Experiment 0: Analysis of the absorption spectrum of plant pigments

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Abstract

The UV-Visible spectrum of the pigments contained within a plant leaf obtained and then the sum of various Gaussian curves was used to generate a fit line for the spectrum. It was found that modelling the absorptions at different wavelengths with Gaussian curves yielded poor results and it is likely that the use of Voigt curves will give better agreement with the experimental data due to the inclusion of lifetime broadening effects in addition to Doppler broadening effects on the spectra linewidths.

1   Results and Analysis

A number of Gaussian curves were added together to generate a fit line for the UV-Visible spectroscopic data collected. Gaussian curves were used since it was assumed that the major contributor to the spectral linewidth would be Doppler broadening due to the Doppler effect, thus resulting in a Gaussian form for the absorption peaks due to the molecular speeds being distributed according to the Maxwell-Boltzmann distribution.[1]

1.1   Chlorophyll a Sample Data

Within the data for Chlorophyll a three clear absorption peaks can be identified. Due to this three separate Gaussian functions were added together to produce an overall curve to fit to the data as shown in figure 1.
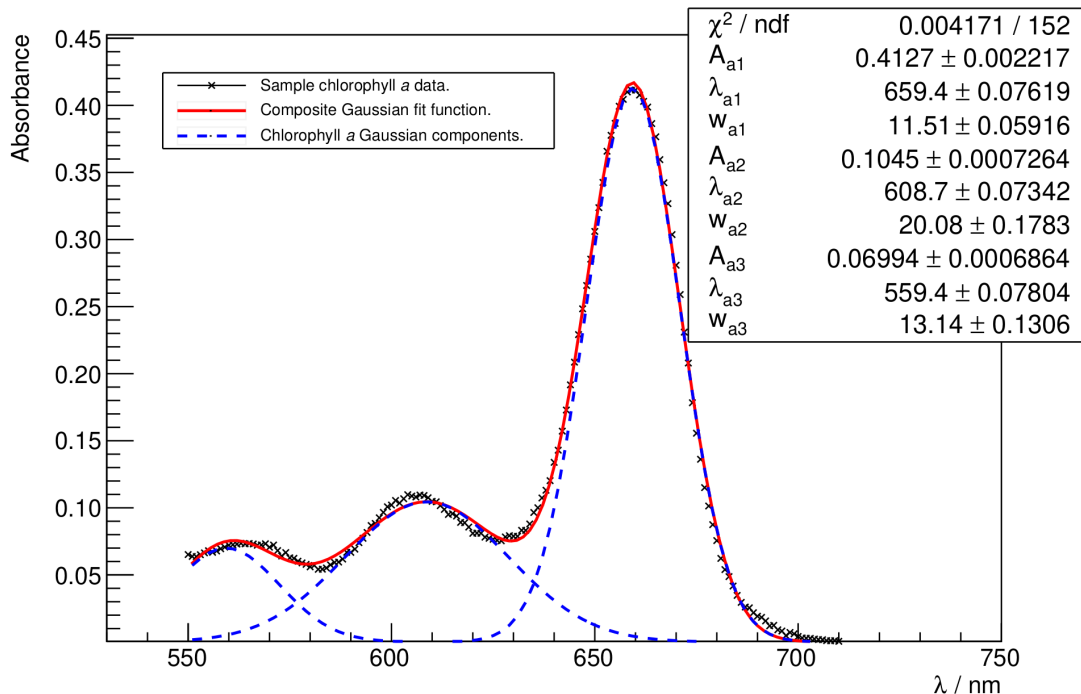

Figure 1: Graph showing the generated fit line for the chlorophyll a UV-Visible absorption data and the component Gaussian curves used to generate the fit.

The parameters for each Gaussian curve shown in the fit statistics box on the figure correspond to those in equation 1.1.1 where x is used to differentiate between the different curves.

$$f_{ax}(\lambda) = A_{ax}e^{-\frac{1}{2}\left(\frac{\lambda - \lambda_{ax}}{w_{ax}}\right)^2} \tag{1.1.1}$$

The residuals for this fitting (χ_1) were then plotted as a histogram and their residuals (χ_2) relative to another fitted Gaussian curve (used as the residuals should occur independently and randomly, thus by the central limit theorem they should be normally distributed) were also plotted (as shown in figure 2). These residuals (χ_2) were plotted as a ratio of the uncertainty of the absorption residual value for which they corresponded (i.e. as a ratio of the uncertainty in χ_1) with this uncertainty estimated to be $\sqrt{N}$ where N is the number of occurrences of the residual value. Furthermore the yellow area on the plot represents a distance within one standard deviation to the fitted Gaussian and the green area represents a distance between one and two standard deviations.
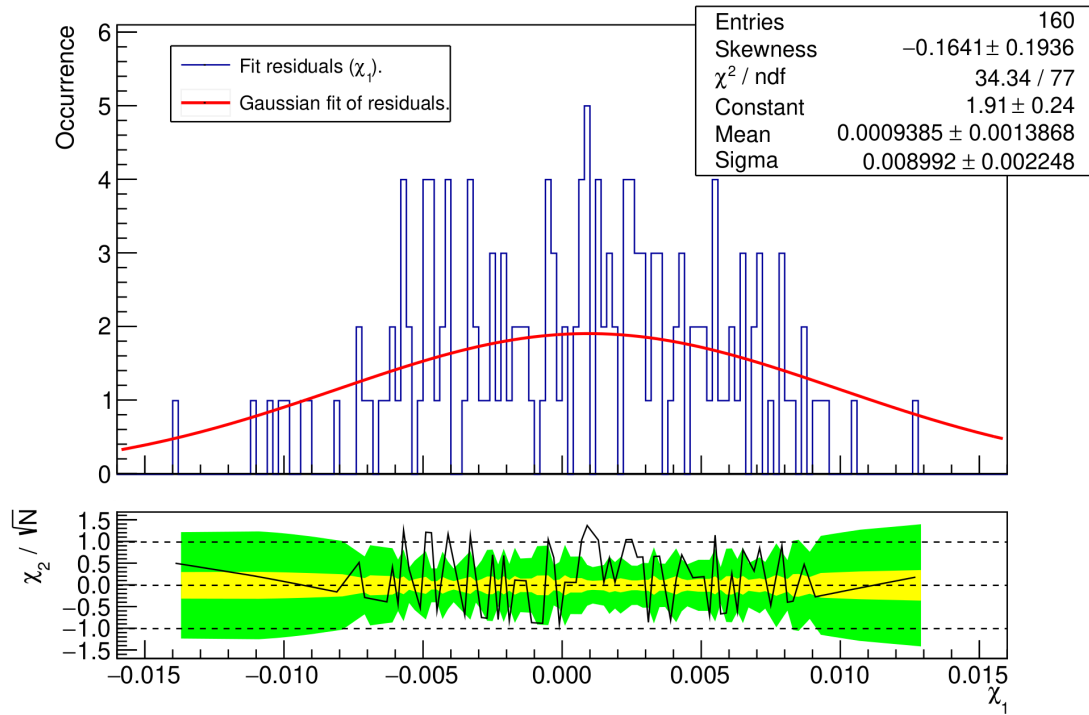

Figure 2: Histogram showing the frequency of each residual value for the fit in figure 1 with a plot of the residuals for this with respect to a fitted Gaussian curve.

1.2 Collected Experimental Data

In the raw data collected the absorption decayed to between 0.056 and 0.051 for all wavelengths greater than 700 nm. This was identified as a systematic error and was corrected by subtracting an absorbance of 0.051 from all of the collected data.

The absorption peaks for the UV-Visible spectrum obtained are less well defined than those for the pure chlorophyll a spectrum and when a fit line was constructed using three Gaussian curves the corresponding fit to the data was quite poor with the maxima of the Gaussian curves used for the fit clearly not matching the maxima in the experimental data.

It was found that plant pigments naturally contain another form of chlorophyll called chlorophyll b in addition to chlorophyll a.[2] Chlorophyll b absorbs UV radiation at a slightly lower frequency than chlorophyll a (principle peak absorption at 642 nm)[3] and has two absorption peaks in total within the 550–750 nm region. To account for the presence of chlorophyll b in the sample two additional Gaussian curves were fitted to the data (represented by violet dash-dotted lines in figure 3) to create the fit line seen. In figure

3 the three Gaussian curves representing the contribution to the absorption caused by the chlorophyll *a* are shown as as blue dashed lines.

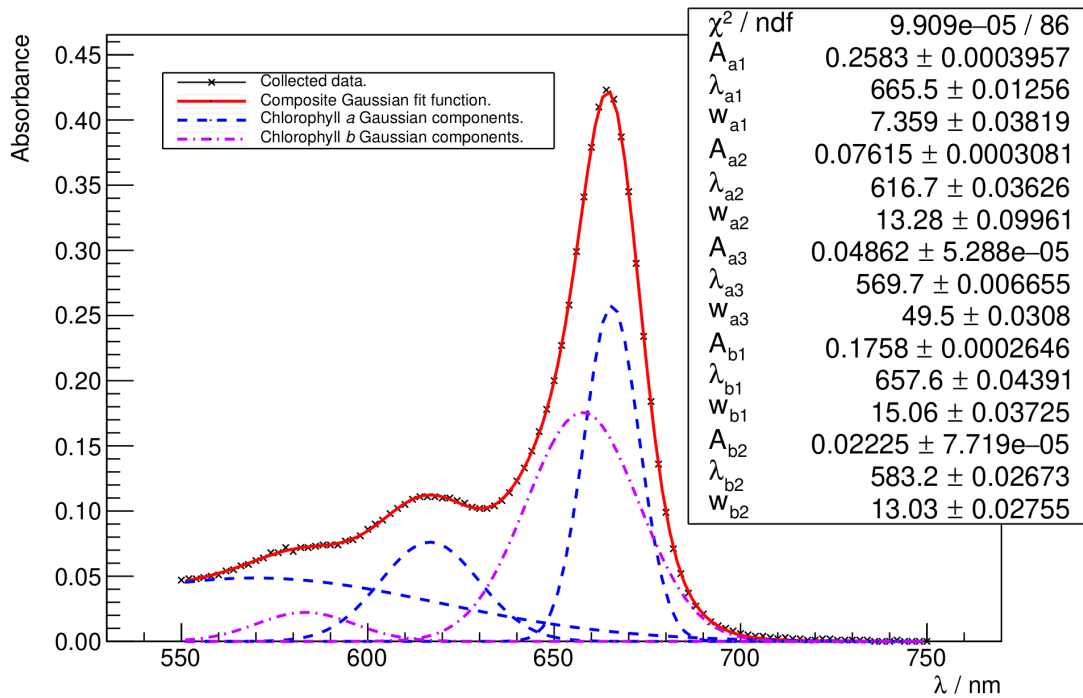| χ² / ndf	9.909e–05 / 86
A_{a1}	0.2583 ± 0.0003957
λ_{a1}	665.5 ± 0.01256
w_{a1}	7.359 ± 0.03819
A_{a2}	0.07615 ± 0.0003081
λ_{a2}	616.7 ± 0.03626
w_{a2}	13.28 ± 0.09961
A_{a3}	0.04862 ± 5.288e–05
λ_{a3}	569.7 ± 0.006655
w_{a3}	49.5 ± 0.0308
A_{b1}	0.1758 ± 0.0002646
λ_{b1}	657.6 ± 0.04391
w_{b1}	15.06 ± 0.03725
A_{b2}	0.02225 ± 7.719e–05
λ_{b2}	583.2 ± 0.02673
w_{b2}	13.03 ± 0.02755

Figure 3: Graph showing the generated fit line for the experimental UV-Visible absorption data and the five component Gaussian curves used to generate the fit.

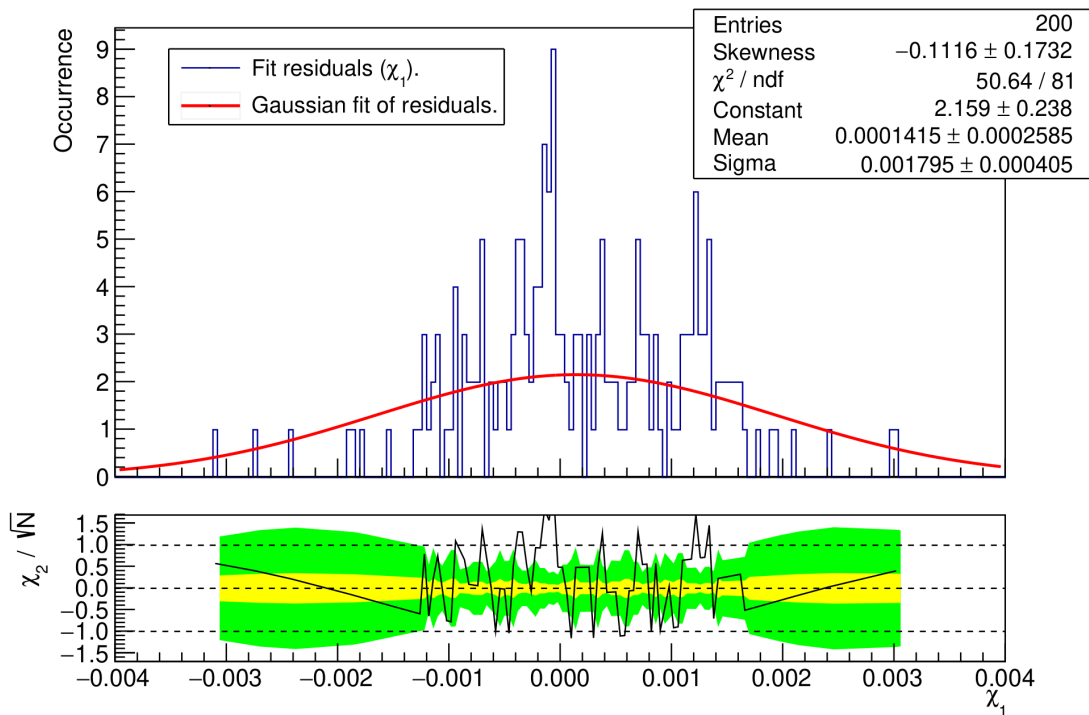| Entries	200
Skewness	−0.1116 ± 0.1732
χ² / ndf	50.64 / 81
Constant	2.159 ± 0.238
Mean	0.0001415 ± 0.0002585
Sigma	0.001795 ± 0.000405

Figure 4: Histogram showing the frequency of each residual value for the fit in figure 3 with a plot of the residuals for this with respect to a fitted Gaussian curve.

2 Discussion

The χ^2 value for the experimental plot in figure 3 is much lower than that for the chlorophyll a plot shown in figure 1 (9.909×10^{-5} compared to 4.171×10^{-3}) which suggests a better fit. In addition to this is can clearly be seen that the fit line on the experimental plot follows the data more closely than that for the chlorophyll a plot where the peaks on the fit line do not completely coincide with the peaks in the data points which, while resulting in a lower χ^2 value (in comparison to setting the λ_{ax} value exactly to the peak wavelength), does not accurately follow the assumed absorption model (i.e. that the total absorption results from the linear combination of various Gaussian curves generated due to chromophores absorbing radiation at a small range around a specific wavelength).

Despite this it can be seen from the residual plots (figures 2 and 4) that the residuals for the chlorophyll a fit line appear to be better described by a Gaussian curve (used to fit the residuals due to application of the central limit theorem) than the residuals for the experimental plot fit line. This can be seen from the lower χ^2 value, and also from how $\chi_2/\sqrt{N}$ has a smaller magnitude maximum and minimum in addition to how it is more concentrated within two standard deviations (the criteria for "reasonable agreement")[4] of zero. This is unexpected since from the visual appearance of the fit lines the residuals for the experimental plot were expected to exhibit random behaviour better modelled by the normal distribution than those for the chlorophyll a plot since the fit line for the chlorophyll a plot is clearly inadequate in places. This suggests that that Gaussian curves used to generate the fit for the experimental data may not accurately describe the underlying physical system.

In figure 1 it can be seen that the fit curve decays faster than data (most apparent by inspection of the bottom of the major absorption peak). In the fitting used for the experimental data this is accounted for by the wider Gaussian curve used to represent the principle chlorophyll a absorption (i.e. by the relatively large value of w_{b1}), however it is possibly unrealistic to expect that the width of the principle absorption peak for chlorophyll b will be almost double the width of the curve for chlorophyll a (seen from how $w_{a1} = 7.36 \pm 0.04$ while $w_{b1} = 15.06 \pm 0.04$). Furthermore the third Gaussian curve used to describe the chlorophyll a absorption in the experimental data is very wide ($w_{a3} = 49.50 \pm 0.03$) compared to the rest of the curves which while allowing the generation of a better fit curve for the data is also unlikely to represent the underlying absorption distribution at that wavelength. This (in conjunction with the irregularities in the fitting of the residuals to a normal distribution) may suggest that the model of using Gaussian curves for the absorption is not fully correct and a different waveform (which doesn't decay as fast as a Gaussian curve) might better represent the absorption of the physical system.

This is likely to be the cause of the slight discrepancies between the peak wavelengths of the Gaussian curves used for fitting the chlorophyll a data and the corresponding curves used to fit the chlorophyll a absorptions in the experimental data, although the fact that these discrepancies are quite small (the largest is $10.3\,\mathrm{nm}$) suggests that Gaussian curves are not totally inadequate for modelling UV-Visible absorptions.

Spectral linewidths are affected by lifetime broadening effects in addition to Doppler broadening effects which were considered.[1,5] Lifetime broadening effects contribute an Lorentzian curve component for the spectral lines,[5] thus explaining why Gaussian curves appear to be unable to fully describe the absorption peaks. Voigt curves are a composite of Gaussian and Lorentzian curves, thus the use of linear combinations of Voigt curves to fit the absorption data collected is likely to result in an improved fit over that which was obtained with Gaussian curves.

References

(1)   P. Atkins and J. de Paula, *Physical Chemistry*, Oxford University Press, Oxford, 10th edn., 2014.
(2)   B. R. Speer, *Photosynthetic Pigments*, 1997, `http://www.ucmp.berkeley.edu/glossary/gloss3/pigments.html` (visited on 06/11/2018).
(3)   H. K. Lichtenthaler and C. Buschmann, *Current Protocols in Food Analytical Chemistry*, 2001, **1**, F4.3.1–F4.3.8.
(4)   I. G. Hughes and T. P. A. Hase, *Measurements and their Uncertainties*, Oxford University Press, Oxford, 2010.
(5)   B. H. Bransden and C. J. Joachain, *Quantum Mechanics*, Pearson Education Limited, 2nd edn., 2000.