Optics

Dispersion and chromatics

Absorption spectra

Absorption and fluorescence spectra of coloured liquids - Recording and evaluating with a spectrophotometer

Description from SpectraLab (467 250)

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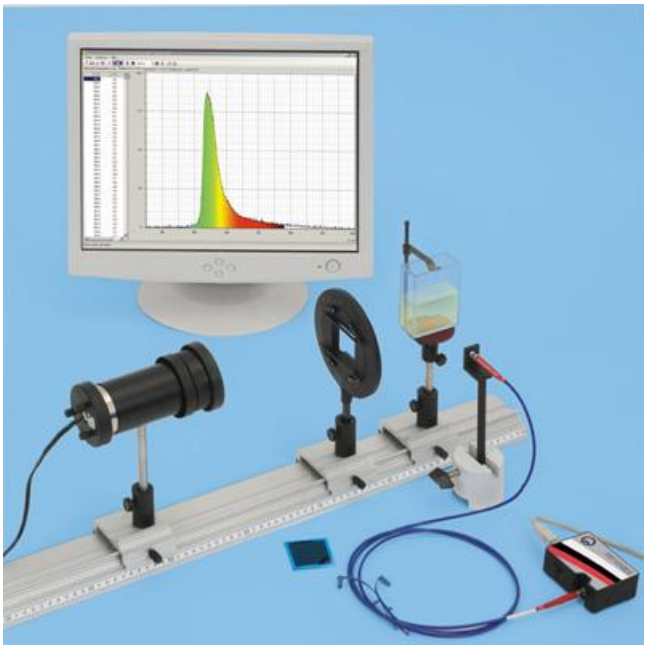
Transmission and fluorescence spectra of coloured liquids



Experiment description

This experiment explores how fluorescence responds by recording the transmission spectrum for absorption and the emission spectrum for scattering. The emitted light from a liquid coloured with fluoresceine is viewed at right angles. A blue filter is used to distinguish the fluorescence from the scattered light.

Required equipment

1	Compact spectrometer, physics	467 251
1	Fibre holder	460 251
1	Lamp housing with cable	450 60
1	Bulb, 6 V/30 W, E14, set of 2	450 511
1	Condenser with diaphragm holder	460 20
1	Transformer, 6/12 V	521 210
1	Holder with spring clamps	460 22
1	Light filter, blue with violet	468 11
1	Plate glass cell (cuvette)	477 14
1	Prism table	460 25
1	Fluoresceine, 25 g	672 0110
1	Microspatula	604 5672
1	Optical bench, S1 profile, 1 m	460 310
4	Clamp rider with clamp	460 311
1	Saddle base	300 11
1	PC with Windows 2000/XP/Vista/7/8	

Experiment setup (see picture)

Place the lamp in the housing but do not yet make a connection to the 6 V transformer output. Fill the plate glass cell with water and dye the water with a very small trace of fluoresceine. Dip the tip of a spatula into fluoresceine powder and tap off whatever clings into the water.

Initially you should not set up either the filter (clipped into a holder with spring clips) or the plate glass cell on the prism table.

Performing the experiment

Recording the transmission spectrum

- Activate ☐ to begin a new measurement.
- Select the **Intensity I1** display.
- Start the measurement with ▶.

- Connect the lamp to the 6-V output of the transformer and move it in its housing till the optical fibres are well illuminated.
- Align the fibre optic waveguide to maximise intensity. Adapt the integration time, either directly or with ⊖ or ⊕, such that maximum intensity lies between 75 % and 100 %. Do not change the integration time again after this.
- Switch off the light again to record the background spectrum.
- Open the **Offset I0** display.
- The displayed spectrum will be removed from subsequent measurements as the background spectrum.
- Change to the **Reference I2** display.
- Connect the lamp again to the 6 V transformer output.
- The displayed spectrum serves as a reference spectrum for the following measurement. Suspend reference measurement with ▮▮.
- Put the plate glass cell on the prism table.
- The light spectrum passing through the liquid can now be seen in the **Intensity I1** display. The reference spectrum is also displayed in grey.
- The liquid's spectrum's relationship to the reference curve is calculated and presented in the **Transmission T** display.
- Extinction (optical density) will be calculated and presented in the **Extinction E** display.
- The ● control can be used to save the transmission spectrum for all displays simultaneously.

Recording the emission spectrum

- Set up the fibre holder in such a way that the fibre is perpendicular to the side and pointing towards the plate glass cell. It may be necessary to move the lamp housing in order to shift the focal point into the plane of the cell in such a way that the fluoresceine fluoresces clearly. Point the optical fibre at the brightest area possible.
- View the spectrum on the **Intensity I1** display. Use ⊕ to increase the integration time till the spectrum is clearly visible.

Recording the transmission spectrum with light filter

- Move the fibre holder back into the direct path of the beam.
- Introduce a blue/violet filter into the beam.
- Alter the display to **Reference I2** and use ▶ to start a reference measurement. Adapt the integration time, either directly or with ⊖ or ⊕, such that maximum intensity lies between 75 % and 100 %. Do not change the integration time again after this.
- The displayed spectrum serves as a reference spectrum for the following measurement. Suspend reference measurement with ▮▮.
- Put the plate glass cell on the prism table.
- The light spectrum passing through the liquid can now be seen in the **Intensity I1** display. The reference spectrum is also displayed in grey.

Evaluation

A minimum appears in the transmission spectrum from the cell filled with fluoresceine at about 490 nm. You may need to reduce the absorption by diluting the fluoresceine or shortening the path taken by the light by rotating the plate glass cell.

In the emission spectrum for the light emitted from the side, there is a clear peak in the green region with a maximum at 520 nm.

In the spectrum for the lamp with the blue/violet filter in the path in **I2 display**, there is a clear peak with a maximum at about 460 nm. In the spectrum of the light, as shown in the **I2 display**, for the light beyond the cell filled with fluoresceine there is considerable absorption of the blue/violet peak, while the intensity in the green region is increased. This can be made more obvious by dragging with the mouse along the y-axis, for example.

Fluoresceine is excited by blue light (value of absorption maximum quoted in literature: 485 nm at pH 9) and then it emits green light (520 to 530 nm).

Notes

It would be more precise in terms of the physics if the reference spectrum were to be recorded first with the plate glass cell filled only with water (no fluoresceine).

Fluorescence emitted due to ambient light can be minimised by darkening the room.

Absorption spectra and transmission spectra are in fact equivalent: T = 1 - A. Where the transmission spectrum exhibits a minimum, the absorption spectrum exhibits a maximum.

In the **Transmission** and **Extinction** displays, only those ranges are evaluated whose intensities in the reference curve amount to at least 2 %.

The Σ control can be used to reduce noise by averaging multiple individual spectra (also Offset and Reference). Alternatively, Smoothing to 1 nm resolution can be set in the settings options.