Meditsiiniline keemia/Medical chemistry LOKT.00.009

Spectroscopy

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http://tera.chem.ut.ee/~koit/arstpr/spe_en.pdf

1 Introduction

Spectroscopy is a general term for methods that investigate interactions between electromagnetic radiation and matter. Spectroscopy uses electromagnetic radiation (or waves) to investigate properties of substances and for quantitative analysis of the substance.

2 Light and its interaction with matter

Light has dual nature:

1. Light can be modeled as flux of photons ("particles of light") with energy E:

$$E = h * \nu$$

In this equation ν is frequency (Hz) and *h* is Planck's constant $h = 6{,}6254 \cdot 10^{-34}$ J·s.

2. At the same time, light can be regarded as electromagnetic wave.

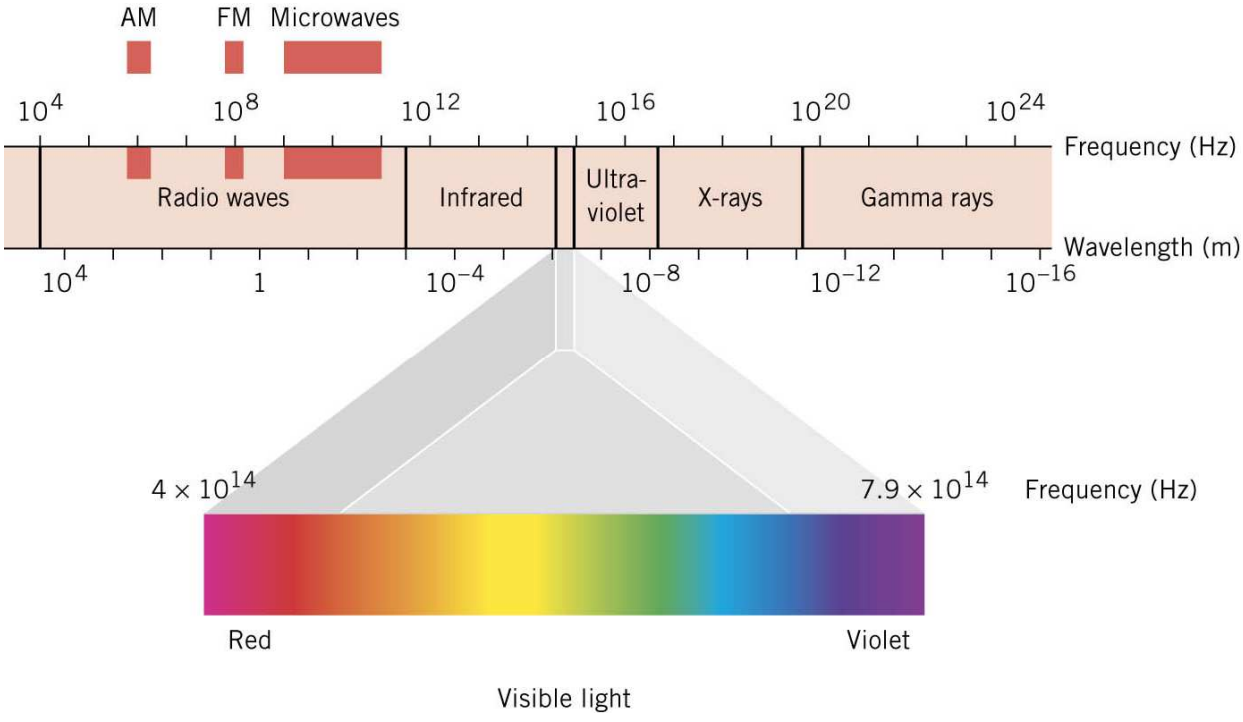

Figure 1. Scale of electromagnetic waves

Propagation of electromagnetic waves can be depicted in time or space.

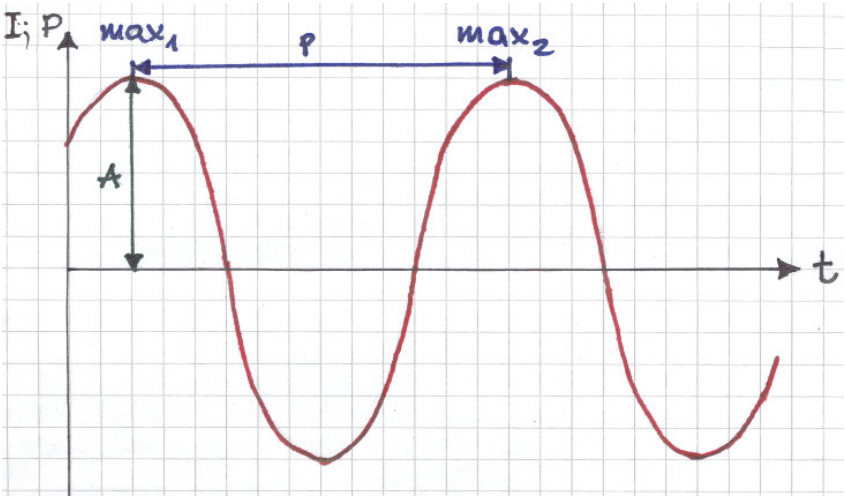

Figure 2. Electromagnetic wave on time scale

Symbols used on Fig. 2:
P – radiant power, energy of radiation impinging unit area per second (unit of measure J/s = W)
I – intensity of light, analogous to radiant power, expresses the brightness of light per unit area (cd - candela).
t – time (s - second).
A – amplitude, maximal value of radiant power of intensity for given wave.
p – period, time between two successive maxima (s - second).
ν – frequency is a number of oscillations of the field per second and it is equal to reciprocal of period (Hz - hertz).

$$\nu = \frac{1}{p} \qquad \left[\frac{1}{s}\right] = [Hz]$$

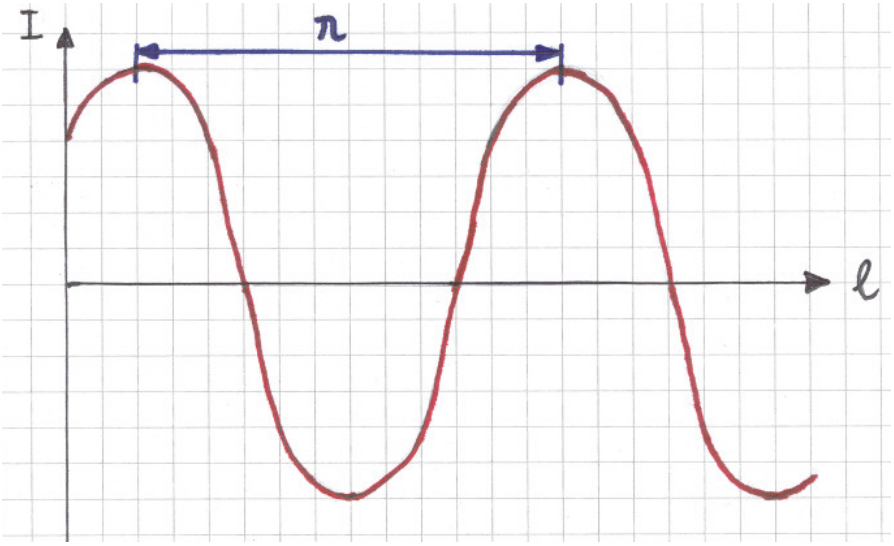

Figure 3. Propagation of electromagnetic waves in space

Symbols used on Fig. 3:
l – distance passed by light wave (m - meter).
λ_i – wavelength of light in medium i, distance between two successive maxima, (1 nm = 10^{-9} m).
v_i – speed of light in medium i (m/s).

$$\mathrm{v}_i = \lambda_i * \nu$$

One part of spectroscopy investigates light absorbed by substances. Atoms and molecules can absorb electromagnetic radiation (light). This can be rationalized as absorption of photons by molecules:

$$M + h\nu \rightarrow M^*$$

In above equation *M* stands for the molecule of substance, $h\nu$ - energy (photon) absorbed by the molecule. *M** represents excited molecule. Planck's constant $h = 6{,}6254 \cdot 10^{-34}$ J·s. Excitation of a molecule means giving it more energy (molecule passes to higher energy state). Lifetime of the excited state is usually very short: about 10^{-12} to 10^{-9} seconds. Excited molecule releases the energy and returns to the ground state. Energy is usually transferred to other molecules as heat, although the rise in temperature is too small to measure.

Different substances absorb photons with different energy. So substances can be identified using their absorption patterns. Amount of light absorbed gives information about concentration of the substance.

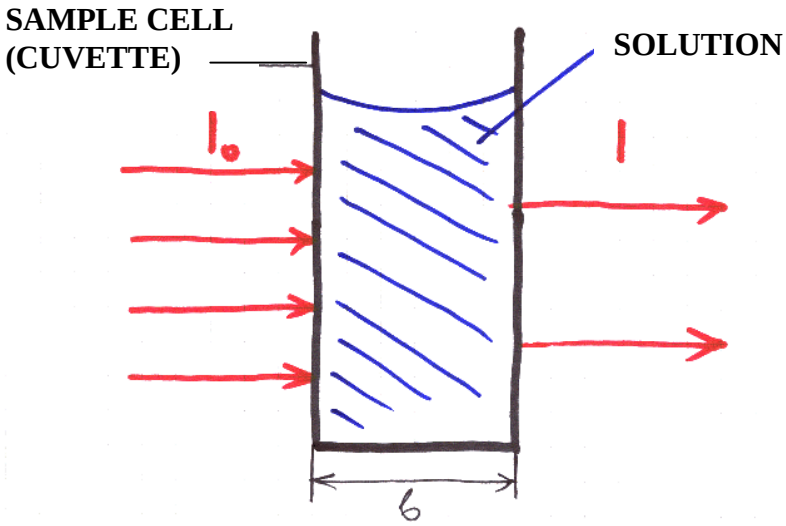

Figure. 3 Absorption of light by a liquid (solution).

On the Figure 3, I_0 is the intensity of the incident (monochromatic) light and *I* is the intensity of light after passage through the solution. Intensity *I* is always smaller than I_0 as a part of the light is absorbed in the solution. *b* is the path length of radiation through cuvette (sample cell), usually expressed in centimeters. Transmittance *T* is calculated as follows:

$$T = \frac{I}{I_0} \text{ or expressed in percentages } T\% = \frac{I}{I_0} * 100\%$$

The bigger the *T*, the more light passes through the sample.
The absorbance *A* of a solution is defined by the equation:

$$A = \log \frac{I_0}{I} = -\log(T)$$

The bigger the *A*, the less light passes through the sample.

Absorbance and transmittance are unitless measures, but absorption value is often followed by symbol AU - absorption unit.

3 Beer's law

Simply, the higher the concentration of substance in a solution and the longer the distance light has to pass in the solution, the bigger is the amount of light absorbed. Beer's law can be written as follows:

$$A_\lambda = a_\lambda * b * c$$

Where A_λ is absorbance (at given wavelength),
a_λ – absorbtivity, parameter which depends on the substance and wavelangth,
b – path length of radiation
c – concentration of the substance, often expressed in moles per liter (mol/l)

4 Absorption spectroscopy

How is spectroscopy performed? In Figure 4 is a scheme of a simple instrument for absorption measurements:

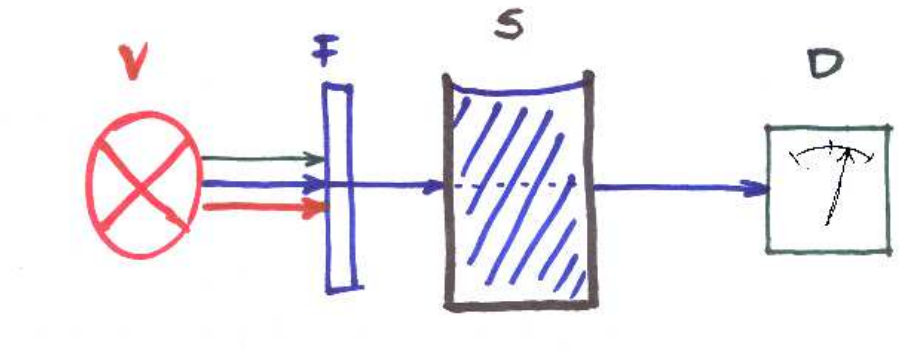

Figure. 4 Simple photometer.

Symbols used on Figure 4:
V – light source (lamp), emits polychromatic radiation
F – filter for selecting one particular wavelength
S – sample in cuvette (sample cell)
D – detector, which detects the amount of light, I passed through the sample
In order to measure I_0, the cuvette with the sample is replaced with the cuvette of pure solvent and the amount of light passed through the cuvette is measured with the detector.

Described instrument is used in practical work "Determination of albumine".

5 Atom emission spectroscopy (flame-photometry)

Practical work "Flame-photometric determination of sodium and potassium" is based on emission of light from excited atoms. On the Figure 5 is a simplified scheme of flame-photometer:

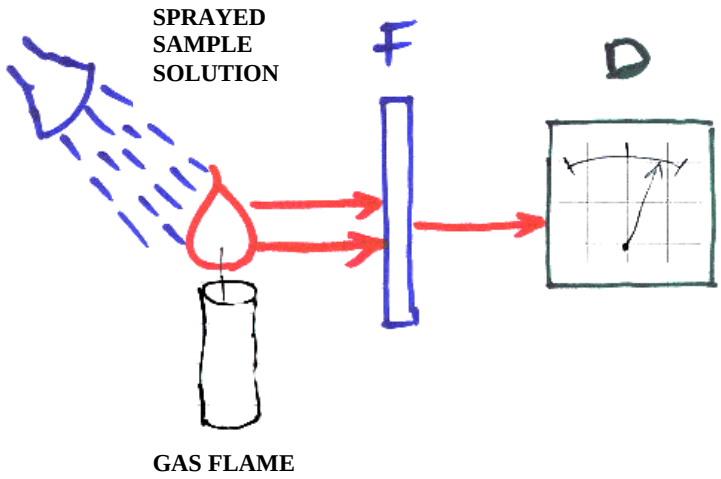

Figure 5. Flame-photometer.

Solution to be tested is sprayed into gas flame, where atoms A undergo excitation by heat. When returning to ground state photons $h\nu$ are emitted.

$$A^* \rightarrow A + h\nu$$

Energy of emitted photons is characteristic to substance. The intensity of light emitted is measured by detector D. Filter F must separate characteristic wavelength from general emission of flame. The bigger the concentration of substance in sample, the higher the intensity of light reaching the detector. So again quantitative analysis is possible.

6 Problems and exercises

1. Compare waves of light and sound. Name some common properties and differences!
2. Name the colors of a rainbow!
3. After passage through a substance intensity of light reduced 100 times. Calculate the absorbance!
4. Concentration of an optically active substance is $1 \cdot 10^{-3}$ M. If the width of the measurement cell is 1 cm, the absorbance is 1.5. Calculate the absorbtivity!

7 Method of calibration graph

In quantitative analysis, the signal measured by an instrument is dependent on analyte concentration. In order to establish which concentration corresponds to any measured signal, calibration graph must be built. Calibration graphs are always present, sometimes created manually by an operator, but modern instruments can create calibration automatically.
As an example, in the case of spectroscopy and Beer's law, the value of absorption coeficent *a* may be unknown and *b* is kept constant by using the same cuvette for all the measurements.

$$c = \frac{A}{a * b}$$

For calibration purposes the absorptions of the solutions with known concentrations of the analyte are measured. In figure 6 the four solutions with known concentrations are marked as c_1, c_2, c_3 and c_4 and measured absorptions are respectively A_1 … A_4. From these data a calibration graph created with known concentrations on abscissa and measured absorptions on ordinate axis. The absorption A_x of the sample with unknown concentration c_x is also measured. Now, after adding the value of A_x on the ordinate axis and using the line on the graph, one can find the concentration of the sample.

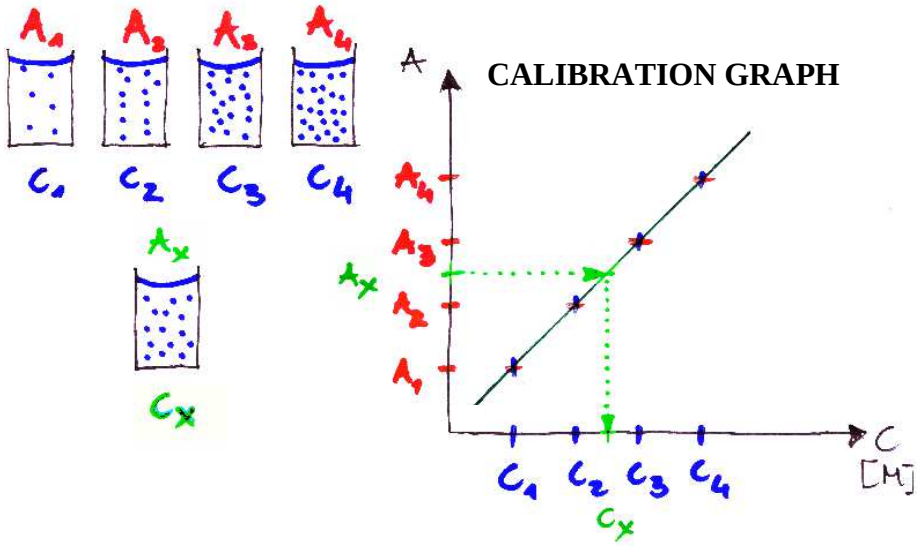

Figure. 6 Method of calibration graph in the case of spectroscopy.

The dependence between physical value and concentration can be linear, but often it is curved and/or logarithmical. To ensure better certainty of the measurement, the solutions with known concentrations are usually made with both lower and higher concentrations compared to c_x.