Optical spectroscopic techniques

What happens if a sample is illuminated by light?

illuminating light (absorbed light) sample transmitted light — Absorption-spectroscopies: UV-VIS, IR

emitted light — Luminescence (Fluorescence and Phosphorescence) spectroscopies

scattered light Raman and Rayleigh scattering — Raman spectroscopy Static and dynamic light scattering
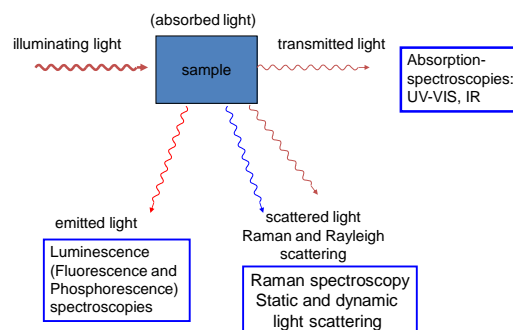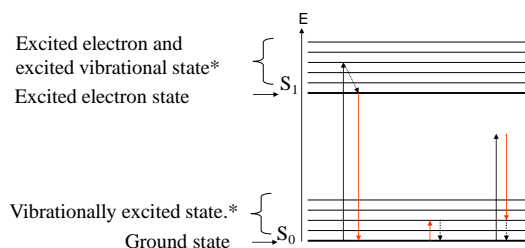

Spectroscopy
(Absorption and emission spectroscopy)

- Analysis of the wavelength dependence of the transmitted or emitted light.
- Information:
  - identification of atoms and molecules,
  - detection of changes in the molecular structure (conformation)
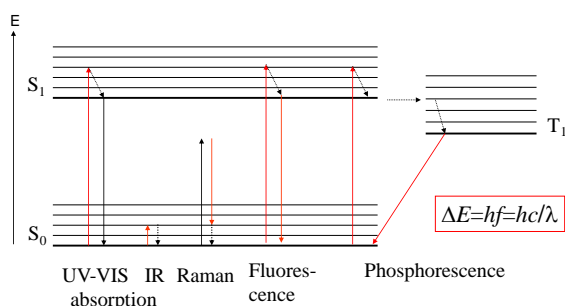 - determination of the concentration

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Why is light absorbed or emitted?

Born-Oppenheimer approximation: $E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$

Excited electron and excited vibrational state*
Excited electron state — S_1

Vibrationally excited state.*
Ground state — S_0

*only for molecules! (not for atoms)

Why is light absorbed or emitted?

S_1

T_1

S_0

UV-VIS absorption   IR   Raman   Fluores-cence   Phosphorescence

$\Delta E = hf = hc/\lambda$

Absorption spectroscopy
(UV-VIS)

As a reminder:

- law of absorption: $J = J_0 \cdot e^{-\mu x}$ where μ(material,c,λ)
- Lambert-Beer law:

- spectrum: $A(\lambda)$

$$A = \lg \frac{J_0}{J} = \varepsilon(\lambda)cx$$

- measurement: spectrophotometer (details: see pract. exc.) reference solution (J_0)
- information: identification (λ_{max}), concentration (A)

Infrared spectroscopy

- Infrared light: λ=800 nm - 1 mm
  - MIR (mid-infrared) : 2,5-50 μm
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:
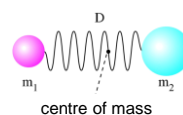 - FT spectrometer

Molecular vibrations

The electrons are light ($m_e << m_{nucleus}$), they can follow the movements of the nuclei easily, therefore the movements of the nuclei are independent of the movements of the electrons.

Classical physical description: the chemical bond is represented by a spring

Molecular vibrations:

known from elementary mechanics:

$$f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$$
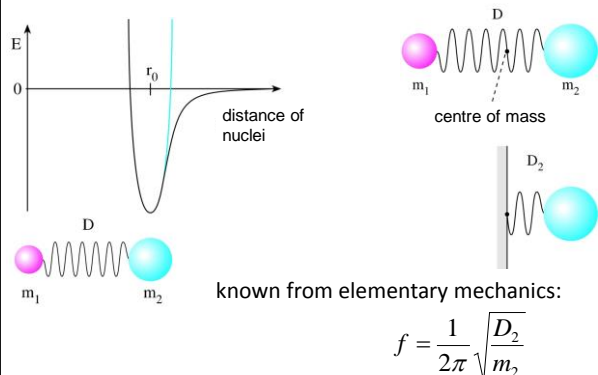
Reduced mass: $m_{red} = \frac{m_1 m_2}{m_1 + m_2}$

centre of mass

Frequency of the vibration with the reduced mass:

$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}} \qquad f = \frac{1}{2\pi}\sqrt{\frac{D(m_1 + m_2)}{m_1 m_2}}$$

The wavelength: $\lambda = \frac{c}{f} = 2\pi c \sqrt{\frac{m_{red}}{D}}$

In the IR spectroscopy the wavenumber (ν) is used, which is the reciprocal of λ:

$$\nu = \frac{1}{\lambda} = \frac{1}{2\pi c}\sqrt{\frac{D}{m_{red}}}$$
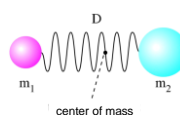
ν: number of waves in a unit length [cm^{-1}]

Example: CO
The measured wavenumber: ν= 2143 cm^{-1}
$\Rightarrow \lambda$=4,67μm $\Rightarrow f$=6,43 10^{13} Hz
m_C=2·10^{-26} kg, m_O=2,7·10^{-26} kg $\Rightarrow D$=1875 N/m

if ν is known, D can be calculated
if D is known, ν can be calculated

Classical vs. quantum physics

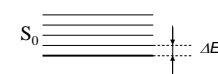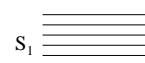Classical physical picture

Quantum mechanical picture

center of mass

$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$$

resonance with the light with frequency f
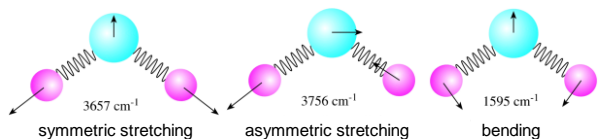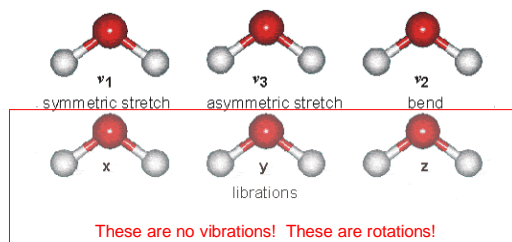
S_1

S_0 ΔE

$\Delta E = hf$

Normal vibrations

All the atoms vibrate
• with the same frequency but
• with different amplitude and
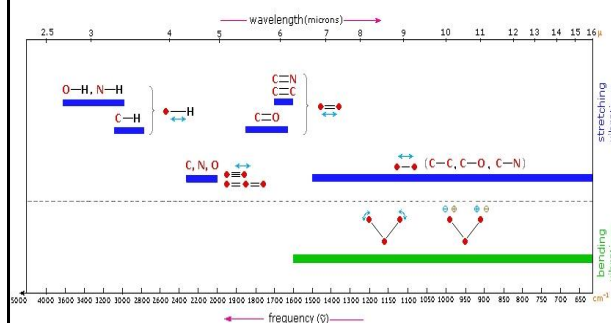• in different direction.

Example: water

3657 cm^{-1} — symmetric stretching
3756 cm^{-1} — asymmetric stretching
1595 cm^{-1} — bending

Normal vibrations of water

ν_1 symmetric stretch
ν_3 asymmetric stretch
ν_2 bend

x
y
z
librations

These are no vibrations! These are rotations!

Vibrations of the large molecules

Molecule consisting of N atoms:
• 3N degree of freedom,
    3-3 are the rotations and translations of the whole molecule
• 3N-6 vibrational degree of freedom (3N-5 for the linear molecules)
• 3N-6 independent normal vibrations

Typical vibrational frequencies (wavenumbers)

Example: Formaldehyde
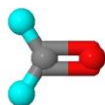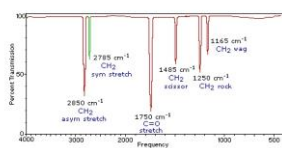
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

Flavin

Benzol

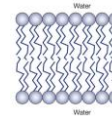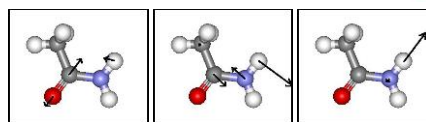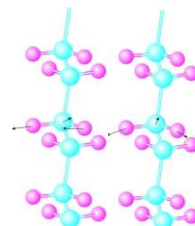antisym. υ   sym. υ   scissoring δ   wagging δ   twisting δ   rocking δ
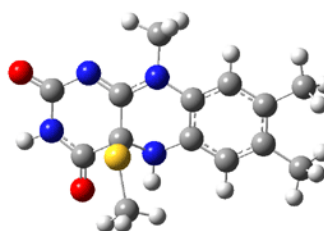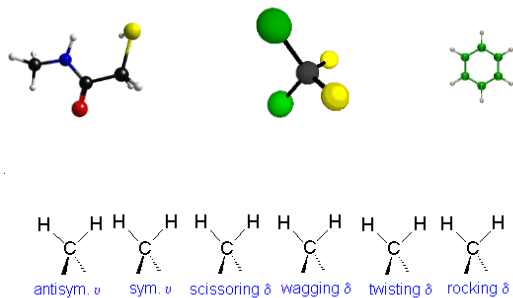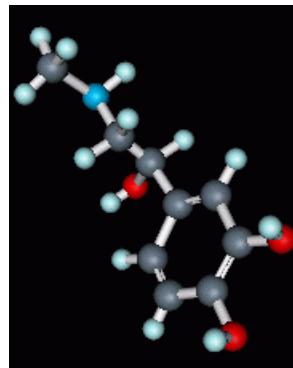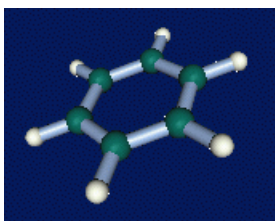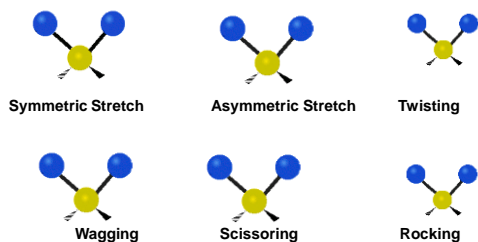
Vibrations of the macromoleclues

Complex global vibrations
Localised vibrations, e.g.:

- CH$_2$ vibrations of the lipids
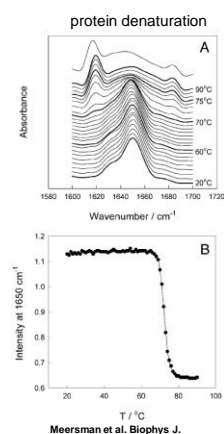
- amid vibrations of proteins
  (acetamide)

Lipids

Symmetric Stretch   **Asymmetric Stretch**   **Twisting**

Wagging   **Scissoring**   **Rocking**

Types of Vibrational Modes. Figure from Wikipedia

Applications

protein denaturation

phase transitions in lipids

Tsvetkova és mtsai PNAS 2002 | vol. 99 | no. 21 | 13504-13509

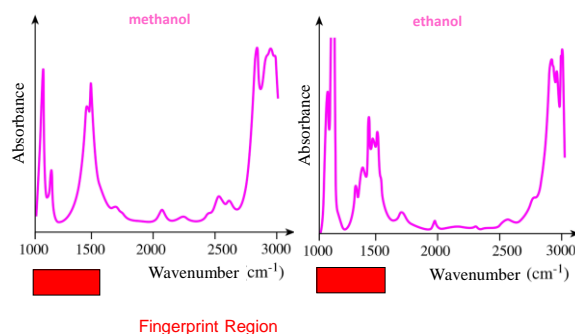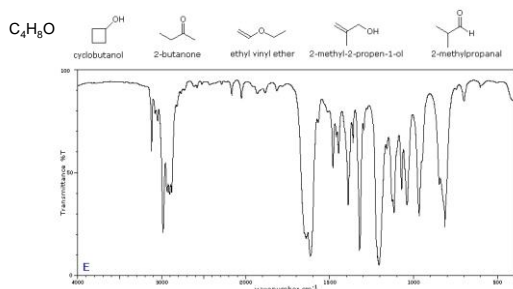Meersman et al. Biophys J.

4
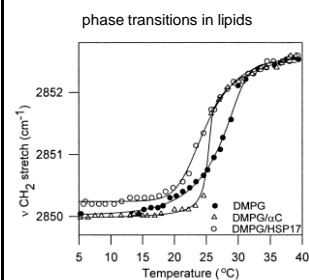
Pharmaceutical applications

- synthesis: identification of the intermediate and the end product
- determination and justification of the molecular structure
- detection of the metabolites
- quality control (purity)

- Remark.: Lambert-Beer law is valid, determination of concentration is possible
- IR active vibration: dipole moment changes

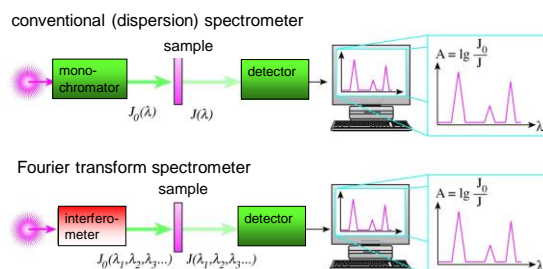## Identification of the molecules

Fingerprint Region
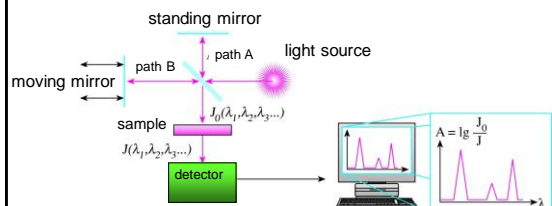
Example: Identification of molecules

C_4H_8O

http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

The technique of the measurement : Fourier transform spectrometer (FTIR)

conventional (dispersion) spectrometer
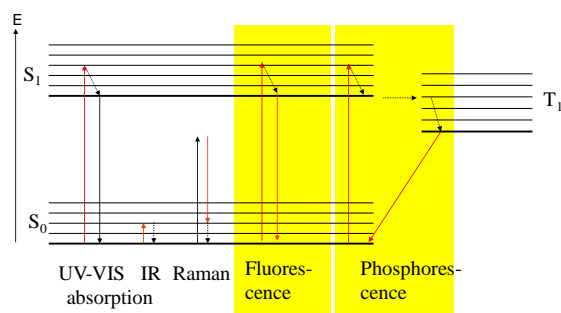

Fourier transform spectrometer

book 6.17

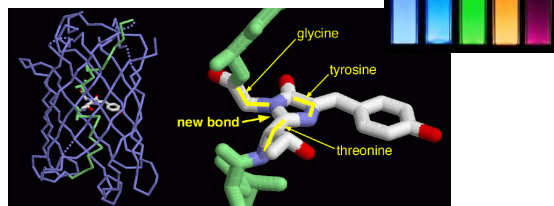## Interferometer

book 6.18

Luminescence spectroscopy

Which molecues are fluorescent?

Amino acids (tryptophan, tyrosine, phenylalanine)
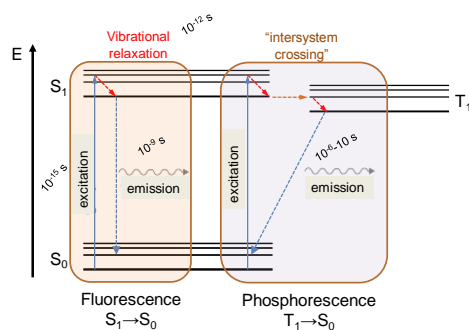Fluorescent dyes
GFP
...

Measurable quantities in Fluorescence Spectroscopy

- Wavelength of the exciting light
- Wavelength of the emitted light (fluor., phosph.)
- Time dependence of the emitted light
- Polarisation of the emitted light
- Intensity of the emitted light

Luminescence emission of molecules

Jablonski diagram

Luminescense

Quantum yield (Q_F):

$$Q_F = \frac{k_f}{k_f + k_{nr}} = \frac{\textit{number of photons emitted}}{\textit{number of photons absorbed}}$$
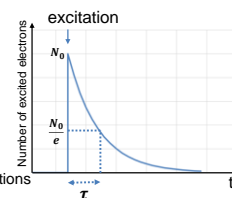
(Dyes, fluorescense markers Q≈1)

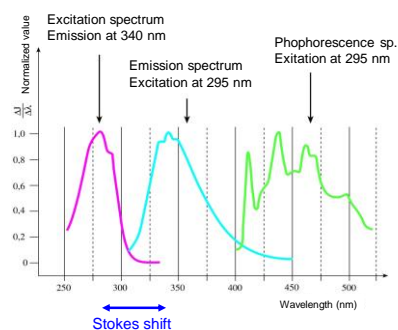**Luminescense lifetime(τ):**

$$N = N_0 e^{-\frac{t}{\tau}}$$

$$\tau = \frac{1}{k_f + k_{nr}}$$

k_f: Rate of photon producing transitions

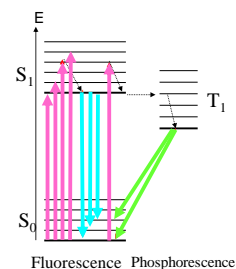k_{nr}: Rate (probability) of non-radiative transitions


Stokes-shift

6.25.

Kasha's rule

Kasha's rule: The excited molecule first reaches the lowest vibrational level and photon emission occurs always from this state to any vibrational level of the ground (S_0) state.
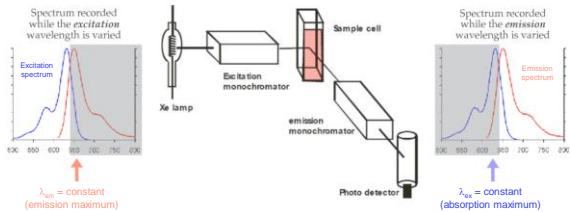
Comparing emission processes

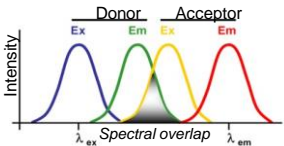|	Fluorescence	Phosphorescence
Relaxation	From singlet state $S_1 \rightarrow S_0$	From triplet state $T_1 \rightarrow S_0$
Lifetime (τ)	Nanoseconds	From microseconds up to seconds
Stokes shift	Smaller	Larger (because T_1 is at lower energy than S_1)
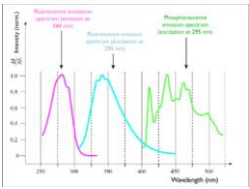
Measurement of luminescence

Spectrofluorimeter

FRET

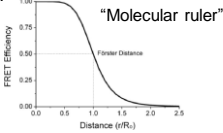**F**örster **R**esonance **E**nergy **T**ransfer

Energy transfers from donor without emission to acceptor in dipole-dipole interactions. Requires spectral overlap between donor emission and acceptor absorption.

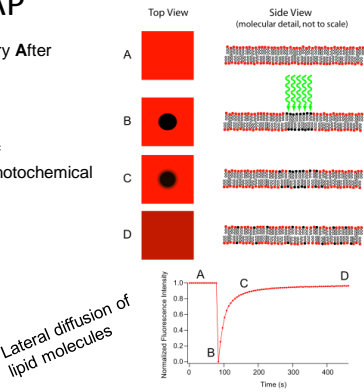FRET efficiency or quantum yield (E):

$$E = \frac{1}{1 + (r/r_0)^6}$$

r_0: Förster distance

"Molecular ruler"

FRAP

Fluorescence **R**ecovery **A**fter **P**hotobleaching

Photobleaching:
The permanent loss of fluorescence due to photochemical reactions.

Lateral diffusion of lipid molecules

Fluorescence microscope
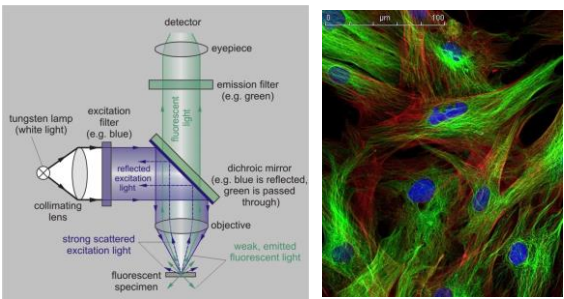
Fluorescence polarization

illumination with polarized light

polarization degree of the emitted light is measured

The fluorescent molecule can rotate between the absorption and the emission ⇨
dynamic information: rotational correlation time
(how fast is the rotational diffusion?)

6.28

View of localized region in peritoneal cavity of an ovarian cancer patient as seen with the naked eye (left) or with the aid of a tumor-targeted fluorescence dye (right).
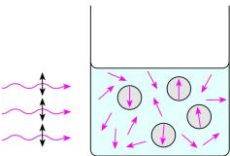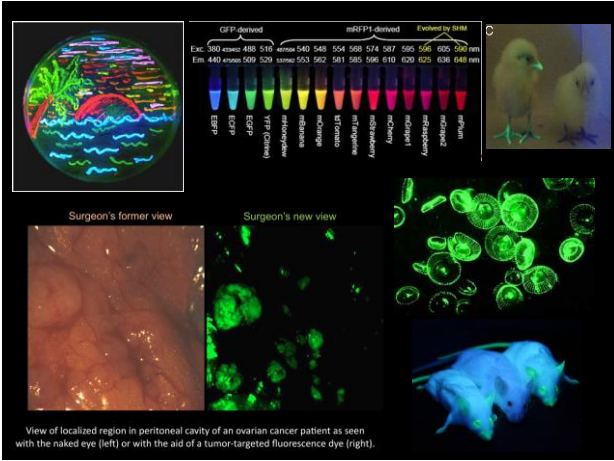
Surgeon's former view   Surgeon's new view

Light scattering

Rayleigh
$\lambda_{scatt} = \lambda_{illum}$
(elastic)

Raman
$\lambda_{scatt} \neq \lambda_{illum}$
(non-elastic)

Chandrasekhra Venkata Raman

Raman scattering:
$\lambda_{scatt} \neq \lambda_{illum} \Rightarrow f_{scatt} \neq f_{illum}$
$\Rightarrow E_{photon,scatt} \neq E_{photon,illum}$

Where is the energy?
Excites vibrations of the molecule (as IR)
very weak (~10^{-8})

E
S_1
S_0

IR   Raman

Raman Spectrometer

Probe

Filter

Laser

Detector

Monochromator

Raman-Scattering

Vibrations are specific for the molecules

⬇

Raman Spectroscopy

E.g.: active agent content of a tablet

Intensität [w. E.]

corn-starch

cellulose

active agent

Ramanshift [cm⁻¹]

http://www.igb.fraunhofer.de/de/kompetenzen/grenzflaechentechnik/oberflaechenanalytik/ausstattung/konfokale-mikroskopie-spektroskopie/raman-spektroskopie-tablette.html

PHARMACEUTICAL APPLICATIONS OF RAMAN SPECTROSCOPY

Edited by SLOBODAN ŠAŠIĆ

WILEY

Handheld Raman spectrometer for identification of materials
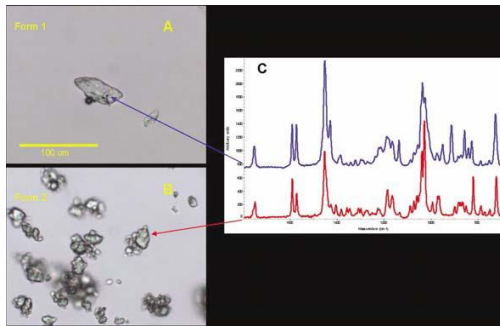

8

Pharmaceutical application

Rayleigh scattering

Size of the particle: $d \ll \lambda$

The scattered intensity:

$$J_{scatt} \sim J_0 N \frac{d^6}{\lambda^4}$$

Information: size, contentration (quantity)
(e.g. colloids)

Meaurement of the Rayleigh scattering

if $J_{scatt} \ll J_0$

J_{scatt} is measured
(Nephelometry)

If $J_{scatt} \approx J_0$

J is measured
(turbidimetry)

The same technique as for the absorption spectrosopy but now J is reduced due to the scattering (and not due to absorption).