HUMBOLDT-UNIVERSITÄT ZU BERLIN
MATHEMATISCH-NATURWISSENSCHAFTLICHE FAKULTÄT I

INSTITUT FÜR PHYSIK • Physik von Makromolekülen

UV-VIS absorption characterization of (macro)molecular solutions

Persons in charge: See announcement in guidelines

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1. General references

[1] P.W. Atkins, *Physical Chemistry*, Oxford University Press.
[2] C.R. Cantor and P.R. Schimmel, *Biophysical Chemistry*, *PartII: Techniques for the study of biological structure and function*, Freeman, New York.
[3] A. Beiser, *Perspectives of modern physics*, Mc-Graw Hill Kogikusha LTD (1969)

2. Topic

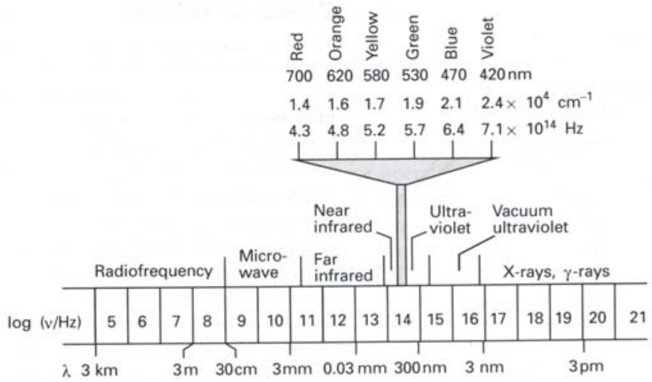
In this experiment the UV-VIS absorption spectroscopy will be used to determine the molar absorption coefficient, the concentration of a solution and the HOMO-LUMO gap of a series of oligo-*para*-phenylene. The experimental results will be interpreted also making use of simple computational models.

3. Duration

The experiment will be carried out during one full day.
The protocol is due after one week at latest !

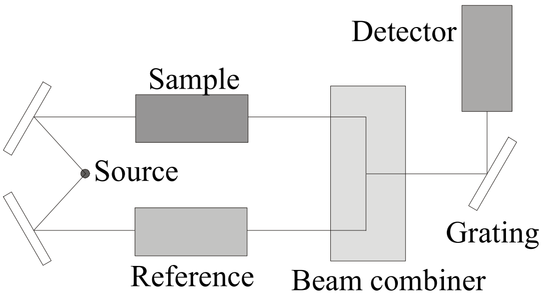
4. Introduction

The interaction of light (photons) with matter can cause a redirection of the radiation and/or a transition between the energy levels of the atoms or molecules [1,2].
Scattering: it represent a redirection of light due to its interaction with the matter; it may or may not occur with transfer of energy, i.e., the scattered radiation has a different or the same wavelength.
Emission: a transition from a higher energy level to a lower energy level is called emission if the energy is transferred to the radiation field (it is called atomic or molecular non-radiative decay if no radiation is emitted).
Absorption: it takes place through a transfer of energy from the radiation field to the atom or molecule. This induces a transition (excitation) from a state of lower energy (E_1) to a state of higher energy (E_2) which can be described by $h\nu = E_2 - E_1$ where:
ν is the frequency of the radiation absorbed;
h= Planck constant = $6.62608 \cdot 10^{-34}$ J*sec=$4.1375 \cdot 10^{-15}$ eV*sec.
The transition can be also expressed in terms of *wave length* $\lambda = c/\nu$.
The type of excitation depends on the wavelength of the light. Electrons are promoted to higher orbitals by ultraviolet or visible light, molecular vibrations are excited by infrared light (see IR experiment), and rotations are excited by microwaves.
The absorption spectrum is the absorption of light plotted as a function of wavelength. The spectrum of an atom or molecule depends on its energy level structure, and absorption spectra can be used to gain insight into the studied compounds.



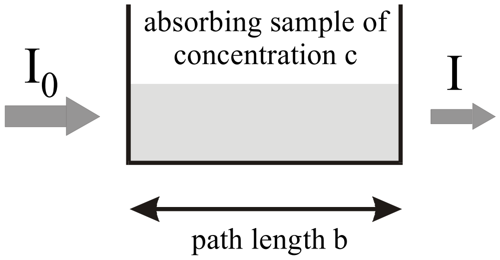
Ultraviolet and visible light are energetic enough to promote electrons to higher energy levels. UV-VIS spectroscopy is usually applied to molecules or complexes in solution. The UV-VIS spectra exhibit broad features that are of limited use for sample identification but are very useful for quantitative measurements. These measurements can be accomplished either in solution or in solid-state. The first type of measurements suffer less from scattering, providing more detailed intrinsic properties of the molecule such as e.g. the one required for the determination of the HOMO-LUMO gap (see below).
Moreover, the measurement of the concentration of an absorbing species in a solution can be accomplished by applying the Beer-Lambert Law (see below).

5. Instrumentation



The light source is usually a hydrogen or deuterium lamp for UV measurements and a tungsten lamp for measurements in the visible range. The wavelengths of these continuous light sources are selected with a wavelength separator such as a prism or grating monochromator. Spectra are recorded by scanning the wavelength separator and quantitative measurements can be obtained from a spectrum or at a single wavelength.

What is of interest is the spectrum of the investigated molecule and not that of the solvent. Thus it is the usual practice to measure the difference in absorbance between the molecule in a solution environment and a solvent blank (reference). It is most convenient the use of a double-beam spectrophotometer which can automatically record this difference.



Experimental measurements are usually executed in terms of transmittance (T), which is defined as:
$T = I / I_o$
where:
I is the light intensity after it passes through the sample and
Io is the initial light intensity.
The relation between the absorbance (A) and T is:
$A = -\log T = -\log (I / I_o)$.

Absorption instruments can usually display the data as either transmittance, %-transmittance, or absorbance.

6. Beer-Lambert law

The Beer-Lambert law rules the linear relationship between the absorbance and the concentration of an absorbing species [1]. It is usually written as:
$A = \varepsilon * b * c$
where ε is the wavelength-dependent molar absorption coefficient with units of $[M^{-1}*cm^{-1}]$;
b is the path length (path of the light in the sample, namely thickness of the cuvette), and
c is the analyte concentration.
For non-interacting molecules this parameter is independent on the concentration, and it contains the wavelength dependence of the absorption spectra.
Hence deviations from the linear relationship between absorbance and concentration (number of chromophores) can be used also as indicator of a molecular aggregation in solution.
Therefore known Limitations of the Beer-Lambert law includes:

- deviations in molar absorption coefficients at high concentrations (typically > 0.01M) due to intermolecular interactions between molecules in close proximity. This is due to changes in refractive index at high analyte concentration
- scattering of light due to particles in the sample
- fluorescence or phosphorescence of the sample
- shifts in chemical equilibrium as a function of concentration

7. Theory of the molecular orbitals: a chemical approach

The electronic levels of a molecule can be described making use of the *Theory of the Molecular Orbitals* which is based on the following [1]:
1) In a molecule composed of *n* atoms, the atomic wave functions (orbitals) interact generating *n* new wave functions, which are extended over the whole molecule. They are called molecular orbitals Ψ_j and can be approximated by a linear combination of the atomic orbitals χ_i (LCAO):

$$\Psi_j = c_1\chi_1 + c_2\chi_2 + \ldots + c_n\chi_n = \sum_{i=1}^{n} c_i\chi_i$$

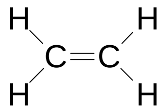
2) Half of these orbitals have an energy that is lower than the starting atomic orbitals, whilst the other half exhibit a higher energy. By analogy with diatomic molecules, the first ones are called bonding orbitals and the latter ones antibonding orbitals.

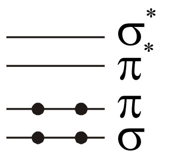
3) A molecular orbital exhibits a character of bonding between two nuclei *p* and *q* when the coefficient C_p and C_q of the related atomic orbitals χ_p and χ_q have the same sign, and antibonding when they have opposite signs.

4) The molecular orbitals are indicated using the Greek alphabet σ and π; they differ with respect to the symmetry of the wave function.; i.e. σ exhibits a high electronic density along the bond between the nuclei, while in the same region π exhibits a zero electronic density. The antibonding orbitals are indicated with a star: σ*, π*, ...

5) The electrons fill the levels in couples, exactly as in the case of the atomic orbitals (first the levels with lower energies are filled; higher spin multiplicity is favored; in each orbital only two electrons can fit, but they must have opposite spin). The total energy of the molecule can be obtained by summing the energies of the occupied molecular orbitals where each factor is multiplied by the number of electrons on the level (1 or 2);

6) In a small molecule such as ethylene the σ orbitals are formed from the interaction between a) the orbital *1s* of the hydrogens and one of the hybrid sp^2 of the carbons; b) between the two sp^2 orbitals of the adjacent carbons. The π orbitals are generated from the interaction between the $2p_z$ orbitals of the carbons.





The energies of these orbitals are quite different and their sequence at increasing energy (from the bottom to the top) is shown here beside.

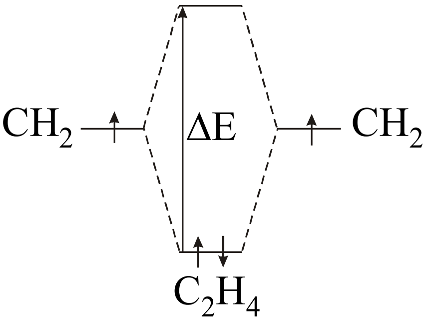
The absorption of energies takes place through transitions of an electron from an occupied level to one that is unoccupied.

7.1 Dependence of energy (λ_{max}) on conjugation

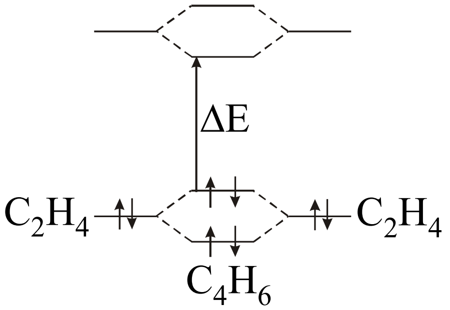
Absorption by a C=C double bond excites a π electron into an antibonding π^* orbital.

The chromophore activity is therefore due to a $\pi \rightarrow \pi^*$ transition. The energy of this transition of a non-conjugated bond is around 7 eV (λ=180 nm). When the double bond is part of a conjugated chain the transition moves to longer wavelengths and, if the chain is long enough, it can lie in the visible range.

The way you can think of this phenomenon, though it is not really 100% correct from a quantum mechanics point of view, is that you create a radical that can move the double bonds around, making resonance structures.



The progressive shift towards higher absorption wavelengths observed with the increasing number of double bounds, can be easily explained on the basis of the Molecular Orbital using the Hückel theory. Focusing only on a molecular orbital of the ethylene possessing a π symmetry, which derives from the interaction between the atomic orbitals $2p_Z$ centered on the carbons of the 2 CH_2 groups, their interaction generates two new orbitals, as mentioned above. The first one possesses lower energy doubly occupied (π known also as bonding orbital or Highest Occupied Molecular Orbital (HOMO)) and the latter one has got a higher energy which is empty (π^*, namely antibonding orbital or Lowest Unoccupied Molecular Orbital (LUMO)). The energy gap between these two levels is called HOMO-LUMO gap (E_g). In analogy the π electrons of the butadiene can be built up from the interaction between the orbital of the two ethylene fragments. One obtains in this way 4 new orbitals, two filled and two empty. The HOMO-LUMO gap is in this case smaller than in ethylene.



Following the same approach one can describe the molecular orbitals of longer alkenes.

The maximum of the absorption spectra (λ_{max}) provides a rough estimation of the HOMO-LUMO gap.

The energy required for this transition is : $E=h\nu=h*(c/\lambda)$ where the radiation that induces the transition is characterized by a frequency (ν)or wavelength (λ).

In Table 1 are listed the λ_{max} and the relative E_g for a series of alkene with increasing # of carbon atoms. It seems clear that as a consequence of the increasing λ_{max} with the increasing number of double bonds, E_g decreases. It is worth noting that also the molar absorption coefficients (and therefore also the absorbance) increases with the number of double bonds. This parameter is indeed related to the intrinsic structural properties of the molecule:

$\varepsilon=0.87*10^{20}*P*a$

where

P= probability of the transition to occur. It depends on the symmetry of the electronic states involved in the transition. It is rather difficult to calculate it; nevertheless for a polyene it is always P = 1;

a= area irradiated by the beam: it increases with the size of the molecule, consequently with the number of double bonds.

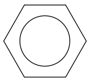
Table 1: evolution of λ_{max}, E_g and ε_{max} for a series of alkene with *n* double bonds

Alkene	**n**	**λ_{max} (nm)**	**E_g (eV)**	**ε_{max}**
Alkene	1	165	7.52	....
1,3-butadiene	2	217	5.72	21000
1,3,5-hexatriene	3	268	4.63	34600
1,3,5,7 octatetraene	4	304	4.08	....
1,3,5,7,9-decacapentaene	5	334	3.72	121000
1,3,5,7,9,11-dodecahexaene	6	364	3.41	138000
1,3,5,7,9,11,13-tetradecaneeptene	7	390	3.18	....
1,3,5,7,9,11,13,15-esadecaneoctaene	8	410	3.03	....
1,3,5,7,9,11,13,15,17-octadecanenonaene	9	447	2.78	....
β-carotene	11	455	2.73	....

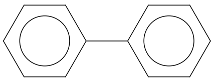
It should be pointed out that λ_{max} in polyenes also depends on other factors such as the number of cis and trans conformations and on the presence of alkyl chains linked to the conjugated system, which induce a shift of λ_{max} to higher wavelengths due to the interaction between the saturated and the unsaturated systems.

If the double bond is replaced by a triple bond λ_{max} does not change much.

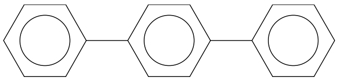
In the case of a system composed of phenyl rings as repeat units, a similar trend can be expected to occur. The system to be studied in the present laboratory is a series of oligo-*para*-phenylenes.



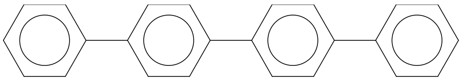
BENZENE



BIPHENYL



TERPHENYL



QUATERPHENYL

8. Particle-in-a-Box Formalism: a physical approach

The optical absorption in the case of a conjugated molecule can be treated theoretically with the "free electron" model, as proposed by Kuhn [3]. It assumes that the spectral characteristics in the visible region are determined solely by the π-electrons, which are "freely" in motion along the chain. In fact the electrons in single bonds (σ-electrons) are localized between the connected atoms, and are excited only by radiation in the UV range. This is effectively an example of a one-dimensional "particle-in-a-box" system, one of the simplest applications of quantum mechanics.

According to quantum mechanical theory, the behavior of a particle is completely specified by its wave function, Ψ, according to the Schrödinger equation. For a one dimensional (x-axis) system the Schroedinger equation is:

$$-\frac{h^2}{8\pi^2 m}\frac{\partial^2\Psi}{\partial x^2}+V\Psi=E\Psi$$

where:

m is the mass of the particle (electron in this case),

h is Planck's constant ($6{,}62608 * 10^{-34}$ J*s), and

V is the potential energy of the electron.

For this application the electron moves freely (without forces acting upon it) between two walls at x=0 and x=L. Between the walls the potential energy is constant and may be taken as zero. At the walls V rises steeply to infinity and the wavefunction must vanish. The set of wavefunctions which satisfy these boundary conditions with real phase may be written analytically:

$$\Psi=\sqrt{\frac{2}{L}}\sin\left(\frac{n\pi x}{L}\right)$$

where n (a quantum number) labels each allowed stationary state of the system and is an integer greater than zero (n = 1, 2, 3, ...). The corresponding energies are given by:

$$E=\frac{h^2}{8mL^2}n^2 \text{ ; n= 1 , 2 , etc..}$$

In the case of polyene:

m is the mass of the electron ($m_{e-}=9{,}10939 * 10^{-31}$ Kg) and *L* is roughly the length of the molecule. In the following we consider *L* as an effective length.

The effective length *L* of the box can be estimated using the C=C double, C-C single, and C-H bond lengths. The number of π-electrons (N) is 2 times the number of double bonds in the conjugated polyene. The N electrons are filling one half the number of π-electron states. The highest occupied state, the HOMO, is the one with quantum number $n_{\text{HOMO}} = N/2$ and the first unoccupied level is found at $n_{\text{HOMO}} = N/2+1$. The excitation energy corresponding to the HOMO-LUMO transition is than given by:

$$\Delta E=\frac{h^2}{8mL^2}(n_{LUMO}^2-n_{HOMO}^2)=\frac{h^2}{8mL^2}(N+1)$$

The wavelength corresponding to the transition is calculated as:

$\lambda_{\max} = hc/\Delta E$ (in nanometer).
From this one can obtain the E_g expressed in eV and vice versa.
For linear polyenes the effective length L of the molecule can be expressed in terms of the number of π electrons as
$L = Nd_0$,
where d_0 is of the order of a typical bond length.

9. Experiment and set-up

9.1. Questions to be answered

1) Determine the molar absorption coefficient (ε) by plotting the absorbance vs. the concentration of the solution. What is the range of linearity? Try to give a brief interpretation.
2) Determine the HOMO-LUMO gap for solutions with the same concentration of oligo-*para*-phenylene at increasing number of phenyl rings along the conjugated backbone. Plot the evolution of the HOMO-LUMO gap vs.number of phenyl rings *k*.
3) Using the particle in a box model determine the HOMO-LUMO gap of the three oligo-*para*-phenylenes investigated and for the series of the alkenes listed in Table 1. Compare the true HOMO-LUMO gaps with the calculated ones. Discuss how the particle in a box approximation fits in the two cases.

9.2. Materials

The system that will be investigated is a series of commercially available oligo-*para*-phenylenes including biphenyl, terphenyl and quaterphenyl.
Solvent: hexane or THF.
Crucible: quartz with a path length (b) of 2 mm. It is important to make use of a crucible that does not absorb in the range of wavelength of interest. Since it is expected that our molecules will absorb in the UV spectral range, one can not make use of glass crucibles. The capacity of the 2 mm cuvette is ca. 0.4 ml.

9.3. Sample preparation

Starting from the known concentration of the biphenyl, prepare samples at concentration of 0.5 mM, 0.2 mM, 0.1 mM, 0.07 mM, 0.05 mM in hexane.
Prepare also solutions of terphenyl and quaterphenyl at a concentration of 0.2 mM in tetrahydrofuran (THF).

9.4. How to use the spectrophotometer

The spectrometer used in this experiment is Shimadzu UV 2101 PC UV-VIS.
Turn on the PC and the spectrophotometer.
Run the file "UVPC" inside the directory "Shimadzu".
The software will start an initialization that will require a few minutes.
For the measurement of the absorbance you need to define some parameters:
Absorbance range: 0 – 2.5
Wavelength range: 400 - 190 nm
Rate of measurement: Medium
Slit: 1.0
Interval: Auto

Measure the beamline without inserting any cuvettes in both the 2 beams.
Insert the two cuvettes, in the position more near to the user put the solution cuvette and in the more distant from the user place the solvent cuvette.
Then push start and wait for the spectrum to be recorded.
Typing CTRL-R allows to rescale the absorbance axis properly.
After each spectrum is recorded, it can be exported in ASCII format on a 3.5" floppy disk. Data will be analyzed in the PMM computer lab on the Window NT system, using Origin software.

9.5. Measurement to be performed

1) Measure the absorption spectra for biphenyl samples at different concentrations.
2) Measure the absorption spectra for a constant concentration of 0.2 mM of the 4 different oligo-*para*-phenylenes.
3) Calculate and plot L versus λ_{max} for the series of oligo-*para*-phenylenes and for a series of alkene (Table 1) according to the model "particle in a box".