijski testi

- Kvantitativni testi za vsebnost nečistot

- Limitni testi za kontrolo nečistot

- Kvantitativni testi aktivne spojine v vzorcih spojine in izdelkih, ali drugih izbranih sestavin v izdelkih

KARAKTERISTIKE, KI NAJ SE OVREDNOTIJO

1. Specifičnost

2. Točnost

3. Natančnost

- **repitabilnost**
- **vmesna natančnost**
- **reproducibilnost**

4. Meja detekcije

5. Meja kvantifikacije

6. Linearnost

7. Območje

8. Robustnost

Table 1: Some Examples of Absorption due to n→σ* Transitions[a]

Compound	λ_{max}(nm)	ε_{max}
H_2O	167	1480
CH_3OH	184	150
CH_3Cl	173	200
CH_3I	258	365
$(CH_3)_2S$[b]	229	140
$(CH_3)_2O$	184	2520
CH_3NH_2	215	600
$(CH_3)_2N$	227	900

[a]Samples in vapor state

[b]In ethanol solvent

Table 2: Solvents for the Ultraviolet and the Visible Region

Solvent	Approximate[a] Transparency Minimum (nm)
Water	190
Ethanol	210
n-hexane	195
Cyclohexane	210
Benzene	280
Diethyl ether	210
Acetone	330
1,4-Dioxane	220

[a]For 1-cm cells

Table 3: Absorption Characteristics of Aromatic Compounds

Compound		E_2 Band		B Band	
		λ_{max}(nm)	ε_{max}	λ_{max}(nm)	ε_{max}
Benzene	C_6H_6	204	7,900	256	200
Toluene	$C_6H_5CH_3$	207	7,000	261	300
M- Xylene	$C_6H_4(CH_3)_2$	–	–	263	300
Chlorobenzene	C_6H_5Cl	210	7,600	265	240
Phenol	C_6H_5OH	211	6,200	270	1,450
Phenolate ion	$C_6H_5O^-$	235	9,400	287	2,600
Aniline	$C_6H_5NH_2$	230	8,600	280	1,430
Anilinium ion	$C_6H_5NH_2^+$	203	7,500	254	160
Thiophenol	C_6H_5SH	236	10,000	269	700
Naphthalene	$C_{10}H_8$	286	9,300	312	289
Styrene	$C_6H_5CH=CH_2$	244	12,000	282	450

KVANTITATIVNA UV-Vis SPEKTROSKOPIJA

Določanje umeritvene premice, limite detekcije in neznane koncentracije v vzorcu:

conc. st. (x)	absorbanca (y)	conc. vzorca (c_{vz})	absorbanca vzorca	absorbanca praznega vz.
0,010	0,22	?	0,74	0,03
0,020	0,40			0,05
0,030	0,68			0,02
0,040	0,87			0,04
0,050	0,99			0,04
				0,00
				0,01
				0,05
				0,00
				0,01

IZRAČUNI

- Izračunamo enačbo umeritvene premice ($A = a \cdot c + b$) s pomočjo najmanjših kvadratov.
- Izračunamo determinacijski koeficient (r^2).
- Izračunamo standardno deviacijo praznih vzorcev (s) in limito detekcije (LOD).
- Izračunamo neznano koncentracijo v vzorcu (c_{vz}):
  - b) na osnovi izračunane umeritvene premice
  - c) na osnovi najbližje standardne raztopine