11 UV-VIS SPECTROSCOPY

Electromagnetic radiation refers to the waves of the electromagnetic field, propagating through space and carrying electromagnetic radiant energy. In a vacuum, electromagnetic waves travel at the speed of light. The ***electromagnetic spectrum*** is the range of frequencies of electromagnetic radiation and their respective wavelengths and photon energies. The electromagnetic spectrum is classified into the following regions – **Table 1**:

Table 1 Classification of electromagnetic spectra

Radiation range	Wavelength scale
Gamma rays	< 0.1 nm
X – rays	0.1 – 10 nm
Ultraviolet	10 – 400 nm
Visible	400 – 800 nm
Infrared	800 nm – 4 mm
Microwaves	4 mm – 0.25 m
Radio waves	> 0.25 m

Physical quantities characterizing electromagnetic radiation

Electromagnetic radiation propagates linearly, while the electric field intensity (**E**) oscillates ***sinusoidally*** with time (***t***) – **Figure 1**.

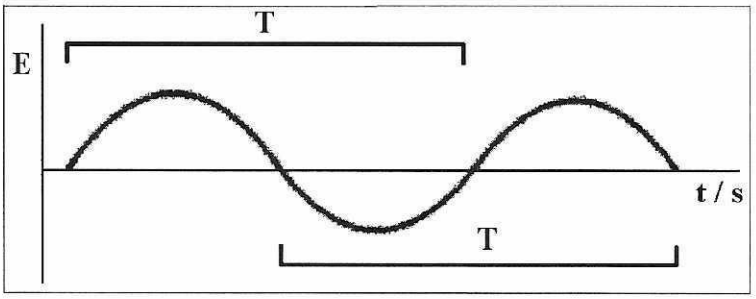


Figure 1 Wave period

A wave consists of successive troughs and crests.

– ***Wave period*** (T) is the time interval for a single wave crest to travel a distance equal to the length of the wave. [s]

– ***Wavelength*** (λ) is the horizontal length of a single wave cycle, i.e. the distance between two adjacent crests or troughs (**Figure 2**). [m]

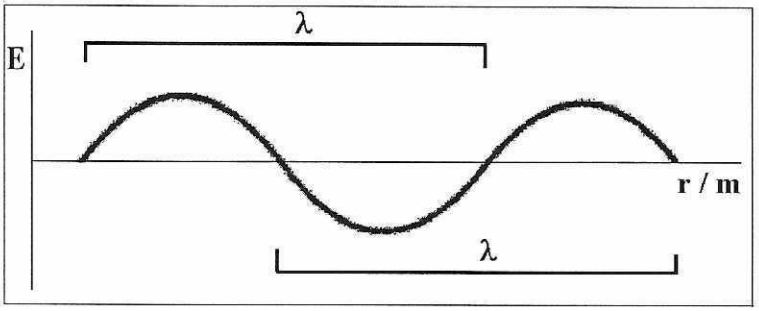


Figure 2 Wavelength

– ***Wavenumber,*** or ***repetency*** ($\tilde{\nu}$) is the number of waves per unit distance.

$$\tilde{\nu} = \frac{1}{\lambda} \qquad [\mathrm{m}^{-1}] \tag{1}$$

– ***Frequency*** (ν) is the number of waves per unit time, i.e. rate of wave oscillation.

$$\nu = \frac{c}{\lambda} \quad \text{or} \quad \nu = c\tilde{\nu} \qquad [\mathrm{s}^{-1} \text{ or Hz}] \tag{2}$$

where c is the speed of light ($c = 3.10^8$ m s^{-1}).

In general, the interaction of electromagnetic radiation with a substance results in several observable effects, like

– ***reflection and refraction*** (studied by refractometry)
– ***polarization*** (polarimetry)
– ***scattering and diffraction*** (scattering methods)
– ***absorption and emission*** (spectral methods)
– ***chemical conversion of matter induced by radiation*** (photochemistry)

The first three effects (reflection, refraction, polarization, scattering and diffraction) are used to be described via wave character of the radiation, while the last two effects (absorption, emission and chemical conversion) are explained via corpuscular (particle) character of the radiation. In this case we assume, that the radiation is formed by flux of ***photons*** – a minimal quantum (amount) of light.

– ***Radiant energy (E)*** is the energy carried by flux of photons:

$$E = h\nu \qquad [\mathrm{J}] \tag{3}$$

where ***h*** is the Planck constant, $h = 6.626 \times 10^{-34}$ J s.

– ***Radiant flux*** (Φ) is the measure of the total power of electromagnetic radiation, defined as the amount of energy that is radiated across a unit area per unit time [W] – Watt.
– ***Luminous flux*** (Φ_L) is the measure of the perceived power of light which is able to induce a visual perception [lm] – lumen.

Transmission and Absorption

If a radiation with flux Φ_0 passes through a transparent substance of thickness ***d***, the flux is attenuated to value Φ. Supposing no reflection (**Figure 3**), the flux difference

$\Delta\Phi = \Phi_0 - \Phi$ is absorbed by the substance.

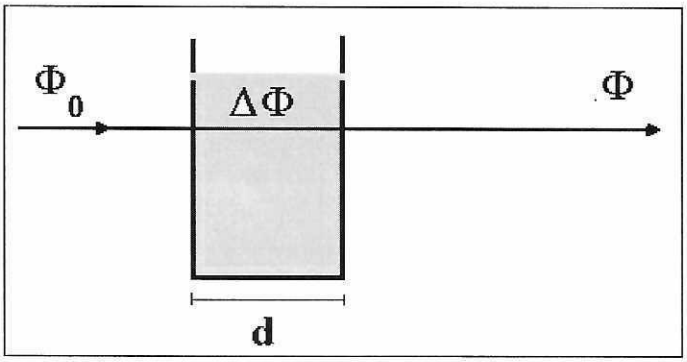


Figure 3 Absorption of radiation

Transmittance (***T***) is defined as the ratio of the flux Φ (the intensity of light after passing through a sample) to the incident flux Φ_0 (the intensity of light before it passes through the sample):

$$T = \frac{\Phi}{\Phi_0} \qquad \text{[unitless]} \tag{4}$$

Transmittance is usually expressed as a percentage, (i.e. multiplied by 100), is ranging between **0** (if $\Phi = 0$) and **100** % (if $\Phi = \Phi_0$).
Transmittance depends on the thickness of matter (***d***), the higher is the thickness the lower is the transmittance.

Absorbance (*A*) is a measure of attenuation of transmitted flux. Attenuation can be caused by absorption, but also reflection, scattering, and other physical processes.
Lambert's law defines attenuation of transmitted flux:

$$\Phi = \Phi_0 10^{-ad} \tag{5}$$

where ***a*** is a coefficient characterizing the absorption medium.
In the case of radiation transmission in solution, absorption coefficient (***a***) is proportional to the concentration (*c*) of the absorber – ***Beer's law***:

$$a = \varepsilon c \tag{6}$$

where ε is the ***molar absorption coefficient (extinction coefficient);*** determines how strongly the solution with unit molarity and unit thickness absorbs light of a particular wavelength. The SI units of ε are m^2mol^{-1} or cm^2mmol^{-1} (when $m^2mol^{-1} = 10\ cm^2mmol^{-1}$).
Joining expressions 5 and 6 we get basic form of ***Lambert-Beer's law***:

$$\Phi = \Phi_0 10^{-\varepsilon cd} \tag{7}$$

Relation between absorption, thickness of absorbing solution (thickness of the measuring cell) and concentration of the substance is commonly given as:

$$A = \varepsilon cd \qquad \text{[unitless]} \tag{8}$$

where A is the absorbance.
The absorbance (A) of the solution is related to the transmittance by following expression:

$$A = \log \frac{\Phi_0}{\Phi} = -\log T \tag{9}$$

For non-absorbing solutions: $A = 0$ and $T = 100\%$
For fully absorbing solutions: $A = \infty$ and $T = 0\%$
The inverse process to absorption is the emission of radiation. On principle, both effects are similar, but range of methods using radiation absorption is wider. Optical methods based on absorption (emission) of radiation, employing Lambert-Beer's law, are broadly used in practice, i.a. in chemical analysis of solutions, providing qualitative and quantitative results.

Spectroscopy – is the study of interactions between matter and electromagnetic radiation.
Spectrum – is a dependence of a quantity of absorbed (emitted) radiation as a function of wavelength (or wavenumber, frequency).
Spectra are classified to: - ***atomic*** (originated from elements) - emission
- absorption
- X-ray
- ***molecular*** (originated from molecules) - electronic (UV and VIS)
- fluorescent
- vibrational-rotational (IR)
- Raman
- NMR, ESR

Electronic spectra

We will focus to spectra originated from molecules with spectral bands in the ultraviolet (**UV**) and visible (**VIS**) region with wavelengths corresponding to 10 – 400 nm and 400 – 800 nm, respectively. However, common UV-VIS spectrometers are built to follow the range of wavelengths 200 – 800 nm.
Spectrum is generated by electron jump between molecular orbitals (MO), such as:

π (bonding MO) and π^* (antibonding MO)
n (nonbonding MO) and π^* (antibonding MO)
n (nonbonding MO) and σ^* (antibonding MO)
σ (antibonding MO) and σ^* (antibonding MO)

When the electron decays back to the original energy state not only electron jump, but also change in rotational and vibrational energy of the molecule takes place. Consequently, on molecular spectrum not only line (as in case of atomic spectra), rather absorption band is detected – **Figure 4**. Atomic spectra consist of series of lines characteristic for the element, while molecular spectra represent a system of one or more spectral bands.

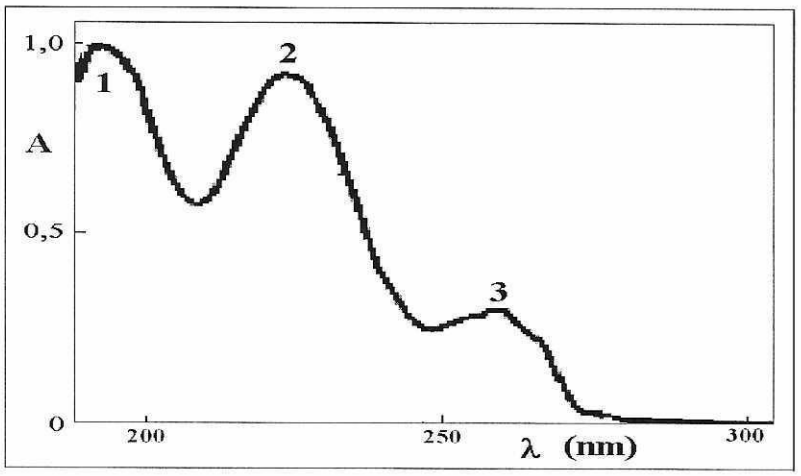


Figure 4 Measured spectrum of an organic compound (cethylpyridinium iodide) in water solution showing three absorption bands (1 – 3) in the UV region

Each spectral band has maximum absorbance at certain wavelength called the *wavelength of maximum absorbance*, designated as λ_{max}. Organic compound shown on **Figure 4** has three absorption maxima.

Absorption of radiation in molecules of organic substances

Spectral bands of molecules of organic substances are generated and specifically localized according to occurrence of some specific atomic groups (mainly atomic groups bonded by multiple bonds – $\pi \rightarrow \pi^*$ transitions), e.g. C=C, C=O, C=S, C=N, N=N, etc. These groups are called ***chromophores***. By substitution of chromophor group by a functional group of atoms with one or more lone pairs of electrons a shift of wavelength of maximum absorbance (λ_{max}) occurs. These substituents are called ***auxochromes***. Auxochromes modify the ability of the chromophore to absorb light, altering both the wavelength (colour, λ_{max}) and intensity of absorption (A). Examples include $-NH_2$, -OH, -COOH, $-SO_3H$ groups.

Types of shifts:
bathochromic - shift to longer wavelengths (red shift)
hypsochromic - shift to shorter wavelengths (blue shift)
hyperchromic - increase of absorbance values
hypochromic - decrease of absorbance values

Values of λ_{max} may be used i.a. for determination of substance structure.

Optical principles of coloured substances

Human eye senses only a small part of the electromagnetic spectrum – within the wavelength range of 380 to 780 nm. Cones of the eye retina serve as photoreceptors, containing photo pigments – colour detecting molecules, that are responsible for colour sensing. White light is made up of all of the colours of the ***visible spectrum*** – **Table 2**.

Table 2 Absorbed and complementary colours of visible light

λ [nm]	**Absorbed colour**	**Complementary colour**
650 – 780	red	blue-green
595 – 650	orange	cyan

560 – 595	yellow-green	violet
500 – 560	green	purple
490 – 500	blue-green	red
480 – 490	cyan	orange
435 – 480	blue	yellow
380 – 435	violet	yellow-green

White light can be divided into three ***primary colours***: blue, green and red. By mixing the light of these primary colours in certain proportions different shade and colour sensation is produced. According to some authors, human eye can discriminate up to 5 million shades of colours.

The ***secondary colours*** of light are magenta, cyan and yellow. These colours are obtained by mixing of any two primary colours in equal proportions. If the mixing of a primary colour with a secondary colour provides white light, the two colours are called ***complementary colours*** – **Table 2**.

Transparent materials transmit all three components to the same degree. White materials reflect incident light without absorption. Grey and black materials absorb all three components of the white light. General feature of all colour materials is the ability to absorb some component of the white light; in case it absorbs two components of the incident light, it appears to have the colour of the third component.

Absorption of radiation in molecules of inorganic substances

Electrons can occupy only specific energy levels in an atom. By absorption of radiation with energies corresponding to UV and VIS region electrons jump from occupied MOs to unoccupied (vacant) MOs with higher energy. Due to this reason, most of anorganic compounds, with ions having incompletely occupied energy levels, are coloured. Examples include compounds of subgroup elements, which oxidation degree differs from the group number or which cations are strongly polarized.

Task

Spectrophotometric determination of molar absorption coefficient (ε) of some prepared substances in solutions.

Goal

Find the absorption maximum of the spectrum obtained for chosen coloured inorganic substance or organic dye and determine the molar absorption coefficient at wavelength of maximum absorbance.

Equipment

Spectrophotometer, cuvette with path length1 cm, 10 ml volumetric flasks, pipettes, beakers.

Chemicals

potassium dichromate ($K_2Cr_2O_7$) – $M_r = 294.18$ ($c = 5 \cdot 10^{-4}$ mol dm^{-3})

potassium manganate ($KMnO_4$) – M_r = 158.29 (c = $7 \cdot 10^{-4}$ mol dm^{-3})
bromophenol blue – M_r = 670.0 (c = $5 \cdot 10^{-5}$ mol dm^{-3})
methylene blue – M_r = 319.9 (c = $5 \cdot 10^{-5}$ mol dm^{-3})
methyl red (sodium salt) – M_r = 291.29 (c = $1 \cdot 10^{-5}$ mol dm^{-3})
distilled water

Procedure

1 Measure the spectrum for the chosen substance according to the manual at the apparatus against distilled water as blank and write the values of the wavelength and absorbance to the **Table 3.**

Table 3 Measured absorbances on the wavelength
substance ....., thickness of the measuring cell d = ...m

λ [nm]	A

2 Prepare a graph of the spectrum, A = f(λ) and find the wavelength of maximum absorbance (λ_{max}) write into **Table 4.**

3 Using the stock solution of chosen substance with concentration (c_0) prepare solutions with lower concentrations (c_i), according to instruction placed at the spectrophotometer.

4 Calculate the volume of stock solution needed for each dilution using the mixing rule:

$$c_i V_i = c_0 V_0 \qquad (10)$$

where c_i is the concentration of the prepared solution
V_i is the total volume of the prepared solution (10 ml)
c_0 is the concentration of the stock solution (mol dm^{-3})
V_0 is the volume of the stock solution (ml)

5 Calculated volumes of stock solution (V_0) write into **Table 4.**

6 Pipette the appropriate volumes V_0 into 10 ml volumetric flasks and fill them with distilled water up to the volume mark.

7 Measure the absorbance (A) of prepared solutions at wavelength of maximum absorbance (λ_{max}). Write measured values into **Table 4.**

8 Prepare an unknown solution by random mixing of stock solution with distilled water. Measure the absorbance of unknown solution and write the value into **Table 4.**

9 When finished, clean properly all the glass and store it in the drawer.

Table 4 Preparation of solutions for spectrophotometric measurements
λ_{max} = ... nm **path length of the cuvette d = ...m**

Measurement	c_i (mol dm^{-3})	c_i (mol m^{-3})	V_0 (ml)	A
1				
2				
3				

4				
5				
Unknown solution			-	

Data evaluation

– Use the given data to draw a graph of spectra for chosen substance – dependence $A = f(\lambda)$.

– Use your measured values (in **Table 4**) to draw a calibration line – dependence $A = f(c_i)$.

– Calculate parameters of the calibration line; in general form:

$$y = Bx + A$$

where B – slope, A – intercept (should be equal to 0) and note also R^2 – coefficient of determination.

– Calculate the concentration of unknown solution (in mol m^{-3}) by interpolation of the calibration line. Write the obtained value into **Table 4** and convert it to mol dm^{-3}.

– Absorbance (A) is directly proportional to path length of the cuvette (d), concentration of the studied substance (c) and molar absorption coefficient (ε) - see **Equation 8**. Therefore, in the dependence $A = f(c_i)$, for given λ_{max}, slope B represents the product of molar absorption coefficient and path length of the cuvette:

$$B = \varepsilon d \qquad (11)$$

Use this formula to calculate the value of molar absorption coefficient (ε); path length of the cuvette (d) should be substituted in meters.

Report must contain:

– Theory

– Equipment and chemicals

– Procedure

– Tables and calculations

– Dependences (graphs) - $A = f(\lambda)$ and $A = f(c_i)$

– Conclusion