Spectroscopic analyses of weakly absorbing samples using an integrating cavity absorption meter

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Introduction

Absorption spectrophotometry, a standard tool for quantitative analysis, suffers from two drawbacks: lack of sensitivity and vulnerability to scattering. It has been pointed out more than once that the solution to these problems lies in using a reflecting cavity as a sample holder. Due to multiple reflections at the cavity wall, the effective pathlength becomes considerably larger than the diameter of the cavity, and scattering losses are eliminated because scattered light is prevented from escaping the detector. Though much effort has been spent in analyzing and improving the performance of such a device, often called an integrating cavity absorption meter (ICAM), a simple strategy for deducing the absorbance of the sample is still lacking [1-4].

Results

Calibration

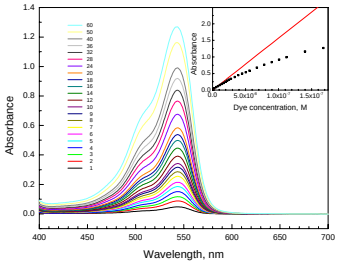


Figure 1. *Absorption spectra of rose bengal, recorded at different concentrations ranging from 2.8 × 10^{-9} M to 1.7 × 10^{-7} M, using an integrating sphere as a cuvette. (The numbers represent relative concentrations) The inset shows the peak absorption values (at 543 nm) as a function of dye concentration. A straight line is fitted to the initial values to demonstrate the linear relationship.*

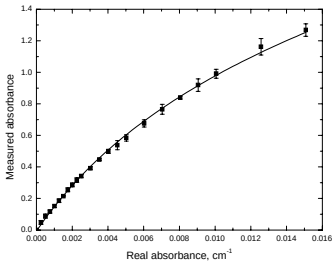


Figure 2. *Peak absorbance values from Figure 1 are plotted against real absorption, i.e. what one would obtain by using a 1 cm ordinary absorption cuvette. The symbols show the average values of 5 independent experiments; standard deviations from the mean values are represented by the error bars. The function* $f(A(\lambda)) = a_0 \ln(1+a_1 A(\lambda))$, *with the parameters* $a_0 = 0.99$ *and* $a_1 = 166.3$, *was fitted to the data points (solid line).*

Once a satisfactory fit is found the expression can be inverted and then the real absorbances can be calculated. This correction method can then be used generally as it is demonstrated on Figure 3. This measuring technique also eliminates losses due to scattering as it is shown on Figure 4.

Applications

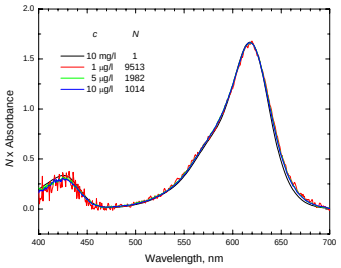


Figure 3. *Corrected and normalised absorption spectra of malachite green, recorded using the integrating sphere, compared with the absorption spectrum of the concentrated solution (10 mg/l, 1.1 × 10^{-5} M) as obtained using a 1 × 1 cm^2 cell in a standard spectrometer (Shimadzu UV-1601 PC). The concentration of the dye was 1 µg/l, 5 µg/l and 10 µg/l (1.1 × 10^{-9}, 5.5 × 10^{-9} and 1.1 × 10^{-8} M), respectively. The curves were normalised to the peak absorption, with N as indicated.*

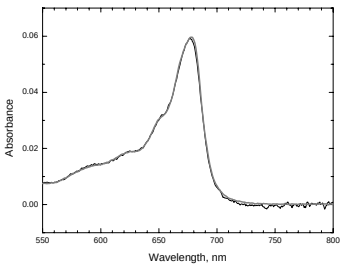
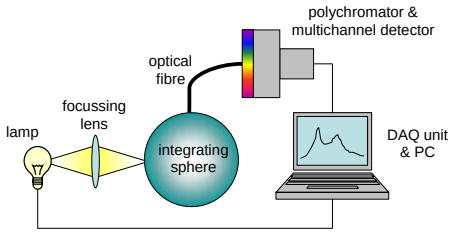


Figure 4. *Absorption spectra of isolated chloroplasts. Thick grey curve, recorded in a Hitachi U-3010 spectrophotometer equipped with a (conventional) integrating sphere, with further corrections to reduce scattering effects [5]. Black curve, recorded using an ICAM, after a dilution of the suspension by a factor of 750. The grey curve was normalised to match the peak absorbance at 677 nm.*

Experimental

A box, with the input and exit ports, containing the integrating sphere. The sphere (about 80 mm diameter) is coated with a reflective silver layer and covered with a white vitrified paint.

The sphere is fixed to the top cover plate of the box. The support for the optical fibre is on the top, the input window is on the front side of the sphere.



Experimental arrangement for recording absorption spectra. Spectral intensities ($I_0(\lambda)$ *and* $I_C(\lambda;C)$) *emerging from the sphere were recorded by a multichannel analyser (Princeton Instruments IRY 512B).* $I_0(\lambda)$ *and* $I_C(\lambda;C)$ *denote the intensity when the sphere is filled up with water only and with the sample at concentration C, respectively. The absorbance is then calculated as* $\log(I_0/I_C)$ *and plotted against the wavelength.*

Conclusions

We have analysed the distortion effects of the ICAM on absorption spectra of non-scattering solutions, and proposed a correction procedure, which depended on the optical density of the solution and on the optical arrangement of the ICAM–detector system. The corrected, true spectra were similar within 5 % difference when the concentration of the solution was changed over a wide range. We also demonstrated that in case of scattering samples the use of the ICAM in addition to enhancing the sensitivity also reduces scattering related spectral distortions. As the sample concentration in the sphere is increasing the intensity of the monitoring light is being attenuated both directly (absorption) and indirectly (effective pathlength), therefore this method is best suited to measure weakly absorbing samples.

References

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Acknowledgements

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