Recording of a fluorescence spectrum with a spectrometer

Aims of the experiment

- To investigate the fluorescent dye fluorescein
- To understand the principle of fluorescence
- To learn about the Stokes shift
- To recognise the relationship between wavelength and energy
- To understand the interaction of photons with absorbing substances

Principles

Fluorescence describes the phenomenon exhibited by certain dyes, so-called fluorescence dyes or fluorophores. When irradiated with light they glow intensely. They not only absorb light to appear a characteristic colour, but they also emit light of a certain wave length.

When irradiated with light, certain electrons of the fluorophore absorb energy from the photons. However, they only absorb photons of certain wavelengths. The electrons get into an excited state. The excited molecule, however, cannot maintain this energy level and rapidly emits the absorbed energy again (within around 10^{-6} seconds). This energy release is also called dissipation.

The dissipation can be radiative or non-radiative. During the energy release via a non-radiative transition, part of the excess energy in the molecule is transferred to other bonds in the molecule by oscillation relaxation. This lowers the excited energy level.

With the second possibility, the energy release is via a radiative transition. Here, the molecule releases the remaining energy by emitting photons, thus returning to its basic state. This release of photons is called emission.

In the case of fluorescence, the energy is always given off radiatively and non-radiatively. Therefore, the emitted photon possesses a lower energy level than the one previously absorbed. Fluorophores can only absorb light of certain wavelengths (energy levels) and can therefore only emit photons of defined wavelengths (energy levels). The energy difference between absorption and emission is referred to as the Stokes difference. This energy difference also manifests itself in a shift of the wavelength towards light of longer wavelength. This shift of the wavelength between absorption and emission is called the Stokes shift.

In this experiment, fluorescence will be investigated using the example of fluorescein. For this, transmission and absorption spectra will be recorded. The results will be compared with a non-fluorescent dye.

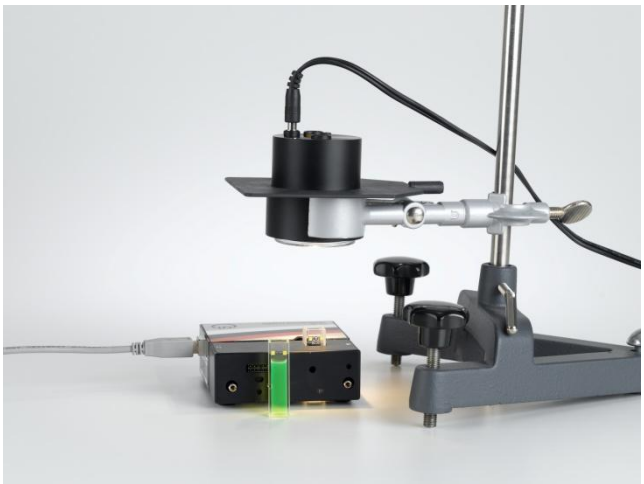

Fig. 1: Experimental apparatus for recording fluorescence spectra with a compact spectrometer.

Risk assessment

Fluorescein must not get into the eyes.

The denatured ethanol is highly flammable and should therefore not be placed near hot surfaces or open flames.

Fluorescein	
 **Signal word: Caution**	**Hazard statements** H319: Causes serious eye irritation. **Precautionary statements** P305+P351+P338: IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
Ethanol	
 **Signal word: Hazard**	**Hazard statements** H225 Highly flammable liquid and vapour. **Precautionary statements** P210 Keep away from heat/sparks/ open flames/hot surfaces. No smoking.

Equipment and chemicals

1 Compact spectrometer USB, complete.....467 252
2 Rectangular cuvette cell, optical glass/10x10mm664 470
1 Halogen spotlight 12 V/20 W 458 100
1 Plug-in power supply 12 V AC 562 791
1 Stand base, V-shaped, small.....300 02
1 Stand rod 25 cm, 10 mm diam.....301 26
1 Clamp with jaw clamp 301 11
1 Double microspatula, steel, 150 mm.....604 5672
2 Beaker, Boro 3.3, 100 mL, squat 602 022
2 Beaker, Boro 3.3, 250 mL, squat 664 130
1 Measuring cylinder 100 mL, with plastic base665 754
1 Graduated pipette 5 mL 665 996
1 Pipetting ball (Peleus ball) 666 003
3 Dropping pipette from set of 10.....665 953
3 Pipette bulbs from set of 10 .....665 954
1 Dye, red, 10 g 309 42
1 Fluorescein, 25 g 672 0110
1 Ethanol, denatured 250 mL.....671 9740
1 Water, pure 675 3400
Also required:
Computer with Windows XP, Vista, 7 or 8

Set-up and preparation of the experiment

Preparing the solutions

Preparation of a fluorescein solution: Measure 45 mL of water and 5 mL of ethanol with a measuring cylinder or graduated pipette and mix in a beaker (100 mL). Dip the spatula into the fluorescein powder and dissolve the fluorescein that remains on the spatula in the water-ethanol mixture. Stir the solution well until the fluorescein has dissolved.

Note: The solution should be prepared 30 minutes before the measurement, as the dye needs time to completely dissolve. Here, the ethanol improves the solubility of fluorescein in water.

Preparing a red dye solution: For a red solution, put a small spatula tip of the dye (red, non-toxic) into a beaker (250 mL) and fill it with 100 mL of water (use a measuring cylinder). Stir the solution well until the dye has completely dissolved. Then remove 10 mL of solution and dilute with 90 mL of water in the second beaker (250 mL). This produces 100 mL of dye solution.

Preparing the reference solution: Using a graduated pipette, add 5 mL of ethanol to 45 mL of water in a 100 mL beaker. Briefly swirl the solution, which can then be used immediately for measuring the reference spectrum.

Construction of the apparatus

From the compact spectrometer with cuvette holder, the lamp with network plug and a stand with clamp and jaw clamp, construct an apparatus for recording the spectra of fluorescent dyes using the SpectraLab software (see Fig. 1).

Note: When setting up the apparatus, make sure that the lamp is placed over the cuvette holder such that the cuvette is well illuminated from above. Only in this way a good recording of the transmission spectrum of the fluorescence is ensured.

Performing the experiment

1. Plug in the network cable and start the SpectraLab software. Make sure that the lamp is turned off.

2. Adjust the integration time with the ⊖ and ⊕ buttons so that the maximum intensity is about 50%.

Note: In order to ensure comparability of the measurement values, the integration time chosen should not be changed during the experiment.

3. Do not open the display Offset I0 in this experiment.

Note: The display Offset I0 must not be opened, as some values in the reference spectrum will otherwise fall to 0. Then calculation of the transmission of these values would no longer be possible, as they would have to be divided by 0.

4. Fill a cuvette with the reference solution (use a dropping pipette). Change the display to Reference I2 and put the reference cuvette into the cuvette holder of the compact spectrometer. Stop recording the spectrum by pressing "Pause". This spectrum will now be used as the reference spectrum.

Note: This display should not be opened again during the entire measurement, otherwise it will be overwritten when the measurement is stopped again.

5. Change to the display Transmission T. The transmission should now be at 100 % throughout.

6. Fill a cuvette with the fluorescein solution using a pipette. Then place the cuvette with the fluorescein solution into the cuvette holder.

7. Note the differences compared with the transmission of the reference solution.

8. Change to the display Absorbance E. The absorbance (optical density) is calculated and shown here.

Note: The solutions should not be too strong, as absorbance values over 2 cannot be adequately displayed.

9. Save the measurement for fluorescein separately under "Save file". By pressing "Stop" it is also possible to save several measurements in one file. Measure the red dye solution in the same way and save the results.

Observation

First of all, the reference spectrum of water with ethanol will be outlined. Then, the transmission and absorbance spectra of the fluorescein and red dye will be considered and explained.

The spectra of water with ethanol

A mixture of water and ethanol was produced for the reference spectrum. The reference spectrum corresponds approximately to the spectrum of the lamp used.

For the transmission spectra in SpectraLab, the transmission T [%] is calculated and plotted against the wavelength λ. The spectrum of the reference solution shows 100 % transmission throughout (see Fig. 2). Here we are dealing with the maximum amount of light which the lamp emits at this wave length.

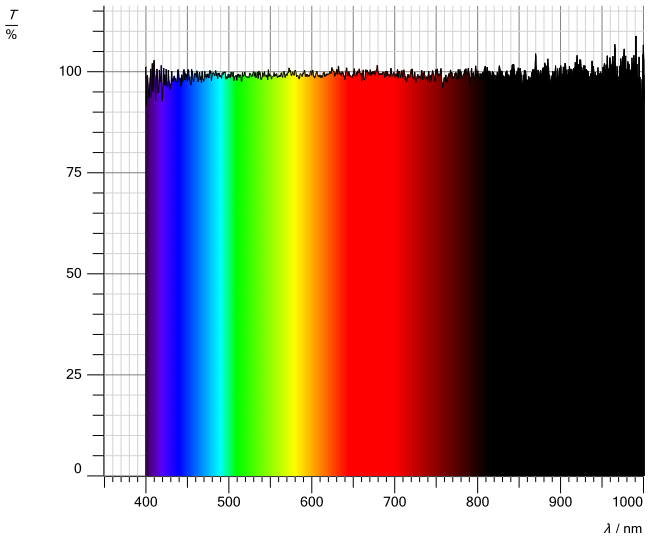

Fig. 1: Transmission spectrum of the reference solution.

The spectra of the fluorescein solution

The transmission spectrum of the fluorescein solution contains a maximum and a minimum. The transmission minimum is at around 480 nm. In addition, a transmission maximum of over 100 % at around 520 nm can be recognised. Here we are dealing with fluorescence (see Fig. 3).

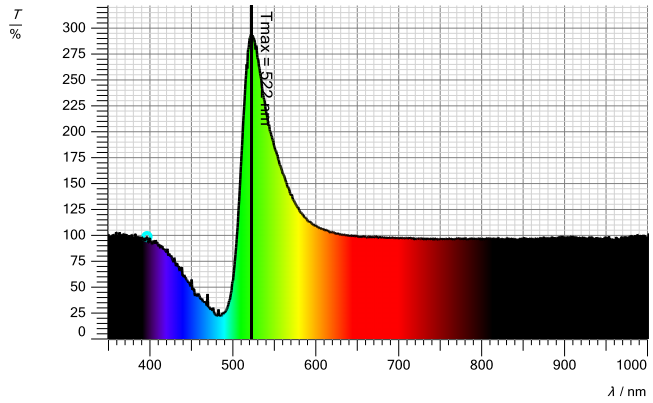

Fig. 2: Transmission spectrum of a fluorescein solution.

In the absorbance spectrum of fluorescein, a maximum at around 480 nm can be identified (see Fig. 4). This corresponds to the minimum in the transmission spectrum. The solution appears orange-yellow, but glows green at the edges.

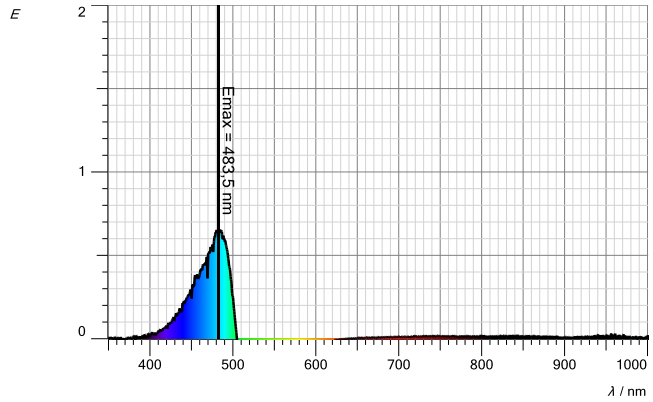

Fig. 3: Absorbance spectrum of a fluorescein solution.

The spectra of the red dye solution

The transmission spectrum of the red dye solution does not show a maximum, but only a minimum at around 500 nm - 600 nm (see Fig. 5). In the absorbance spectrum, the absorbance maximum is in the same range. The solution appears red.

If a red dye solution is in the cuvette, 100 % transmission of the wavelengths 600 - 1000 nm can be seen in the transmission spectrum, i.e. in the range of yellow to red light. In the range of 500 - 350 nm, the transmission increases continuously up to 100 %. On the other hand, at 600 nm - 400 nm the wavelengths of green light are primarily absorbed.

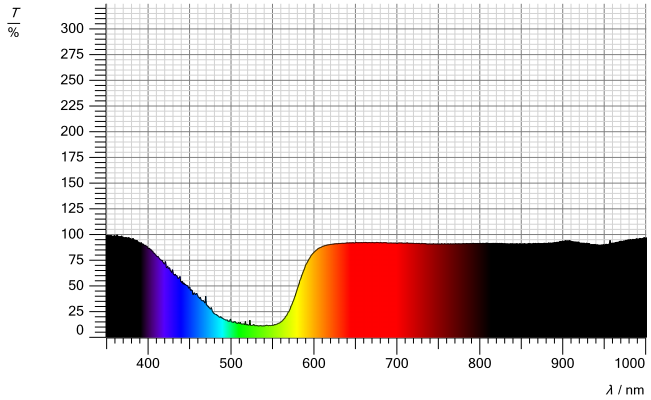

Fig. 4: Transmission spectrum of a red dye solution.

When the absorbance spectrum of a red dye solution is considered, it can be seen that the absorbance lies in the range of 400 nm - 600 nm. The associated colours of light are blue to yellow. The absorbance maximum is at 538 nm which corresponds to green light. As green is the complementary colour of red, the solution appears red (see Fig. 6).

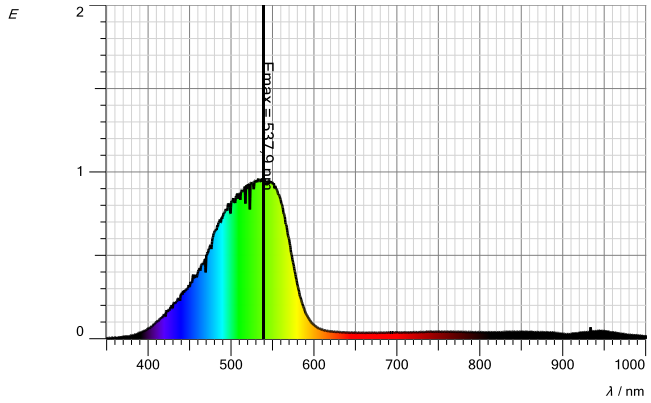

Fig. 5: Extinction spectrum of a red dye solution.

Evaluation

The transmission spectra of fluorescein and the red dye differ distinctly. Both contain a transmission minimum with wavelengths in the complementary colour to the colour of the solution. Therefore both dyes can absorb light energy from the spectrum.

However, the spectrum of fluorescein also contains a maximum of almost 300 %. At these wavelengths, more light is therefore detected than previously emitted from the lamp. This additional amount of light occurs through fluorescence. Fluorescein is therefore able to re-emit the absorbed light energy not only via non-radiative, but also via radiative transitions. It emits the energy as light with a wavelength of around 520 nm.

The absorbance maximum of the dyes can be determined in the absorbance spectra. For fluorescein this is at around 484 nm. Fluorescein therefore absorbs light with a wavelength of around 484 nm and emits light at around 520 nm, which then contains less energy. This wavelength difference is termed the Stokes shift. So for fluorescein this is around 40 nm.

Absorbed light		Transmitted light
Wavelength	**Colour**	**Observed colour**
730 nm	**deep red**	**green**
640 nm	**red**	**blue-green**
590 nm	**orange**	**blue**
550 nm	**yellow**	**blue-violet**
530 nm	**yellow-green**	**violet**
510 nm	**green**	**deep red**
490 nm	**blue-green**	**red**
450 nm	**blue**	**orange**
425 nm	**blue-violet**	**yellow**
400 nm	**violet**	**yellow-green**

Tab. 1: Matching absorbed light to transmitted light.

The perceived colour of the fluorescein solution can be attributed to two effects. Firstly, light with wavelengths of around 480 nm is absorbed. So this produces a perceived orange-red colour according to Table 1. However, yellow light is also emitted so that the solution appears distinctly more yellow.

Results

The comparison of the red dye with fluorescein shows the special characteristics of fluorescein. Fluorescein is a fluorophore. It not only absorbs light, but emits it again at a longer wavelength. The Stokes shift resulting from this is around 40 nm for fluorescein.

Cleaning and disposal

The red dye solution can be poured into the laboratory drain. Place the fluorescein solution into a container for halogen-free liquid organic waste.