Experiment 12
UVVIS SPECTROSCOPY – DETERMINATION OF SALICYLIC ACID IN ASPIRIN

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>
<thead>
<tr>
<th>Radiation range</th>
<th>Wavelength scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma rays</td>
<td>&lt; 0.1 nm</td>
</tr>
<tr>
<td>X-rays</td>
<td>0.1 – 10 nm</td>
</tr>
<tr>
<td>Ultraviolet</td>
<td>10 – 400 nm</td>
</tr>
<tr>
<td>Visible</td>
<td>400 – 800 nm</td>
</tr>
<tr>
<td>Infrared</td>
<td>800 nm – 4 mm</td>
</tr>
<tr>
<td>Microwaves</td>
<td>4 mm – 0.25 m</td>
</tr>
<tr>
<td>Radio waves</td>
<td>&gt; 0.25 m</td>
</tr>
</tbody>
</table>

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

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

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

$$\tilde{\nu} = \frac{1}{\lambda} \quad [m^{-1}]$$  \hspace{1cm} (1)

- **Frequency** ($\nu$) is the number of waves per unit time, i.e. rate of wave oscillation.

$$\nu = \frac{c}{\lambda} \quad \text{or} \quad \nu = c \tilde{\nu} \quad [s^{-1} \text{ or } \text{Hz}]$$ \hspace{1cm} (2)

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

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:

$$E = h\nu \quad [J]$$ \hspace{1cm} (3)

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

- **Radiant flux** ($\Phi$) 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** ($\Phi_L$) 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 \( \Phi_0 \) passes through a transparent substance of thickness \( d \), the flux is attenuated to value \( \Phi \). Supposing no reflection (Figure 3), the flux difference \( \Delta \Phi = \Phi_0 - \Phi \) is absorbed by the substance.

![Figure 3 Absorption of radiation](image)

Transmittance \((T)\) is defined as the ratio of the flux \( \Phi \) (the intensity of light after passing through a sample) to the incident flux \( \Phi_0 \) (the intensity of light before it passes through the sample):

\[
T = \frac{\Phi}{\Phi_0} \text{ [unitless]}
\]

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:

\[
\Phi = \Phi_0 10^{-ad}
\]

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*:

\[
\Phi = \Phi_0 10^{-ac}
\]
\( \alpha = \varepsilon C \) \hspace{1cm} (6)

where \( \varepsilon \) 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 \( \varepsilon \) are \( \text{m}^2\text{mol}^{-1} \) or \( \text{cm}^2\text{mmol}^{-1} \) (when \( \text{m}^2\text{mol}^{-1} = 10 \text{ cm}^2\text{mmol}^{-1} \)).

Joining expressions 5 and 6 we get basic form of Lambert-Beer’s law:

\[ \Phi = \Phi_0 10^{-\varepsilon \alpha d} \] \hspace{1cm} (7)

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

\[ A = \varepsilon \alpha d \] \hspace{1cm} [unitless] \hspace{1cm} (8)

where \( A \) is the absorbance

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

\[ A = \log \frac{\Phi_0}{\Phi} = -\log T \] \hspace{1cm} (9)

For non-absorbing solutions: \( A = 0 \) and \( T = 100\% \)

For totally 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:

- $\pi$ (bonding MO) and $\pi^*$ (antibonding MO)
- $n$ (nonbonding MO) and $\pi^*$ (antibonding MO)
- $n$ (nonbonding MO) and $\sigma^*$ (antibonding MO)
- $\sigma$ (antibonding MO) and $\sigma^*$ (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](image)

**Figure 4** Measured spectrum of an organic compound (cetylpyridiniumiodide) 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 $\lambda_{\text{max}}$. 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 chromophore group by a functional group of atoms with one or more lone pairs of electrons a shift of wavelength of maximum absorbance ($\lambda_{\text{max}}$) occurs. These substituents are called **auxochromes**. Auxochromes modify the ability of the chromophore to absorb light, altering both the wavelength (colour, $\lambda_{\text{max}}$) and intensity of absorption ($A$). Examples include -NH$_2$, -OH, -COOH, -SO$_3$H 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 $\lambda_{\text{max}}$ 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 photopigments – 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.

<table>
<thead>
<tr>
<th>$\lambda$ [nm]</th>
<th>Absorbed colour</th>
<th>Complementary colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>650 – 780</td>
<td>red</td>
<td>blue-green</td>
</tr>
<tr>
<td>595 – 650</td>
<td>orange</td>
<td>cyan</td>
</tr>
<tr>
<td>560 – 595</td>
<td>yellow-green</td>
<td>violet</td>
</tr>
<tr>
<td>500 – 560</td>
<td>green</td>
<td>purple</td>
</tr>
<tr>
<td>490 – 500</td>
<td>blue-green</td>
<td>red</td>
</tr>
<tr>
<td>480 – 490</td>
<td>cyan</td>
<td>orange</td>
</tr>
<tr>
<td>435 – 480</td>
<td>blue</td>
<td>yellow</td>
</tr>
<tr>
<td>380 – 435</td>
<td>violet</td>
<td>yellow-green</td>
</tr>
</tbody>
</table>

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 inorganic 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.

Aspirin
The synthesis of aspirin (acetylsalicylic acid) is classified as an esterification reaction. Salicylic acid is treated with acetic anhydride, an acid derivative, causing a chemical reaction that turns salicylic acid's hydroxyl group into an ester group (R-OH → R-OCOCH₃). This process yields aspirin and acetic acid, which is considered a by-product of this reaction (Figure 5). Aspirin is a medication used to reduce pain (head- and tooth-ache, artrodynia), fever, edema, inflammation (i.a. pericarditis and rheumatic fever), prevents heart attack and stroke.

![Figure 5 Preparation of aspirin](image)

Acetylsalicylic acid hydrolyses in acidic as well as in basic medium giving its active form –
salicylic acid, which will be determined in this experiment using spectrophotometry. Salicylic acid creates in presence of ferric salts complexes, e.g. according to scheme on Figure 6, which composition depends on the pH of the medium. They can be easily distinguished due to their different colours; in acidic medium violet, in neutral medium purple and in basic medium yellow colours.

![Figure 6 Complex of salicylic acid with ferric nitrate \([\text{Fe}^{3+} (\text{KS})_3]^{3+}\)](image)

**Task**
Spectrophotometric determination of salicylic acid in aspirin

**Goal**
Measure the spectrum of salicylic acid – ferric nitrate complex (violet solution) and evaluate the wavelength of maximum absorbance \((\lambda_{\text{max}})\). Measure calibration dependence of absorbance on concentration of salicylic acid in solution and determine the amount of salicylic acid in commonly used aspirin tablet.

**Equipments and chemicals**
Spectrophotometer, electromagnetic stirrer, stirring rod, 10 ml volumetric flasks, pipettes, beakers, funnel, filter paper,
salicylic acid (2-hydroxybenzoic acid \(\text{C}_7\text{H}_6\text{O}_3\), \(M_r = 138.12\), stock solution with concentration of \(c = 6 \times 10^{-4} \text{ mol dm}^{-3}\),
ferric nitrate nonahydrate – \(\text{Fe(NO}_3)_3\cdot 9 \text{ H}_2\text{O}\), \(M_r = 404\), stock solution with concentration of \(c = 2.475 \times 10^{-2} \text{ mol dm}^{-3}\),
distilled water.

**Procedure**

**A Preparation of the aspirin for measurement**
1 Break one tablet of aspirin into halves. Put a small beaker on the analytical balance. Press
the button "TARE" and add one half of the aspirin into the beaker. Determine its mass \(m_{\text{Aspirin}}\) and write the result to Table 3.

2 Add 8 ml of distilled water into the beaker, put a stirring rod inside and place the beaker on the electromagnetic stirrer. Stir it at least 10 minutes.

**B Preparation of mixtures of salicylic acid and ferric nitrate**

3 Transfer appropriate volume \((V_0)\) of salicylic acid and ferric nitrate stock solutions \((V_{Fe})\) into 10 ml volumetric flasks according to Table 3. Fill the volumetric flask with distilled water up to the volume mark.

4 Using the mixing rule (Equation 10) calculate the concentrations of salicylic acid in the respective volumetric flasks and write the results into Table 3.

**Table 3 Preparation of solutions for spectrophotometric measurements**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>(V_0) [ml]</th>
<th>(V_{Fe}) [ml]</th>
<th>(c_i) [mol dm(^{-3})]</th>
<th>(A) at (\lambda_{\text{max}}) = nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

\(c_iV_i = c_0V_0\) \hspace{1cm} (10)

where \(c_i\) is the concentration of salicylic acid in prepared solutions

\(V_i\) is the total volume of the prepared solutions (10 ml)

\(c_0\) is the concentration of the salicylic acid stock solution \((c_0 = 6 \times 10^{-4} \text{ mol dm}^{-3})\)

\(V_0\) is the volume of the salicylic acid stock solution pipetted according to Table 3

**C Determination of absorption maximum – \(\lambda_{\text{max}}\)**

5 Measure the spectrum of the solution with the highest concentration of salicylic acid within the wavelength range of 380 – 600 nm and 5 nm step, use solution "0" (without salicylic acid) as a reference (blank). For details regarding handling of the spectrometer follow the instructions placed at the device. Write the measured values of absorbance \((A)\) at selected wavelengths \((\lambda)\) into Table 4.
Table 4 Measured absorbance values at selected wavelengths

cuvette thickness \( d \) = ............cm

<table>
<thead>
<tr>
<th>( \lambda ) [nm]</th>
<th>( A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>380</td>
<td></td>
</tr>
<tr>
<td>385</td>
<td></td>
</tr>
</tbody>
</table>

6 Use MS Excel to plot the dependence \( A = f(\lambda) \). Find the wavelength of maximum absorbance (\( \lambda_{\text{max}} \)) and write it with corresponding absorbance value into Table 3.

D Construction of calibration curve

7 Measure the absorbance (\( A \)) of remaining prepared solutions (solutions 1 – 7) at determined absorption maximum (\( \lambda_{\text{max}} \)), use solution "0" as blank. Write the measured values into Table 3.

8 Use MS Excel to plot the calibration curve \( A = f(c_i) \), where \( c_i \) denotes respective concentrations of salicylic acid in solutions. Calculate the parameter (slope) of this dependence with corresponding standard error. The intercept of the line should be set to zero according to the Lambert-Beer law.

E Determination of salicylic acid in aspirin

9 Carefully filter the solution from the beaker with partly dissolved aspirin (the whole amount will never dissolve) into 10 ml volumetric flask. Do not lose any solution!

10 Add 1 ml of ferric nitrate solution into the filtrate and fill up the gained solution with distilled water up to 10 ml volume mark.

11 Measure the absorbance of the prepared aspirin solution at determined absorption maximum (\( \lambda_{\text{max}} \)), use solution "0" as blank.

12 Using the calibration curve calculate the concentration of salicylic acid in aspirin and write the result into Table 3.

13 Using the relation for molar concentration, calculate the mass of salicylic acid in solution and write the result into Table 3.

14 Using the known mass of aspirin used for the experiment (represent 100%), calculate the percentual amount of salicylic acid in a tablet.

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

16 Prepare the experimental report.