Basics in steady state and time resolved spectroscopy

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Synthesis of Phthalocyanines and Nanoparticles
Sensors/Electrochemistry
Photodynamic Therapy
Nonlinear Optical Materials
Content

• Steady state absorption spectroscopy
  Absorbance, purity, extinction coefficient $\varepsilon$, aggregation
• Steady state fluorescence spectroscopy
  Fluorescence, Investigation of FRET
• Time correlated single photon counting (TCSPC)
  Lifetime of first excited state, Investigation of FRET
• Laser flash photolysis
  Population and lifetime of triplet state
• Singlet oxygen luminescence detection
  Singlet oxygen quantum yield
• X-ray diffraction spectroscopy
  Structure and size of samples
• X-ray photoelectron spectroscopy
  elemental composition, chemical state and electronic state of the elements in a material
# Electromagnetic radiation

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<th>Spin change: NMR/ESR spectroscopy</th>
<th>Rotation and vibration: IR spectroscopy</th>
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<tr>
<td>Identification</td>
<td>Radio waves</td>
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<tr>
<td></td>
<td>nuclear magnetic resonance</td>
<td>electrons</td>
<td>mol. rot.</td>
<td>outer atomic shell</td>
<td>inner atomic shell</td>
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<tr>
<td></td>
<td></td>
<td>mol. vibr.</td>
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<td>atomic core</td>
<td>beta-tron</td>
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<td>synchrotron</td>
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</table>

<table>
<thead>
<tr>
<th>( \lambda / \text{cm} )</th>
<th>( 10^4 )</th>
<th>( 10^2 )</th>
<th>1</th>
<th>( 10^{-2} )</th>
<th>( 10^{-4} )</th>
<th>( 10^{-6} )</th>
<th>( 10^{-8} )</th>
<th>( 10^{-10} )</th>
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</thead>
<tbody>
<tr>
<td>( \nu / \text{Hz} )</td>
<td>( 10^6 )</td>
<td>( 10^8 )</td>
<td>( 10^{10} )</td>
<td>( 10^{12} )</td>
<td>( 10^{14} )</td>
<td>( 10^{16} )</td>
<td>( 10^{18} )</td>
<td>( 10^{20} )</td>
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<tr>
<td>( E / \text{eV} )</td>
<td>( 10^{-6} )</td>
<td>( 10^{-4} )</td>
<td>( 10^{-2} )</td>
<td>1</td>
<td>( 10^2 )</td>
<td>( 10^4 )</td>
<td>1MeV</td>
<td>( 10^8 )</td>
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</table>
Jabłoński Diagram

Absorption

Internal Conversion

Intersystem crossing

Fluorescence

Phosphorescence

Absorption

T-T Absorption

Absorption

10^{-12} s..10^{-11} s

10^{-8} s

10^{-9} s..10^{-8} s

10^{-11} s..10^{-10} s

10^{-6} s..10^{-5} s

(10^{-5} s..10^{-6} s)

(10^{-6} s..10^{-7} s)

(10^{-7} s..10^{-8} s)

(10^{-8} s..10^{-9} s)

(10^{-9} s..10^{-10} s)

(10^{-10} s..10^{-11} s)

(10^{-11} s..10^{-12} s)
Phthalocyanine

- Porphin
- Tetraaza-Porphyrin
- Phthalocyanin
- Tetrabenzoporphyrin
- Tetrapyrazino-tetraazaporphyrin
- Naphthalocyanin
- Anthracocyanin
Absorption spectrum shows the fraction of incident light absorbed by the material over a range of frequencies.
Lambert-Beer-Law

\[
I(s) = I_0 - I_A
\]

\[
I = I_0 \cdot 10^{(-\varepsilon \cdot c \cdot s)} \quad \text{or} \quad I = I_0 \cdot e^{(-\sigma \cdot n \cdot s)}
\]

\(\varepsilon\) = molar extinction coefficient, \([\varepsilon]\) = \(\text{M}^{-1} \cdot \text{cm}^{-1}\)

\(c\) = concentration, \([c]\) = mol

\(\sigma\) = absorption cross section of one mol, \([\sigma]\) = \(\text{cm}^2\)

\(s\) = pathway, \([s]\) = cm

\(n\) = number of mol

picture by University of Bremen
Determinaton of $\varepsilon$

$$I = I_0 \cdot 10^{(-\varepsilon \cdot c \cdot s)}$$

OD = $-\varepsilon \cdot c \cdot s$
Origin of spectra in Pcs

Metallated Pc ($D_{4h}$)
Metallated Pc ($D_{4h}$)

Also N, L and C bands at high energy in transparent solvents: chloroform, dichloromethane

Absorbance

Charge transfer transition

Wavelength (nm)
Unmetallated Pc complexes ($D_{2h}$)

Metallated Pc ($D_{4h}$)

e_g split - hence Q band split

$e_g$  $a_{1u}$  $a_{2u}$

Absorbance

Wavelength (nm)
H$_2$Pc spectra – Not split in basic solvents (eg DMSO, pyridine)

![Graphs showing spectra comparison between toluene and DMSO](image)
Expansion of the $\pi$ electron system

The Q band splitting of $\text{H}_2\text{Pcs}$ becomes smaller at longer wavelength.
J aggregates – edge to edge: red shifted – NOT COMMON

H aggregates – face to face: blue shifted - COMMON
Plurality of ligands and aggregation

![Graph showing absorbance against wavelength (nm)]

Absorbance

Wavelength (nm)

Not aggregated
Effects of solvents on aggregation

\[ R_2 = \text{SCH(CH}_2\text{O(CH}_2\text{CH}_2\text{O})_2\text{C}_2\text{H}_5}_2 \]

Proof of non-aggregation: Beer’s law
Proof of non-aggregation: Beer’s law

Proof of aggregation: dimer peaks decreases faster than monomer on dilution
Proof of aggregation: Effects of Surfactants

(absorbance vs. wavelength (nm))

No surfactant:

(pH 7.4)

(e.g. Cremophore EL )

surfactant:
Steady State Fluorescence

Fluorescence spectrum is plot of fluorescence intensity vs. registration wavelength (frequency, energy) at one excitation wavelength.
Steady State Fluorescence
Fluorescence

1. **Stokes Law**: a maximum of fluorescence spectrum is red-shifted compared to a maximum of the corresponding absorption spectrum. *(Reasons: Franck-Condon rule)*

2. **Mirror Image Rule**: a fluorescence spectrum (plotted in energy scale) strongly resembles the mirror image of the absorption spectrum. *(Reason: the vibrational energy level spacing is similar for the ground and excited states)*

3. **Universal Relationship (between Abs-Flu)**:
   \[ W_f(\nu) = C(T) \cdot K(\nu)_{Abs} \cdot \exp(-\hbar \nu / kT) \]

4. **Kasha-Vavilov Rule**: the fluorescence spectrum shows very little dependence on the wavelength of the excitation. *(Reasons: the emission occurs exclusively from the lowest singlet excited electronic state)*
Fluorescence

Dependency of the spectral position of a fluorescence band on time

\[ v_{fl}^t = v_{fl}^\infty + \left(v_{fl}^0 - v_{fl}^\infty\right) \cdot e^{-\frac{t}{\tau_R}} \]

\( \tau_R \) is solvent relaxation time

Low solvent polarity

High solvent polarity
Quantum Yield

- yield of product relative to amount of photons absorbed
- sum off all quantum yields in a process is \( \leq 1 \)

\[
\Phi = \frac{\text{number of product formed molecules}}{\text{number of photons absorbed}}
\]

Examples:
\( \Phi_R \) = Quantum yield of reaction
\( \Phi_{fl} \) = Fluorescence quantum yield
\( \Phi_{Ph} \) = Phosphorescence quantum yield
\( \Phi_{ISC} \) = Intersystem crossing quantum yield
\( \Phi_{IC} \) = Internal conversion quantum yield
\( \Phi_\Delta \) = Singlet oxygen quantum yield
Fluorescence Quantum Yield $\Phi_f$

- absolute measurements: spectrometer with Integrating sphere (Ulbricht Sphere)
- comparative measurements: optical spectrometer

$$\Phi_{fl} = \frac{\text{number of fluorescence photons}}{\text{number of photons absorbed}}$$
Example: comparative measurement

\[ \Phi_{sample} = \Phi_{std} \frac{I_{Sample} \, OD_{std} \, n_{sample}^2}{I_{std} \, OD_{sample} \, n_{std}^2} \]
Polarization of Fluorescence

**Polarization**

\[ P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \]

\[ r = \frac{2P}{3 - P}; \quad P = \frac{3r}{2 + r} \]

**Anisotropy**

\[ r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2 \cdot I_{\perp}} \]

**Perrin expression**

\[ P_0 = \frac{3\cos^2 \theta - 1}{3 + \cos^2 \theta}; \quad r_0 = \frac{3\cos^2 \theta - 1}{5} \]

\[ -\frac{1}{3} \leq P_0 \leq \frac{1}{2}; \quad -0.2 \leq r_0 \leq 0.4 \]

At \( \beta = 54.7^\circ \) (Magic Angle)

\[ r = r_0 \frac{3\cos^2 \beta - 1}{5} = 0 \]

Foerster Resonance Energy Transfer

- **Dipole-dipole** resonance interaction between photoexcited donor molecule (D) and acceptor molecule (A) (in the most cases A is in the ground state);

Energy of dipole-dipole interaction between donor and acceptor molecules:

\[
M_{dd} = \frac{1}{R^3} \left\{ (\mu_D \mu_A) - \frac{3}{R^2} (\mu_D \mathbf{R})(\mu_A \mathbf{R}) \right\}
\]
Foerster Resonance Energy Transfer

Rate of dipole-dipole EET

\[
k_{DD}^{DA} = \frac{9000 \ln 10 \cdot \chi^2 \Phi_0^D}{128\pi^5 n^4 N_a \tau_0^D R^6} \int I_D^n(\tilde{\nu}) \varepsilon_A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4},
\]

\[
\int I_D^n(\tilde{\nu}) d\tilde{\nu} = 1 \quad \chi^2 = (\cos \alpha - 3 \cos \theta_D \cos \theta_A)^2
\]

High probability of EET if:

- Overlap of the fluorescence spectrum of D and the absorption spectrum of A;
- High extinction coefficient of A molecule;
- Short distance between D and A molecules;
- Right orientation of transition dipole moments \((\chi^2 \neq 0)\);
- High fluorescence quantum yield of D.
Foerster Resonance Energy Transfer

Rate of dipole-dipole EET

\[ k_{DA} = \frac{1}{\tau_0^D} \left( \frac{R_0}{R} \right)^6, \]

\[ R_0^6 = \frac{9000 \ln 10 \cdot \chi^2 \Phi_0^D}{128 \pi^5 n^4 N_a} \int I_D^n(\tilde{\nu}) \varepsilon_A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4}, \]

If \( R = R_0 \), then the fluorescence of the D is quenched by a factor 2:
Factors influencing FRET Efficiency

Good spectral overlap

\[ J = \int f_{QD}(\lambda)\varepsilon_{Pc}(\lambda)\lambda^4 d\lambda \]

Förster radius

\[ R_0^6 = 8.8 \times 10^{23} \kappa^2 n^{-4} \Phi_F^D J \]

FRET efficiency

\[ \text{Eff}_{ss} = 1 - \frac{\Phi_F^{DA}}{\Phi_F} \]

\[ \text{Eff} = \frac{R_0^6}{R_0^6 + r^6} \]

\[ \text{Eff}_{tr} = 1 - \frac{\tau_F^{DA}}{\tau_F} \]

\[ \tau_i = \sum \alpha_i \tau_i \]
TCSPC

- Determination of fluorescence lifetime
- Time resolution: 60 ps up to 1 μs
- Measurements with low fluorescence quantum yield possible (<1%)
TCSPC Principle

⇒ repeat the dwell time measurement many times, “count how many photons arrived after what time”, i.e. build a histogram

⇒ time axis is not continuous, but divided into “time bins”

⇒ Note: not more than one photon per laser pulse can be registered!

Photon emission is a stochastic process!
TCSPC principle

- detection of single photons caused by a *periodic* light signal
- light intensity is so low that probability to detect one photon in one period is very low
- thus periods with more than one photon are very rare
- the time difference between laser pulse and every detected photon will be measured
- with many pulses (millions) one gets a distribution of time differences which correspond to the fluorescence lifetime
- time resolution up to 20 ps with a MCP-PMT and 150 ps with a PMT
“Start” “Stop1” “Stop2”

Laser Luminescence

N

“Pile-up” effect

TCSPC principle
TCSPC setup

Diagram showing components:
- Laser
- Beam splitter
- Sample
- Filter
- Monochromator
- (MCP)-PMT
- Photo diode
- PC
- Histogram electronic
- "Start"
- "Stop"
• PMT: pulses from photomultiplier
• SYNC: synchron pulses
• CFD: Constant-Fraction Discriminator measures the exact time of detection
• TAC: Time-to-Amplitude Converter condensator which loads up in the time between SYNC signal and PMT signal
• PGA: Programmable Gain Amplifier amplifies the TAC- output voltage with tunable factor
• ADC: Analog-to-Digital-Converter convert voltage to a number between 0 (fastest photons) and e.g. 4096 (latest)
• MEM: Memory has in our case 4096 counter, which will be increased by 1 if a photon is detected in the certain time period
Data Analysis

Fluoreszenz

Norm. Intensität

Wellenlänge [nm]
Data Analysis

2 4 6 8 10 12 14 16 18 20 22

0
2000
4000
6000
8000
10000

Zählrate

Zeit [ns]

Rodamin 6G

Apparatefunktion

45 ps

Zeit [ns]

Zählrate

Zeit [ns]
Data Analysis

Data:

Model function:

\[ f(p, t) = \left( \sum_i a_i \exp \left( \frac{t}{\tau_i} \right) \right) \otimes IRF(t - z) \]

\( d(t_i) \)

\( \chi^2 \)-value:

\[ \chi^2 = \sum_{i=1}^{N} \left( \frac{f(p, t_i) - d(t_i)}{\sigma(t_i)} \right)^2 \]
Data Analysis

\[ \tau = 3.73 \pm 0.01 \]
**Example**

![Chemical structures and graphs with data](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative $A_1$</th>
<th>$\tau_{F-1}$ (ns) $\pm 0.5$</th>
<th>Relative $A_2$</th>
<th>$\tau_{F-2}$ (ns) $\pm 0.3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdTe MPA QD</td>
<td>0.57</td>
<td>26.4</td>
<td>0.43</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>$(0.61^a)$</td>
<td>$(26.3^a)$</td>
<td>$(0.39^a)$</td>
<td>$(4.3^a)$</td>
</tr>
<tr>
<td>QD-ZnttbIPc-linked</td>
<td>0.21</td>
<td>9.6</td>
<td>0.79</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Decay Associated Fluorescence Spectroscopy (DAFS)

• Fluorescence lifetime of a homogenous species is independent from the detection wavelength

• Measurement of time resolved fluorescence at different detection wavelengths

• Data analysis at different detection wavelength is given by the model

• Global fit over all data sets

Result: Fluorescence spectra of different species with different fluorescence lifetimes
DAFS

Fluoreszenz

\[ \tau_{\text{fl}} \]
Data Analysis

Data:

Matrix of amplitude coefficients:

\[
D_\omega = \begin{pmatrix}
I_1^1 & K & I_L^1 \\
I_1^\omega & I_L^\omega \\
I_1^M & I_L^M 
\end{pmatrix}
\]

\[
S(\lambda) = \begin{pmatrix}
a_1^1 & K & a_N^1 \\
M & a_n^\omega & M \\
a_1^M & K & a_N^M 
\end{pmatrix}
\]

Matrix of lifetimes:

\[
\Pi(\tau_n, \delta t_n) = \begin{pmatrix}
\exp\left(-\frac{t_i}{\tau_1}\right) \otimes IRF(t, \delta t_1) \\
K \\
\exp\left(-\frac{t_i}{\tau_N}\right) \otimes IRF(t, \delta t_N)
\end{pmatrix}
\]

N species \quad 1 \langle i \langle N
M Wavelength \quad 1 \langle w \langle M
L time channels \quad 1 \langle l \langle L
Data Analysis

Model matrix:

\[ \tilde{D} = S(\lambda) \cdot \Pi(\tau_n, \delta_n) \]

\( \chi^2 \)-Value:

\[ \chi^2_{\text{global}} = \frac{1}{M} \sum_{\omega} \frac{1}{L} \| (D_{\omega} - \tilde{D}_{\omega}) \circ \sigma_{\omega} \|^2 \]
Example

\[ M = H_2 : H_2Pc \]

\[ M = Zn : ZnPc \]

\[ M = H_2 : H_2Pc-H_2Pc \]

\[ M = Zn : ZnPc-ZnPc \]
Example

Fluoreszenz

ZnPc-ZnPc

Anregung bei
- 740 nm
- 750 nm
- 790 nm
Example

ZnPc-ZnPc

$\tau_{fl} = 0.76 \pm 0.02 \text{ ns}$
Example

\[ \tau_{fl} = 0.76 \pm 0.02 \text{ ns} \]
Example

Fluoreszenz

$H_2Pc-H_2Pc$

![Fluoreszenzdiagramm](image.png)

Anregung bei
- 836 nm
- 860 nm
- 882 nm

Wellenlänge [nm]

Norm. Intensität
H$_2$Pc-H$_2$Pc

Amplitude [a.u.]

Wellenlänge [nm]

$\tau_1 = 0.43 \pm 0.02$ ns

$\tau_2 = 0.81 \pm 0.02$ ns

$\tau_3 = 0.87 \pm 0.02$ ns