Transmission and Absorbance of Liquid Samples

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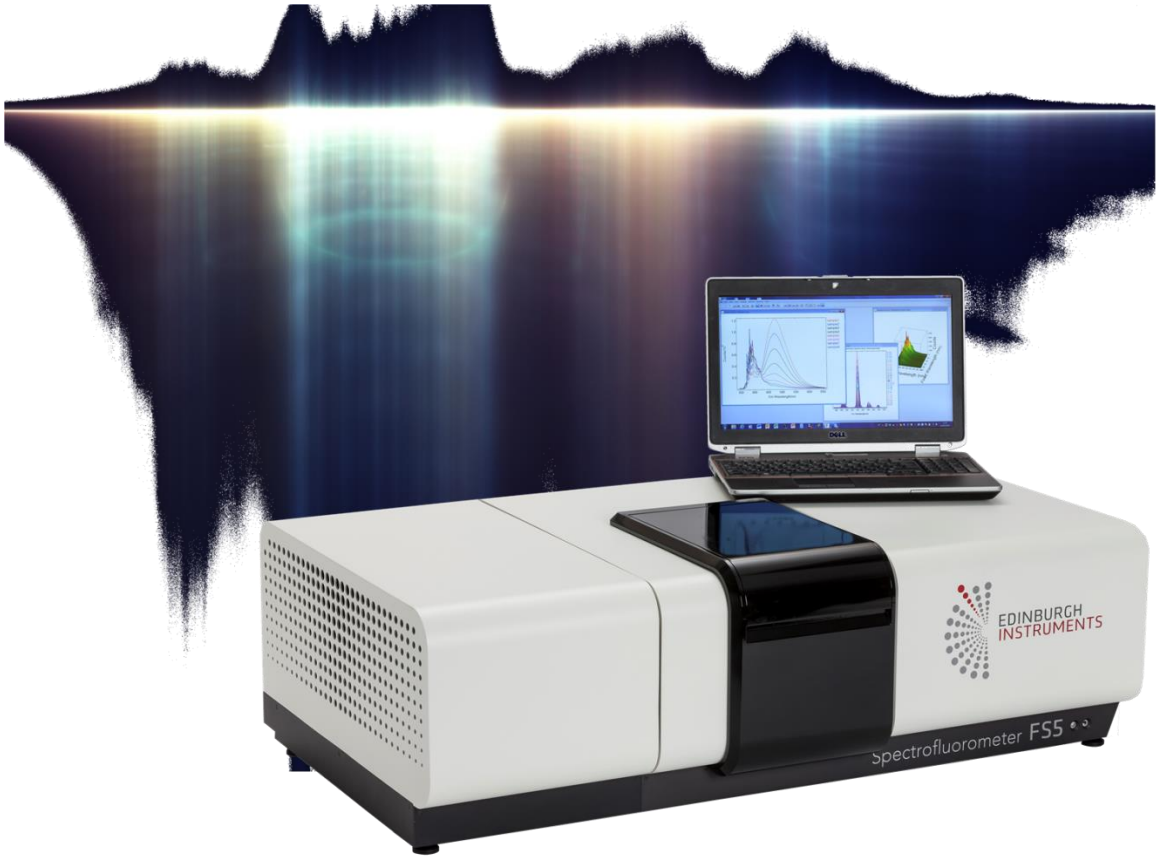

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Introduction

The absorbance *A* or optical density (OD) of a substance is inherent in all aspects of spectroscopy and follows the Beer-Lambert law[1], $A(\lambda)=log[Tref(\lambda)/Ts(\lambda)]$, where $Tref(\lambda)$ is the transmission of the reference sample and $Ts(\lambda)$ the transmission of the sample at the measured wavelength.

Instrument and procedure

The FS5 Fluorescence Spectrometer is equipped with a silicon detector that allows for transmission and absorbance measurements. Liquid samples can be measured in the standard cuvette holder (SC-05), cuvette holder for coolant circulation (SC-20) and thermoelectrically cooled/heated cuvette holder (SC-25) which can be interchangeably installed in the FS5. Fluorescein solutions were prepared in phosphate buffered saline (PBS) in 1 cm plastic cuvettes. After the transmission scans of the sample and reference are complete, they can be joined to calculate the absorbance through the absorption wizard in the operating software, Fluoracle.

Results

Figure 1 shows the transmission spectra of fluorescein samples at different concentrations measured in an FS5 fluorescence spectrometer.

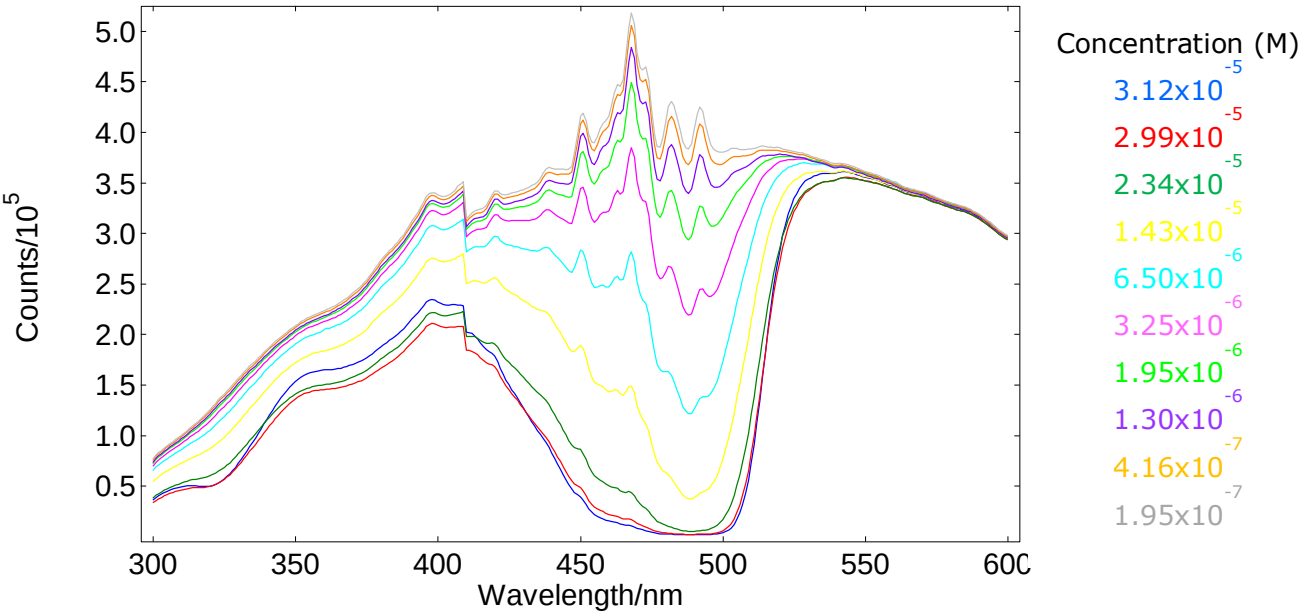

Figure 1: Transmission spectra for different concentrations of fluorescein in PBS.

The transmission spectra were recorded with a band-pass of 3 nm, a step of 1 nm and integration time of 0.2 s. The repeatability of the measurements was confirmed by reference transmission scans to less than 1% relative deviation.

From the transmission spectra, the absorbance spectra of the fluorescein samples in PBS shown in Figure 2 were calculated in the Fluoracle software. The spectra reveal the dianion form[2] of the solution with the absorption peak at 490 nm. The concentration c was calculated according to the Beer-Lambert law from the peak absorbance as $c = A / \varepsilon l$, with the molar attenuation coefficient ε of fluorescein[2].

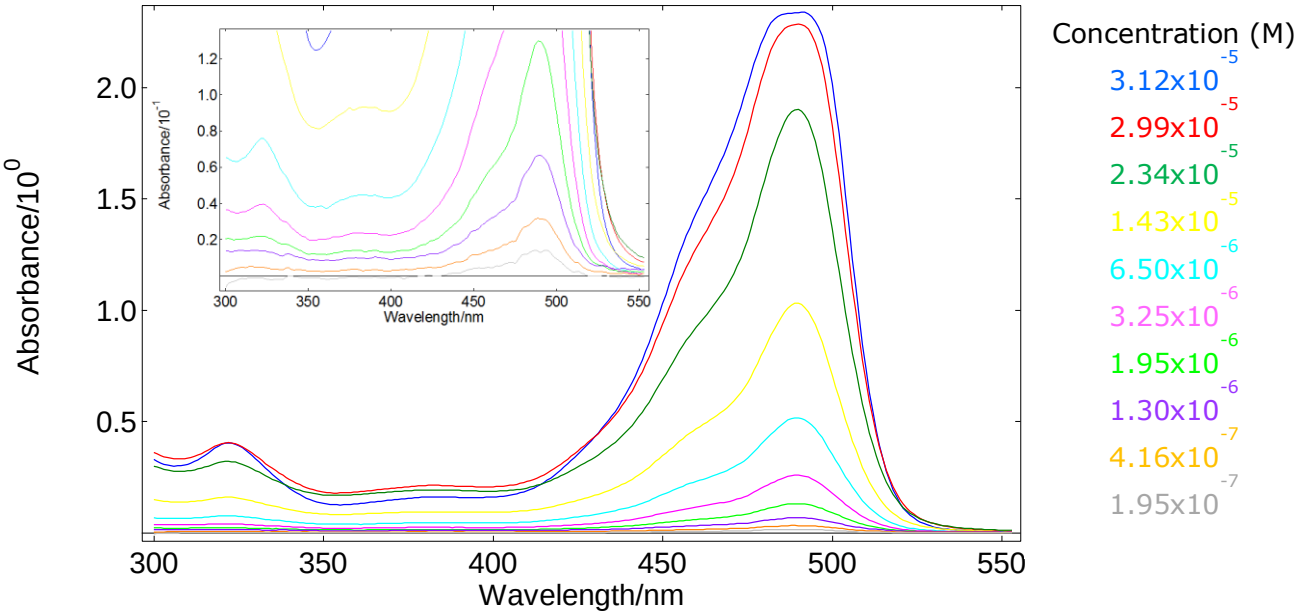

Figure 2: Absorbance spectra for different concentrations of fluorescein in PBS.

Conclusion

The FS5 Fluorescence Spectrometer enables transmission and absorbance measurements of liquid samples in the wavelength range 230 nm-1000 nm and optical densities of OD 0.01 to OD 2.

References

1. Ball, D. W. *The Basics of Spectroscopy*. (SPIE Press, 2001).
2. Sjöback, R., Nygren, J. & Kubista, M. Absorption and fluorescence properties of fluorescein. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **51,** L7–L21 (1995).

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