5.10pt

UV-VIS spectroscopy

Methods of biophysical chemistry - seminar

Jan Novotný
novotnyjan@mail.muni.cz

Spectroscopic methods - context

Complete missing items in the table:

Spectral region	Observed phenomena	Method
X-rays		
UV-VIS		
IR		
MW		
RW		

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X-rays	transitions of core e^-	absorption, diffraction, SAXS
UV-VIS	valence e^-	absorption,CD,luminescence
IR	molecular vibrations	IR spectroscopy, Raman scattering
MW	e^- spin transitions, molecular rotations	EPR, rotation spectroscopy
RW	nuclear spin transitions	NMR

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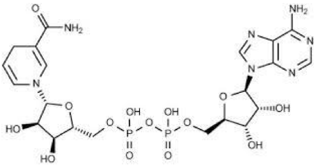
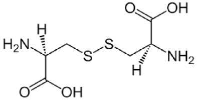
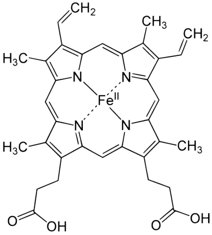
7. Two maxima: 450nm and 700nm are found in VIS spectrum of chlorophyll.
8. Measurements of electron transition in gas phase allows to observe fine rotational-vibrational splitting of resonance bands.

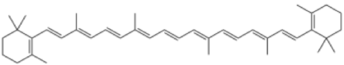
Exercise 1

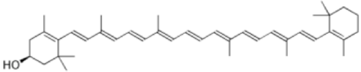
Assign the name, biochemical activity and type of transition to the following compounds:











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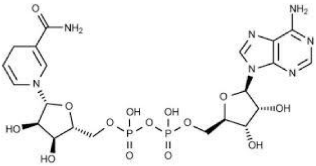
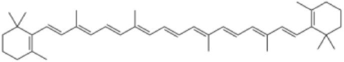
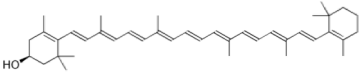


NADH - redox coffactor, absorption at 340 nm, π-π*

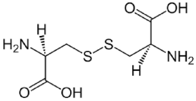


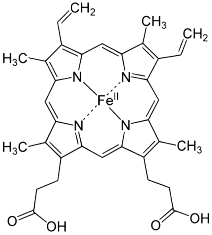
caroten - accessory orange photosyntetic pigment, protection from free radicals, π-π*



xanthophyll - accessory yellow photosyntetic pigment, protection from free radicals, π-π*

cistin - stabilisation of tertiary protein structures by disulfide bonds, n-σ*

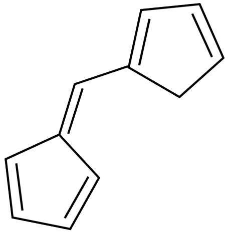


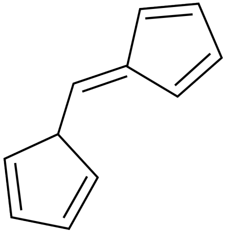


hem - hemo/myoglobin, cytochrome: d-d

Exercise 2: Conjugation

Which of the isomers is characterized by higher resonance absorption frequency?





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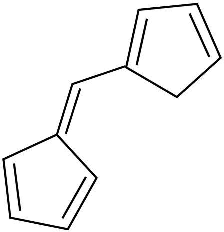

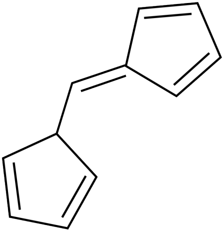

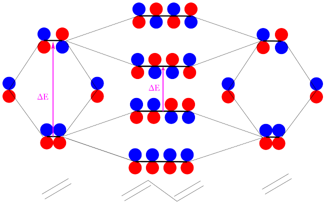

HOMO-LUMO gap gets smaller upon lengthening of conjugated system.

Exercise 3: Effect of pH on absorption spectrum

Identify the resonance wavelength and extinct coefficient corresponding to $pH < pK_a$ a $pH > pK_a$. Explain your decision.

$$A_1, \varepsilon_1 = 270nm,\ 1450$$
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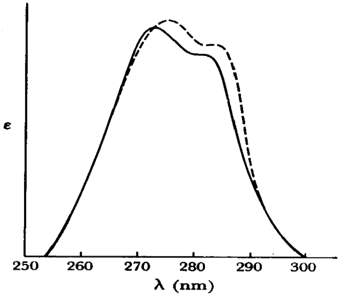
Attached absorption spectrum of tyrosine shows the effect of adding of ethylene-glykolu (resulting concentration 15%). Interpret observation using models of electronic levels.



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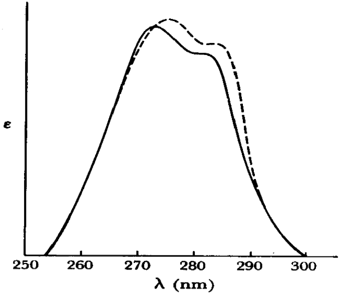
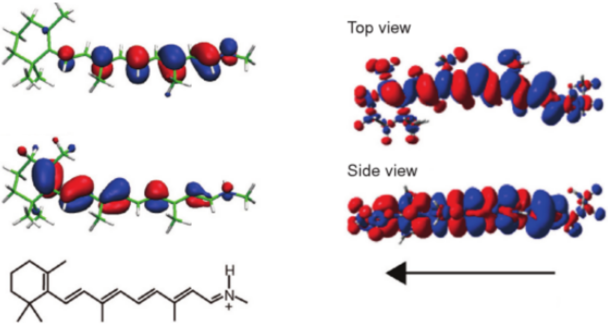


Bathochromic, Hyperchromic shift. In general, decrease in polarity of environment leads to destabilisation of more polar antibonding $\pi*$ (blueshift $\pi \rightarrow \pi*$) and lone pair n (redshift $n \rightarrow \pi*$)

Exercise 5: Retinal

The attached scheme shows the structure of vision pigment retinal. Identify HOMO a LUMO orbitals (left column). Try to estimate effect of bond to quaternary nitrogen on value of λ_{max}. Based on analysis of electron differential density (right column) determine the orientation of transition dipole moment.

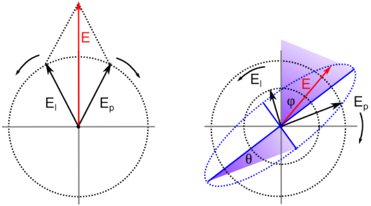


M. Mohseni, Y. Omar, G. S. Engel, M. B. Plenio: Quantum effects in biology

Exercise 6: Circular dichroism

In CD spectroscopy several quantities are used to characterize the interaction of polarised light with chiral molecules. Fill in corresponding physical relationship and unit.

Optical rotation dispersion
Circular dichroism
Elipticity



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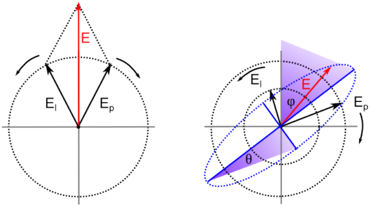
Optical rotation dispersion	$\varphi = \frac{2\pi . x}{\lambda}(n_l - n_p)$ [rad]
Circular dichroism	$\Delta\varepsilon = \varepsilon_l - \varepsilon_p$ [$\mathrm{M^{-1}cm^{-1}}$]
Elipticity	$\mathrm{tg}\theta = \frac{E_l - E_p}{E_l + E_p}$



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Molar extinct coefficient of sample at 540 nm is 268 $dm^3 mol^{-1} cm^{-1}$. 52.3% of intensity was absorbed upon passing the optical distance 7.5 mm. What was the concentration of detected compound?

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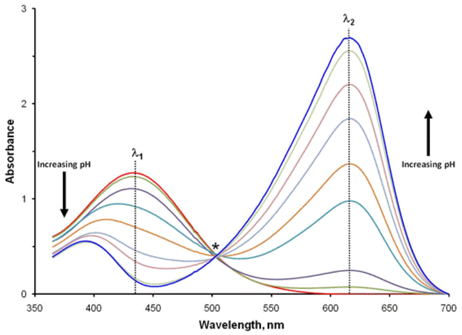
Řešení

$A = \log \frac{I_{in}}{I_{out}}$, $A = \varepsilon.c.l$

$c = \frac{\log \frac{1}{0.477}}{\varepsilon.l} = \frac{0.321}{298{,}0.75} = \mathbf{1.6}$ mM

Exercise 8: Monitoring the reaction using VIS-spectrum

The scheme shows series of VIS-spectra of bromthymol blue upon changing the pH.
a) What is the name of labelled point and how is the point defined?
b) Assign *HInd* a Ind^- entities to absorption bands.
c) Express ratio of conjageted base and acid by means of actual values of $A(\lambda_1)$, $A(\lambda_2)$ a their limited counterparts $A(\lambda_1)_{max}$ a $A(\lambda_2)_{max}$.



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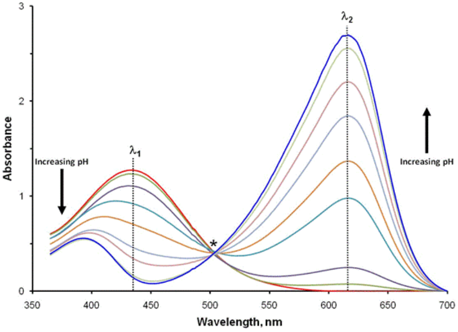

Řešení

a) isosbestic point: $\varepsilon(HInd)_{\lambda *} = \varepsilon(Ind^-)_{\lambda *}$

b) $HInd$ $\lambda_1 = 430$**nm,** Ind^- $\lambda_2 = 620$**nm**

c) $\frac{[HInd]}{[Ind^-]} = \frac{A(\lambda_1).A(\lambda_2)_{max}}{A(\lambda_2).A(\lambda_1)_{max}}$

Exercise 9: Transition moment

Assume that conjugated system of π electrons can be approximated as particle in 1D- box of infinity potential borders. A) Show relations beteween energy of excitation and the length of the box. B) Derive either analytically or graphically, that the probability of transition $n = 1 \rightarrow 2$ is non-zero whereas $n = 1 \rightarrow 3$ is forbidden. *Hint: Eigenfunction of free electron in potential box with length L has a form* $\psi_n = A.\sin\frac{\pi.n}{L}x$

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$A = \sqrt{1/\int_0^L \sin^2\frac{\pi.n}{L}x\mathrm{d}x} = \sqrt{\frac{2}{L}}$

$E_n = A^2\int_0^L \sin\frac{\pi.n}{L}x\left(-\frac{\hbar^2}{2m}\frac{\partial^2}{\partial x^2}\right)\sin\frac{\pi.n}{L}x\mathrm{d}x =$

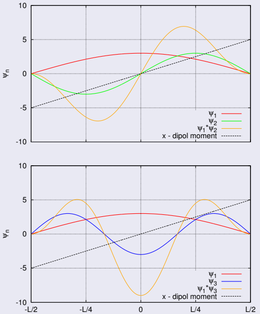
$\frac{\hbar^2}{2m}\frac{\pi^2 n^2}{L^2}\int_0^L A^2\sin\frac{\pi.n}{L}x\mathrm{d}x = \frac{\hbar^2}{2m}\frac{\pi^2 n^2}{L^2}$

$\mu_{1\rightarrow 2} = eA^2\int_0^L \sin\frac{\pi.1}{L}x.x.\sin\frac{\pi.2}{L}x\mathrm{d}x = 1$ g*u*u=g

$\mu_{1\rightarrow 3} = eA^2\int_0^L \sin\frac{\pi.1}{L}x.x.\sin\frac{\pi.3}{L}x\mathrm{d}x = 0$ g*u*g=u

$\sin\alpha.\sin\beta = \frac{1}{2}[cos(\alpha-\beta) - cos(\alpha+\beta)]$

$\int x.\cos ax = \frac{x}{a}\sin ax + \frac{1}{a^2}\cos ax$



Excercise 10: Frank-Condon factor

The ground state of model system is described by vibration wavefunction $N_0.e^{-a(x-x_0)^2}$. Calculate the Frank-Condon factor into excited state $N_1.e^{-a(x-x_1)^2}$.

Futher reading

Franz-Xaver Schmid: **Biological Macromolecules: UV-visible Spectrophotometry**
M. Mohseni, Y. Omar, G. S. Engel, M. B. Plenio: **Quantum effects in biology**
P. Atkins, J. de Paula: **Physical Chemistry**
http://www.cyut.edu.tw/w̃jchien/BiopolymerSpect/text/absorption.pdf