

A 60 Fluorescence Spectroscopy

Duty:

The main purpose in this experiment is to determine the absorption and fluorescence spectra of Rhodamine 6B dissolved in ethanol. In the related spectra the wavelengths should be determined at which the absorbance and fluorescence reach the maximum values, λ_A and λ_F , respectively. The corresponding photon energies $h\nu_A$ and $h\nu_F$ are significantly shifted. You have to explain this shift in terms of the molecular structure of the absorbing/emitting species.

Operational safety

Chemicals used here: Rhodamin 6G, Ethanol

The components of the experimental setup: spectrometer, optical fiber, light emitting diodes, UV-light source (deuterium lamp), computer.

Theoretical Backgrounds

Absorption:

A molecule absorbing a photon (in visible or ultraviolet spectral range) becomes transferred from the ground into an electronically excited state. In terms of the quantum mechanics the intensity of this transition (\sim the probability for the $|g\rangle \rightarrow |e\rangle$ event) is directly related to the transition matrix element which bridges the vibrational wave functions of the electronic ground und excited states. The intensity of the photon absorption scales with the overlap integral (Franck-Condon factor). The quenching of the light intensity as observed when the light beam passes a cuvette filled with dissolved dye, $I_0 \rightarrow I$, is well described by the Lambert-Beer's formula (see also the experiment A34): The absorbance A defined as $\log(I_0/I(d))$ is proportional to the product of the optical path length d (the width of the absorbing layer) and the concentration of dye molecules in the solution c . The proportionality constant ϵ represents the molar extinction coefficient. (see formula 1)

$$A = \log(I_0/I) = \epsilon c d \quad (1)$$

Please note the difference between the absorbance A defined by equation (1) and the absorption which can be directly related to the transmission T : $A = 1 - T$ where $T = I/I_0$. The absorbance is also termed by *optical density* (OD). A similar distinction has to be pointed for the terms *Extinction* and *Absorbance*. Whereas the extinction mirrors the whole quenching of the light intensity as originated from (1) the resonant absorption, (2) the resonant and non-resonant light scattering, and (3) the back reflection, the absorbance stays for wavelength-dependent light absorption exclusively. Note, the experiments performed with monochromatic light (nearly one wavelength λ) evidence that both A and ϵ strongly depend on the wavelength λ , $A(\lambda)$ and $\epsilon(\lambda)$.

Fluorescence

After absorbing optical radiation (visible light) the dissolved molecules become transferred into an electronically excited state. After completing the vibrational relaxation cascade the molecules undergo a radiative back transition between the electronic excited $|e\rangle$ and the ground $|g\rangle$ states. According to Kasha's rules the radiative transition takes place from the vibrational ground state belonging to the lowest excited electronic state.^[5] Due to the fact that usually the interatomic distances (binding lengths) in the excited molecules are significantly longer than in the ground state the related potential surfaces are more separated from each other. Consequently, one usually observes mirror-image-like distributed absorption and emission spectra. Usually a simple fluorescent transition has to compete with the inner conversion as well as with the "inter-combination" processes. The later can occur when the so called *intersystem crossing* is involved, ISC, e.g. when spin inversion takes place between a singlet and triplet state and both are non-radiative processes. A simple measure of the fluorescence efficiency is the quantum yield.

Realization of the Experiment

The measurements will be performed by means of a modular constructed fluorescence spectrometer which can be simply rebuilt for recording the absorption spectra as well. The excitation of the fluorescence is provided by several light emitting diodes which are used here

as the nearly monochromatic light sources (differing by the wavelength λ_{ex} of the exciting light). The absorption will be measured for light emitted by two lamps: Tungsten-Halogen and deuterium lamp. The cuvette filled with the proper solution (rhodamine 6G in ethanol) is ready to be used. Both the absorption and fluorescence spectra have to be recorded first and further processed according to the following scheme:

- (1) The spectra will be recorded by means of the software package *SpectraWiz*. The related data should be stored on the USB-Stick and for further analysis transferred into a data processing program (e.g. Origin).
- (2) Prior to start the measurements you have to install a new folder in the directory **►Fluoreszenzspektroskopie►A60**. The folder should be named by the current date and your *Gruppennummer*. The data gained during the experiment can be easily stored in this folder.
- (3) In the next step the program *SpectraWiz* should be activated and the spectrometer should be rebuilt in such a way that the absorption spectra can be detected.
- (4) Prior to record the absorption spectra one has to perform a so called *dark* and a *reference* measurement.
- (5) Switch the program located in the directory **►View** to **►Scope** and chose the recommended exposition time of the sensor in **►Setup►Detector integration time** (see Fig. 1) (a value around 70). The value chosen has to be kept constant in all following experiments otherwise all foregoing steps have to be repeated!
- (6) The next measurement will be performed without the sample (cuvette) and without light (see Fig. 2) simply by activating the **Dark Scan**.
- (7) Now the cuvette filled with the dye solution should be positioned in the sample holder and the reference measurement can be started by activating the **Reference Scan** (see Fig. 3).
- (8) By switching the view under **►View►Absorbance** the absorption spectrum of right sample will be recorded.
- (9) The data can be stored in the directory previously installed under **►File►Save►Sample**.

Recording the fluorescence spectra

- (1) First the spectrometer has to be rebuilt for recording the fluorescence spectra. Similar to the absorption measurements the fluorescence spectra have to be recorded after taking the related *dark* and *reference* spectra. However, before starting the reference measurement one has to set a new exposition time in ► **Detector integration time** (a value around 500).
- (2) During recording the fluorescence spectrum one has to keep the ► **Scope-Mode** activated.
- (3) The spectrum can be stored in the directory previously installed and further converted onto the under your personal USB stick.

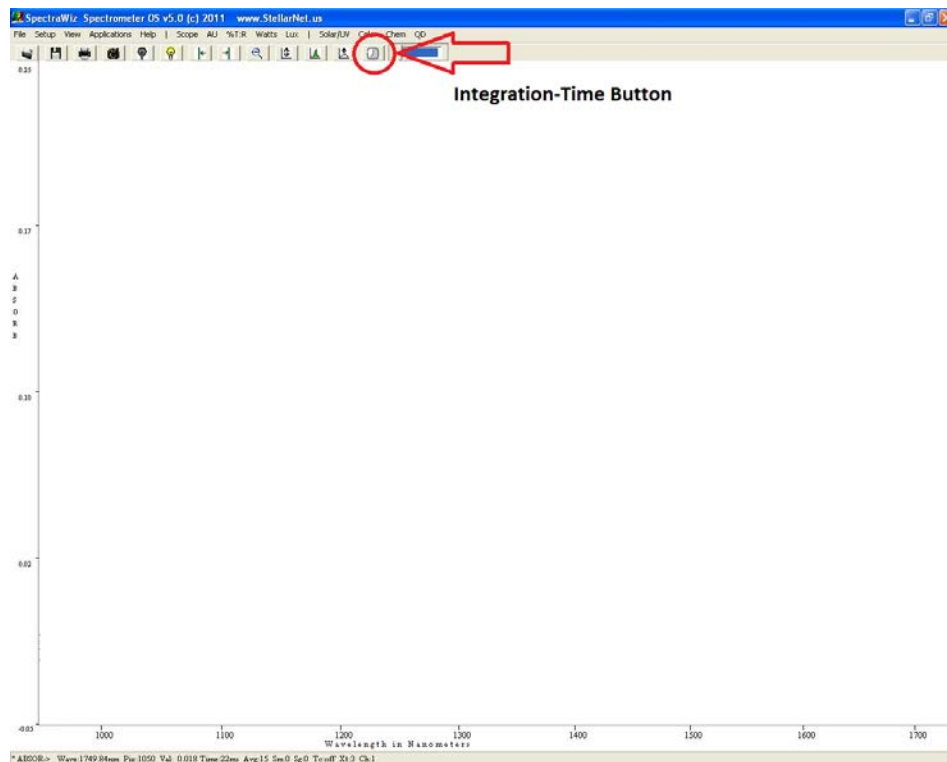


Fig. 1: Change the integration time

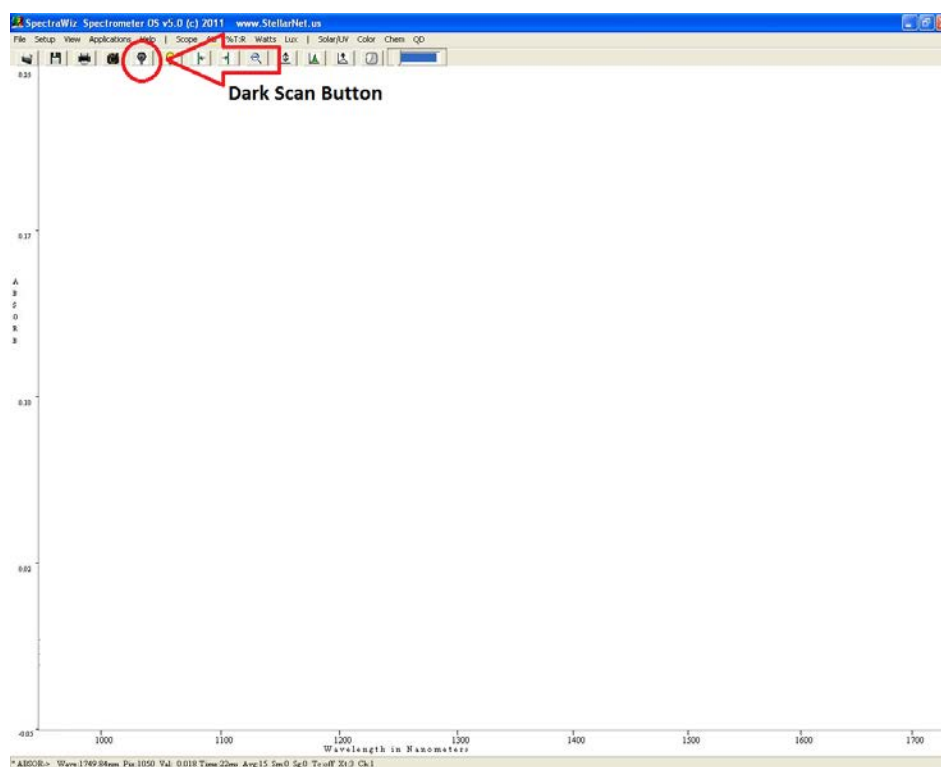


Fig. 2: Recording the *dark* scan

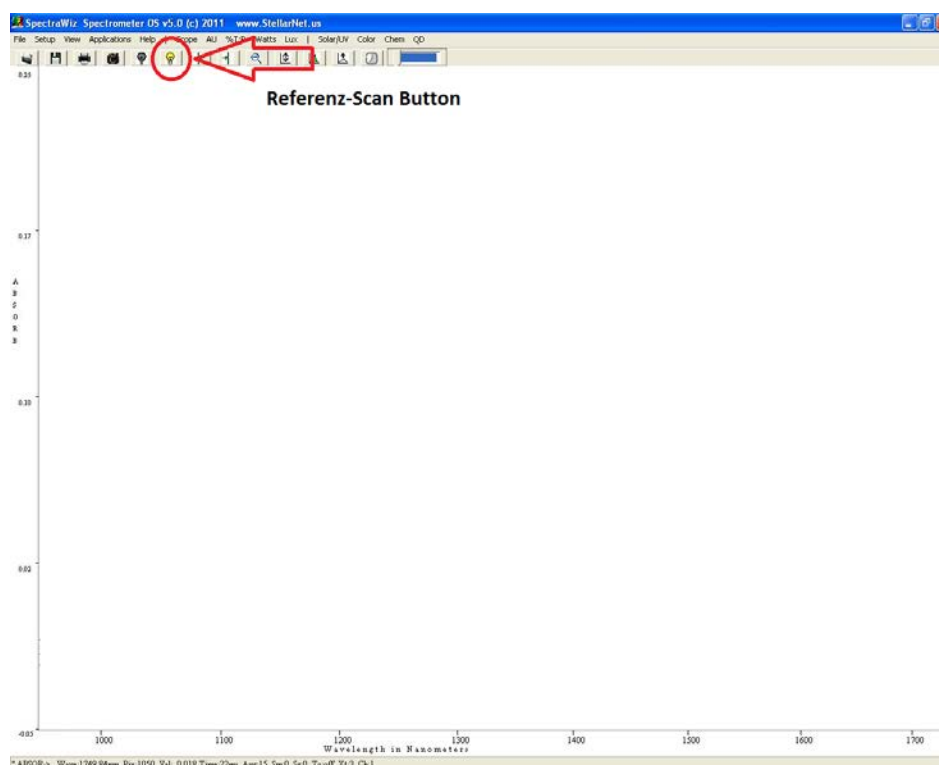


Fig. 3: Recording the *reference* scan

Data analysis and discussion

We start with two drawings of the setup used for recording the absorption and fluorescence spectra.

1. Basing on the absorption measurements one has to prepare a plot, the optical density (OD) versus wavelength λ (in nm.) and determine the dye concentration in the solution. The optical path length in the cuvette used is 1 cm and the extinction coefficient for Rhodamine 6B can be taken from the related literature.
2. The absorption spectrum should be replotted as a function of the photon energy $h\nu$, $I(h\nu)$. In the next step you should find the photon energy $E_A = h\nu_A$ corresponding to the maximum in the absorption spectrum, $I_{\max}(E_A)$. Please calculate also the corresponding wavelength λ_A .
3. The intensity of the fluorescence spectrum should be plotted both, as a function of the photon energy $E=h\nu$ and as a function of the wavelength λ . For both the intensity maximum I_{\max} should be determined and the related photon energy E_F and the wavelength λ_F derived.
4. Now the difference between the E_A and E_F as well as between the related λ_A and λ_F values should be determined. (ΔE and $\Delta\lambda$, Stokes's shift)
5. The error in the determination of the ΔE and $\Delta\lambda$ should be estimated.
6. The results obtained here should be discussed in terms of the related literature data.
7. One should explain the mirror image symmetry found here for the shape of the absorption and emission spectra taken for the same substance.

Please do not forget to cite the literature sources you used in the data analysis and discussion. (It is simply forbidden to cite internet sources, allowed are scientific journals and regular textbooks)

Further Questions

A scheme of the Jabłoński diagram should be prepared. Basing on this diagram one should describe the relaxations cascades triggered by the light absorption event. The following topics should be described and explained in detail: (1) the absorption process (matrix element, transition probability, symmetry of the involved wave functions, selection rules); (2)

vibrational relaxation in the electronic ground and excited states; (3) the internal conversion, IC; (4) the fluorescence, (5) the intersystem crossing, ISC; and (6) the phosphorescence.

Knowledge required

Topics: Absorption; Franck-Condon transition und F-C factors; the relation between the overlap integral and the extinction coefficient; Lambert-Beer relation; Fluorescence; Kasha's rules; optical radiation; radiative and non-radiative processes; quantum yield, Stokes's shift, Boltzmann distribution; the relations between the energy, wavenumber and wavelength; operation of an optical wave guide.

*This experiment has been financed by the **KIT-Fakultätslehrpreis 2014** gained by the organizers of the practical courses in the physical chemistry for beginners (Dozenten des A-Praktikums).*

Literature

- [1] ATKINS, DE PAULA; Physikalische Chemie, 5. Auflage, Wiley-VCH Verlag GmbH & Co. KGaA, 2013
- [2] WEDLER; Lehrbuch der physikalischen Chemie, 6. Auflage, Wiley-VCH Verlag GmbH & Co. KGaA, 2012
- [3] SCHMIDT; Optische Spektroskopie: Eine Einführung, 2. Auflage, Wiley-VCH Verlag GmbH & Co. KGaA, 2000
- [4] U. Brackmann, *Lambdachrome® Laser Dyes*, 2nd Edition, Lambda Physik GmbH, Göttingen, 1994
- [5] M. Kasha, Characterization of electronic transitions in complex molecules, Discuss. Faraday Soc., 1950, 9, 14-19
- [6] M. Kasha, Collisional Perturbation of Spin-Orbital Coupling and the Mechanism of Fluorescence Quenching. A Visual Demonstration of the Perturbation, J. Chem. Phys., 20, 71, 1952