Ultraviolet and visible spectrometry

Theoretical overview

Molecular absorption of electromagnetic radiation

- changes of energy state of the molecule include
  - electronic state ΔE_e =150-600 kJ/mol
    (electron transitions between orbitals)
  - vibrational state ΔE_v =2-60 kJ/mol
  - rotational state $\Delta E_r \approx 3$ kJ/mol
- relation to the absorbed radiation wavelength
  $\Delta E = \Delta E_e + \Delta E_v + \Delta E_r = h \cdot \nu = h \cdot c / \lambda$
  $h = 6.626 \cdot 10^{-34}$ J s (Planck's constant)

Spectral regions

Region	λ	Absorbing compounds
Far ultraviolet (vacuum UV region)	<190 nm	saturated and mono-unsaturated
(Near) ultraviolet	190-380 nm	poly-unsaturated and aromatic
Visible light region	380-780 nm	coloured

Visible light absorption

Table of complementary colours:

λ (nm)	Colour of light	Colour of absorbing body
400–435	violet	yellow-green
435–480	blue	yellow
480–490	green-blue	orange
490–500	blue-green	red-orange
500–560	green	red
560–580	green-yellow	violet
580–595	yellow-orange	blue
595–620	red-orange	green-blue
620–760	red	blue-green

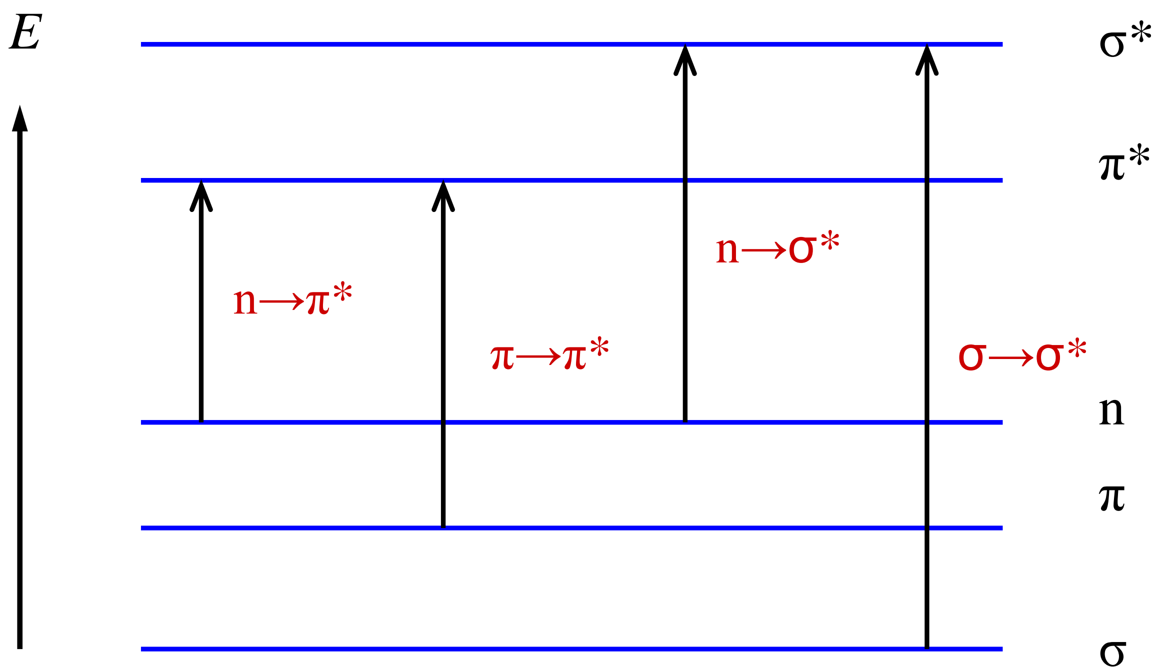
Labert-Beer law

transmittance $T = I/I_0$

in a diluted solution the value of absorbance A measured at the specific wavelength is proportional to the concentration of absorbing compound

$A_\lambda = -\log T = \log(I_0/I) = \varepsilon_\lambda \cdot b \cdot c$

Energy changes of electronic transitions



Probability of transition influences the value of absorption coefficient

relation to spin state of excited electron

1) transition S0 (ground singlet) →S1 (upper singlet) is allowed
   $\varepsilon_{max} \approx 10^3\text{–}10^5$ l.mol^{-1}.cm^{-1}
2) transition S0 → T1 (triplet) is forbidden
   $\varepsilon_{max} \approx 10^0$ l.mol^{-1}.cm^{-1}

Terms used in UV/VIS spectrometry

chromophore — a group of atoms responsible for UV/VIS absorption of the molecule, e.g. double bonds C=C, C=C-C=C, C=O, N=N, aromatic rings etc.

auxochrome — a substituent that increases absorption of a molecule, typically methyl, hydroxyl, alkoxyl or amino group or an atom of halogen; when the auxochrome is conjugated with a π-electron system, the λ_{max} value is shifted to a longer wavelength (*bathochromic efect*)

bathochromic effect (red shift) — a shift of λ_{max} to longer wavelength caused by molecule modification or a change of solvent

hypsochromic effect (blue shift) — a shift to shorter wavelength

hyperchromic effect — an increase of absorption

hypochromic effect — a decrease of absorption

Some chromophores and the corresponding transitions

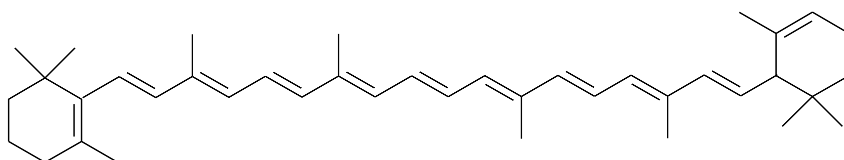
Chromophore an example of compound	Transition	λ_{max} (nm)
H_2O	σ→σ*	183
C-C a C-H, CH_4	σ→σ*	cca 170, 173
C-X, CH_3OH, CH_3NH_2, CH_3I	n→σ*	180-260, 187, 215, 258
C=C, $H_2C{=}CH_2$	π→π*	160-190, 162
$H_2C{=}CH{-}CH{=}CH_2$	π→π*	217
C=O, H−CH=O	n→π*, π→π*	270, 170-200, 270, 185
$H_2C{=}CH{-}CH{=}O$	n→π*, π→π*	328, 208
C=N	n→σ*, n→π*	190, 300
N=N	n→π*	340
C=S	n→π*	500
NO_2	n→π*	420-450
N=O	n→π*	630-700

The effect of conjugation

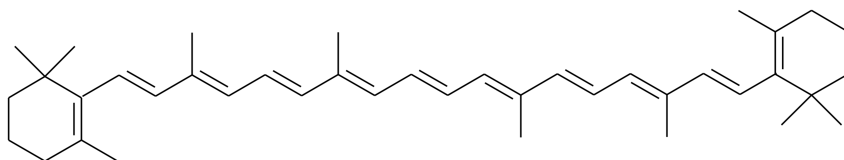
Conjugated polyenes:

n	H−(CH=CH)$_n$−H		CH$_3$−(CH=CH)$_n$−CH$_3$	
	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
2	217	4.3	223	4.4
3	268	4.7	275	4.5
4	304	?	310	4.9
5	334	5.1	341	5.1

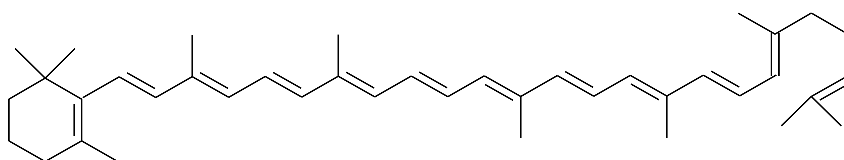
α-carotene, λ_{max}= 447 nm



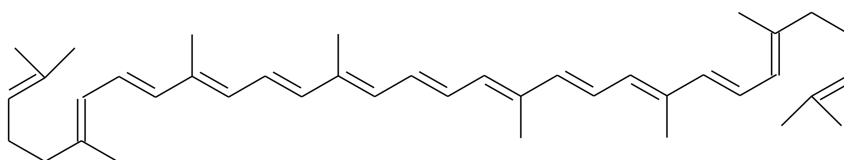
β-carotene, λ_{max}= 451 nm



γ-carotene, λ_{max}= 462 nm



lycopene, λ_{max}= 476 nm



Benzene and its derivatives

Compound	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
benzene	204	3.9	254	2.0	-	-
toluene	207	3.8	261	2.4	-	-
brombenzene	210	3.9	261	2.3	-	-
phenol	211	3.8	270	3.2	-	-
benzaldehyde	250	4.1	280	3.0	320	1.7
acetophenone	246	4.0	280	3.0	320	1.7
benzoic acid	230	4.1	273	3.0	-	-
aniline	230	3.9	280	3.5	-	-
styrene	247	4.0	281	2.0	-	-
cinnamaldehyde	285	4.4	-	-	-	-
cinnamic acid	273	4.3	-	-	-	-
biphenyl	248	4.2	-	-	-	-

Heterocyclic compounds

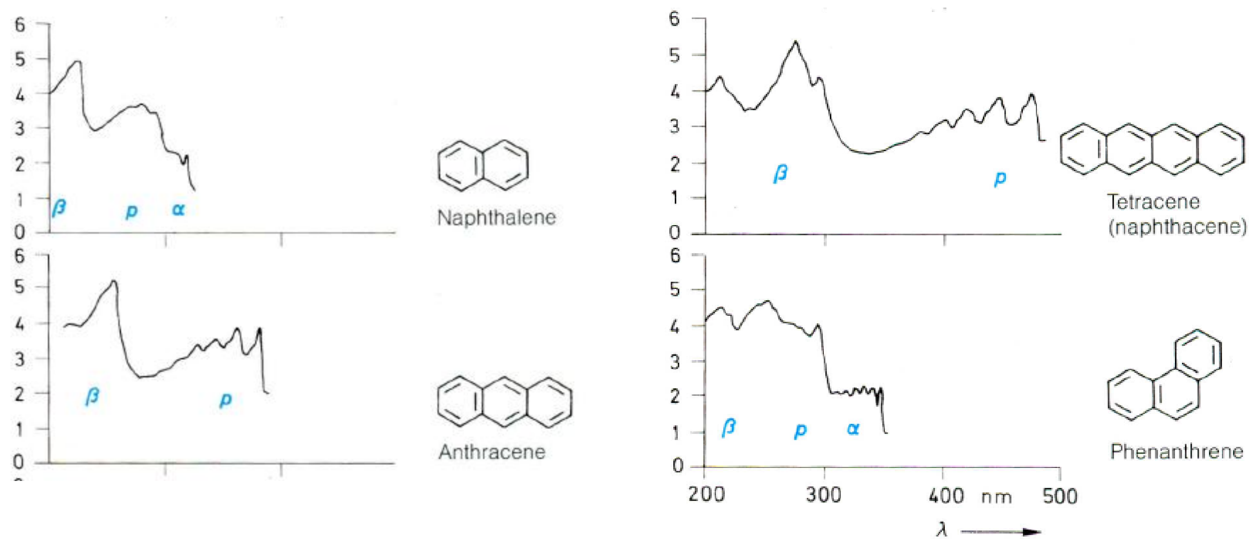
<u>5-membered</u>

Compound	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
furan	200	4.0	-	-
2-furaldehyde	227	3.3	272	4.1
2-acetylfuran	225	3.4	269	4.1
pyrrole	210	4.2	240	2.5
2-acetylpyrrole	250	3.6	287	4.2
thiophene	-	-	235	3.7
2-acetylthiophene	260	3.9	285	3.7
thiazole	-	-	240	3.6

<u>6-membered</u>

Compound	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
Pyridine	195	-	250	3.3	-	-
2-Picoline	-	-	262	3.4	-	-
Pyrazine	-	-	260	3.7	-	-
Quinoline	227	4.6	275	3.7	313	3.4
Isoquinoline	218	4.9	262	3.6	317	3.5
Pyrimidine	-	-	-	-	343	3.3

Polycyclic aromatic hydrocarbons



Practical rules for spectrophotometric measurement

- choice of a measuring cell
  - quartz: for UV
  - glass: for VIS
  - plastic: for some routine measurement in VIS
  - length of a cell: most commonly 0.1–5 cm ⇒ optimum absorbance 0.1–2
- choice of a solvent
  the kind of solvent may influence the position of spectral band and the maximum absorbance
- spectrum recording
  - scan rate
    very fast scan ⇒ higher noise of the spectrum
  - spectral band-width
    narrow SBW (0.2–0.5 nm) ⇒ better resolution and higher noise of the spectrum
    wide SBW (2–4 nm) low resolution, low noise; suitable for the recording of wide bands (VIS region) and the highly precise measurement of a single absorbance value
- sample dilution
  allowed only for stable species

Solvents for UV spectrometry

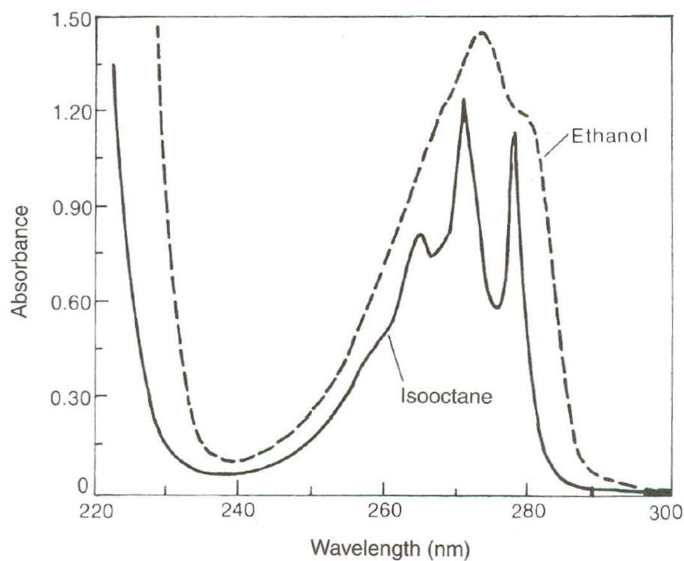
Table the lowest wavelengths of measurement with the solvent

Solvent	λ (nm)	**Solvent**	λ (nm)
acetonitrile, water	190	chloroform	240
isooctane, cyclohexane	195	ethylacetate	260
hexane	201	dimethylformamide	270
methanol, ethanol	205	acetic acid.	270
1,4-dioxane	215	benzene	280
diethylether	220	toluene	285
glycerol	230	pyridine	300
dichloromethane	233	acetone	330

Effect of solvent on the absorption spectrum

The kind of solvent slightly affects

- values of λ_{max}, ε
- shape of the spectrum



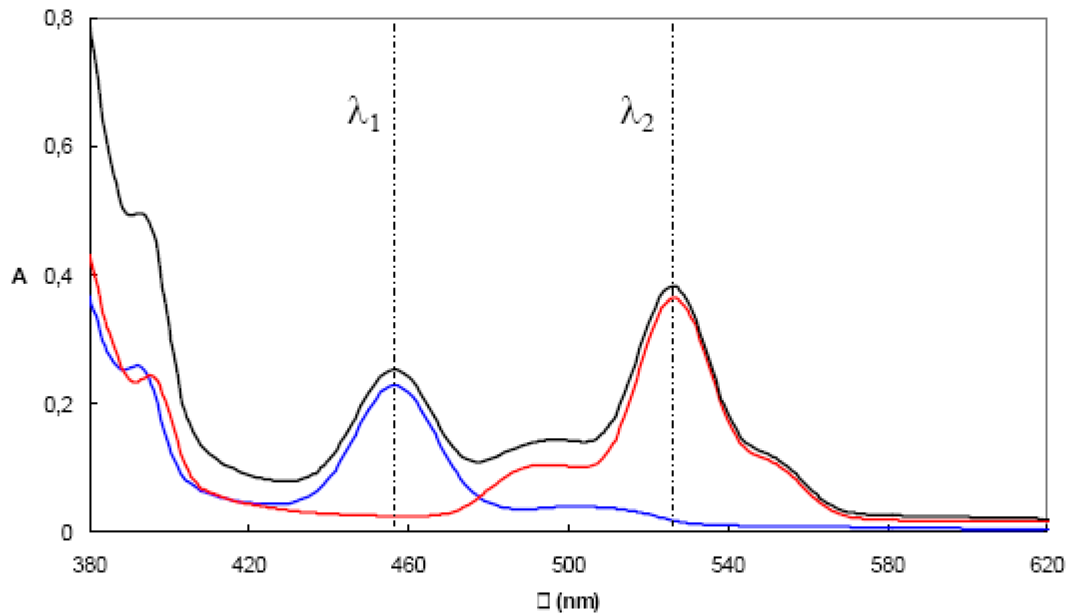
spectra of phenol measured in isooctane and ethanol

Spectra of biologically important compounds

Compound	λ_{max} (nm)	ε ($l.mol^{-1}.cm^{-1}$)
NAD, NADP	260	15 000
NADH, NADPH	260	15 000
	340	6 200
FMN, FAD	260	15 000
	375	10 000 (FMN) 9 000 (FAD)
	445	12 500 (FMN)
	450	11 000 (FAD)
pyridoxal	250	3 000
	320	6 000

Compound	λ_{max} (nm)	ε (l.mol-1.cm-1)
cholesterol	235	20 000
calciferols	265	18 300
β-carotene	450	120 000
retinol	330	45 000
trans, *trans*-9,12-octadecenoic acid.	231	35 000
adenosine	267	12 300
guanosine	248	11 000
cytidine	271	9 100
thymidine	267	9 650
uridine	262	8 500

Two-component analysis



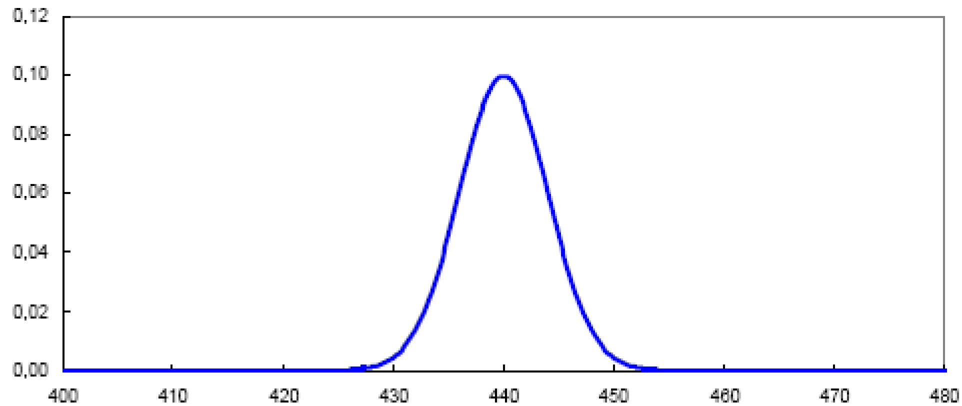
Rule of absorbance additivity:

$$A_{\lambda 1} = b \,.\, (\varepsilon_{A\lambda 1} \,.\, c_A + \varepsilon_{B\lambda 1} \,.\, c_B)$$
$$A_{\lambda 2} = b \,.\, (\varepsilon_{A\lambda 2} \,.\, c_A + \varepsilon_{B\lambda 2} \,.\, c_B)$$

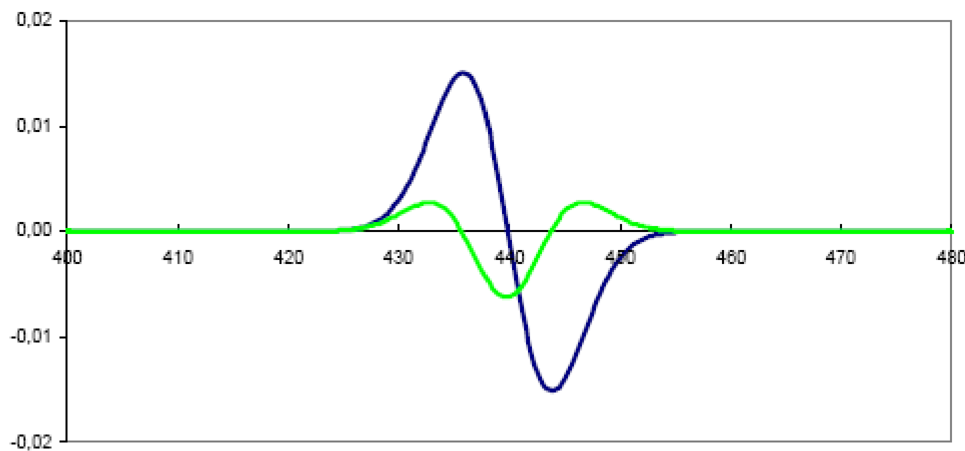
$$c_A = \frac{A_{\lambda 1} - A_{\lambda 2} \,.\, \varepsilon_{B\lambda 1} / \varepsilon_{B\lambda 2}}{b \,.\, (\varepsilon_{A\lambda 1} - \varepsilon_{A\lambda 2} \,.\, \varepsilon_{B\lambda 1} / \varepsilon_{B\lambda 2})}$$

$$c_B = \frac{A_{\lambda 2} - A_{\lambda 1} \,.\, \varepsilon_{A\lambda 2} / \varepsilon_{A\lambda 1}}{b \,.\, (\varepsilon_{B\lambda 2} - \varepsilon_{B\lambda 1} \,.\, \varepsilon_{A\lambda 2} / \varepsilon_{A\lambda 1})}$$

Derivative spectrometry



original spectrum
A *vs.* λ



1st derivative
dA/dλ *vs.* λ

2nd derivative
$d^2A/d\lambda^2$ *vs.* λ

$T = \Phi / \Phi_0$

$A = -\log_{10}T = -2{,}303 \cdot \ln T = \varepsilon \cdot b \cdot c$

$dA/d\lambda = -2.303 \cdot (1/T) \cdot dT/d\lambda = b \cdot c \cdot d\varepsilon/d\lambda$

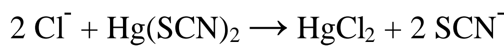
⇒ the first (and also the second) derivative of absorbance is proportional to the concentration of the absorbing compound

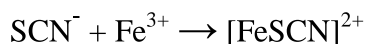
Flow injection analysis – FIA

- an optional arrangement of a (spectrophotometric) measurement
- instead of the batch-preparation of the measured solution the sample is injected into the flow of the carrier solution or the reagent solution and then measured (usually using a spectrophotometer)
- FIA is much faster than traditional batch analysis and can be easily automated

An example of FIA arrangement: determination of chlorides

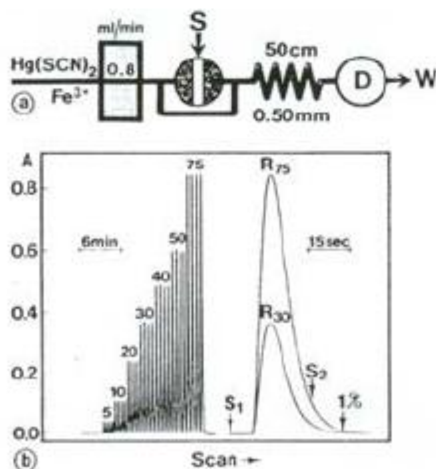
Chemical principle:


$2\ Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2\ SCN^-$


$SCN^- + Fe^{3+} \longrightarrow [FeSCN]^{2+}$

absorbance of a red-coloured solution of ferric-thiocyanate complex is measured

FIA arrangement:



- a peristaltic pump delivers the reagent (a solution of mercury thiocyanate and ferric sulphate) at a constant flow rate
- a sample dose (30 µl) is injected into the flow
- the reactions takes place in the capillary
- the product is measured in a flow-through cell of a spectrophotometric detector operated at 480 nm and an absorbance peak is recorded
- the next injection follows after 40 s
- approx. 100 samples per hour can be analysed

Equipment for FIA

- peristaltic pump (tubes of a diameter of 0.25 to 2 mm, flow rate 0.0005 to 10 ml/min)
- PTFE capillaries, join pieces
- low pressure injection valve (sample loop 5–500 µl)
- additional parts: filters, micro-columns, valves, thermostat
- detector (most often a spectrophotometer with a flow-through cell)