BIOC1015 Experiment 1: Spectrophotometric determination of cytochrome c

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1 Objective

We had a sample of cytochrome c (sample X) of unknown concentration. We aimed to find the concentration by comparing it with that of a known reference solution.

2 Method

2.1 Determining the peak absorption wavelength (λ_{max}) of cyt c

The absorption of a stock solution (0.1mg/ml) of cyt c was measured in a spectrophotometer at wavelengths between 380 and 420nm, at 5nm intervals. Before each reading, the spectrophotometer was zeroed using distilled water. The results were plotted on a graph.

2.2 Plotting a calibration graph

Six 10ml samples of the stock cyt c solution in distilled water were prepared at concentrations between zero and 0.1mg/ml, at 0.02mg/ml intervals. The absorption of each sample was measured at λ_{max}, the results were plotted on a graph, and a line of best fit drawn.

2.3 Measuring the concentration of sample X

The absorption of sample X was measured, and the concentration was read off the graph.

3 Results

3.1 Peak absorbance

The absorption of the stock solution is shown in the table below:

λ (nm)	Absorption
380.0	0.262
385.0	0.253
390.0	0.318
395.0	0.386
400.0	0.463
405.0	0.562
410.0	0.606
415.0	0.544
420.0	0.424
407.5	0.626
412.5	0.599

The result shows clearly that the peak absorbance is at 410nm ($\pm$5nm).

As an afterthought, in an attempt to determine the location of the peak more precisely, two additional readings were taken at 407.5nm and 412.5nm. These showed the peak to be closer to 407.5nm than 410nm, but we nonetheless decided to use the original value of 410nm for λ_{max} in subsequent measurements.

3.2 Calibration graph

The absorption of various dilutions of the stock solution are tabulated below:

Concentration (mg/ml)	0	0.02	0.04	0.06	0.08	0.1
Volume of stock solution (ml)	0	2	4	6	8	10
Volume of distilled water (ml)	10	8	6	4	2	0
Absorption at λ_{max}	0	0.141	0.280	0.443	0.536	0.682

(The zero value for the absorption of distilled water was by definition.)

Happily, these data show good proportionality.

There was an unexplained change in the systematic error of the spectrophotometer between parts 1 and 2 of the experiment, which prompted us to retake a measurement that could otherwise have been done only once, namely the absorbance of undiluted stock solution at 410nm.

3.3 Concentration of sample X

The absorbance of sample X at 410nm was 0.434. From the graph, this indicates that its concentration was 0.062mg/ml (2 s.f.).

4 Accuracy

4.1 Spectrophotometer

The error of the spectrophotometer is ± 0.02. In part 1, the expected normal distribution curve around the peak was marred slightly by the reading at 385nm. Nonetheless, this measurement was within the bounds of expected experimental error.

More worrying was the unexpected and unexplained change in behaviour of the spectrophotometer between parts 1 and 2 of the experiment. The meter was entirely consistent before and after than change; luckily, with the exception of the purity calculation, we were only interested in relative measurements so this did not unduly affect the outcome of the experiment.

The accuracy of the spectrophotometer was by far the largest source of error. Other potential sources of experimental error (e.g. accuracy of the pipette used to prepare dilute samples) were negligible by comparison.

4.2 Purity of the stock solution

The molar absorption coefficient of pure cytochrome c is $125,000\text{molar}^{-1}\text{cm}^{-1}$ at its (unspecified) peak wavelength λ_{ref}, and its relative molecular mass is 13300.

The concentration of the stock solution was $\frac{0.1}{13300} = 7.52\mu\text{M}$ (3 s.f.), and its expected absorbance at the peak wavelength was 0.940 (3 s.f.).

The purity of the stock solution was therefore $\frac{0.682}{0.940} = 73\%$ (2 s.f.). However, a number of assumptions have been made in the above calculation:

1. That λ_{ref} was exactly the same as our value for λ_{max}. This is unlikely to be true, and cannot be fully compensated for even by finding out the value of λ_{ref}, due to systematic error in the wavelength setting of the spectrophotometer we used. (We were advised to use only a single spectrophotometer for the duration of the experiment for this reason: this implies that the error is significant.)

2. That any impurities present in the stock solution did not absorb light in the 410nm region. If they did, this would increase the purity value, thus the value obtained is actually a maximum.

3. That the Beer-Lambert law holds under the conditions of the experiment. In particular, the expected value for the absorbance of pure 0.1mg/ml cytochrome c quoted above is perilously close to 100% absorbance, and it is unclear to me whether proportionality holds all the way to 100%.

4. That, of the two behaviours exhibited by the spectrophotometer, the latter was correct (thus yielding the 0.682 figure instead of 0.606). I have no basis for assuming this, but the previous behaviour was unreproducible.

In this case, I assume sample X was prepared from the stock solution. Were this not the case, the lack of purity of the stock may have adversely affected the accuracy of the value obtained for the concentration of sample X.

4.3 Accuracy of the measured concentration of sample X

The accuracy of the spectrophotometer resulted in an error of ± 0.02 in the absorbance of sample X, which equates to approximately $\pm 4.6\%$ or ± 0.003mg/ml in the resulting concentration.

The calibration graph is assumed to be relatively accurate due to amortization of errors over several samples. The error in λ_{max} is not relevant, nor is the purity of the stock solution assuming that sample X was prepared from it. We assume, however, that the known concentration of the stock solution was accurate.

5 Conclusions

The concentration of sample X was 0.062 ± 0.003mg/ml (2 s.f.).

The purity of the stock solution could not be determined to any reasonable accuracy due to significant sources of error, but was likely less than 80%.

Spectrophotometers are fickle.