Absorption spectroscopy
(Miklós Nyitrai, 2nd of March 2016)

Content
Light;
Electromagnetic waves and interactions;
Definition of absorption;
Absorption measurements;
Applications.

Reminder
The light is electromagnetic radiation.

What is the correlation between frequency, energy and wavelength?

Absorption in diluted solutions
- transmittance
- absorption
**Transmittance**

- Light source
- Substance
- Detector

Transmittance ($T$) is given by:

$$T = \frac{I}{I_0}$$

Usually given in %

**Absorption or light absorption**

- Sample
- Light intensity
- Distance

Absorption follows an exponential function:

$$I = I_0 \exp(-kx)$$

or

$$I = I_0 e^{-kx}$$

**Similar to the radioactive decay low!**

$$N = N_0 \exp(-\lambda t)$$

or

$$N = N_0 e^{-\lambda t}$$

**A different presentation**

- Sample
- Light intensity
- ln(distance)

Absorption also follows an exponential function:

$$\ln(I) = \ln(I_0) - kx$$

**What are the bases?**

The Born-Oppenheimer approximation; Nuclear vs. Electron motions.
Example: water molecule

The splitting of the energy levels

\[ E_{\text{sum}} = E_{\text{elec}} + E_{\text{vib}} + E_{\text{rot}} \]

The changes can be then written as:

\[ \Delta E_{\text{sum}} = \Delta E_{\text{elec}} + \Delta E_{\text{vib}} + \Delta E_{\text{rot}} \]

The splitting of energy levels

Energy scheme: band spectra

The energy scale

The magnitude of the energy types:

\[ \Delta E_{\text{elec}} \sim 1,000 \times \Delta E_{\text{vib}} \sim 1,000,000 \times \Delta E_{\text{rot}} \]

Spin multiplicity within the molecules

The number of possible quantum states (n) of a system based on the spin quantum number S:

\[ M = 2S + 1 \]

Singulet \( \rightarrow \) \( S = 0; M = 1 \)

Triplet \( \rightarrow \) \( S = 1; M = 3 \)

Excited state

Vibrational relaxation (10^{-14}s)

\[ S_0 \rightarrow S_1 \text{; intersystem crossing} \quad (10^{-10} - 10^{-8}s) \]

Excitation (10^{-9}s)

\[ S_0 \rightarrow S_1 \text{; intersystem crossing} \quad (10^{-10} - 10^{-8}s) \]

\[ T_1 \quad \text{vibrational levels} \]

Interpretation of absorption
The definition of absorption

Aim:
- easy understanding
- measurable
- additive

Note: transmittance \( T = \frac{I}{I_0} \) is not additive. A solution of 50% transmittance is mixed with a solution of 60% transmittance (keeping the concentrations) will never give a solution of 110% transmittance.

Distance dependence

\[ I = I_0 \times 10^{-\varepsilon(\lambda) c x} \]

Define parameters! Why \( \varepsilon(\lambda) \) and not only \( \varepsilon \)?

Why \( \varepsilon(\lambda) \) and not only \( \varepsilon \)?

\[ \varepsilon \text{ depends on } \lambda! \]

The definition of absorption

\[ \text{OD} = A = -\log \left( \frac{I}{I_0} \right) = \varepsilon(\lambda) c x \]

"optical density"

Absorption measurements

photometry = absorption spectroscopy

How to measure absorption?
A scheme of a photometer.
Spektrophotometer

Main components:

1. Light source
   • UV lamp (~180-350nm): Deuterium lamp
   • Visible source (~350-800nm): Wolfram
2. Monochromator: to select wavelength
3. Sample holder: e.g. a cuvette
4. Photodetektor: PMT, diode...
5. Other components: lenses, filters, slits.

How does the prism work?

The wavelength dependence of the refraction index

Photometers

Why we use reference?
The absorption of proteins

Applications: protein concentration

Measured absorption: $A$
Extinkciós coefficient: $\varepsilon$
Usual units for $\varepsilon$: $M^{-1} \text{cm}^{-1}$, or $(\text{mg/ml})^{-1} \text{cm}^{-1}$

If $A = 0.55$ and $\varepsilon = 1.1 \text{ (mg/ml)}^{-1} \text{cm}^{-1}$

$c = (A/\varepsilon)$ in mg/ml; $c = 0.5$ mg/ml

Application: gel electrophoresis

What is the advantage of additivity? (example)
Summary

- interpretation of absorption;
- definition of absorption;
- absorption measurements;
- applications.

Thank you!