Colorimetry in the school laboratory

Over recent years, colorimeters have become essential pieces of equipment to support secondary biology and chemistry practical work and their use is specifically mentioned in the Course/Unit Support notes for both *CfE* Advanced Higher Biology and *CfE* Advanced Higher Chemistry [1].

Figure 1 *- WPA CO7500 and Mystrica colorimeters.*

In addition colorimetry is a valuable technique for use in Higher Biology and Higher Human Biology (in particular for enzyme assays) and Higher Chemistry (for example in kinetic studies). As noted previously [2], we routinely use WPA CO7500 and Mystrica colorimeters (both types of device are available from Scientific and Chemical, http://www.scichem.com/) for practical activities (Figure 1).

Figure 2 *- Semi-micro (left) and standard (right) cuvettes (1 cm pathlength).*

We have produced a simple activity called 'How good is your colorimeter?' and details are available on the SSERC website [3]. As an extension to that activity we present here another simple protocol which should allow users to use their colorimeter more confidently and effectively.

In common with many pieces of equipment, failure to follow some basic rules means that the data obtained are unreliable and/or incorrect. Over several years of running professional development courses across the sciences we have noticed that some teachers and technicians would not qualify for what might be termed the 'full colorimeter driving licence'. It is entirely possible that poor technique is then transferred to pupils.

For most school colorimeters there are two cuvettes which are in common use as shown in Figure 2.

Both of the cuvettes shown have a pathlength (i.e. the distance through which the light beam passes) of 1 cm. The cuvette on the left has a maximum volume of ~2.7 cm^3 and the one on the right a maximum volume of ~4.5 cm^3. A common problem which we encounter is the volume of solution used for measurements. The basic question one needs to ask (or know the answer to) is how much solution is needed for reliable measurements? Alternatively 'Can you have too little or too much in the cuvette?'

To generate answers to the question, a solution of $CuSO_4.5H_2O$ (approximate concentration was 0.2 mol dm^{-3}) was prepared in distilled water. The colorimeter (in this case a WPA CO7500) was set up to measure absorbance at 680 nm and distilled water was used as the blank. 2.5 cm^3 of the $CuSO_4.5H_2O$ was put into a standard cuvette and the absorbance measured to be 0.66.

For the purposes of the activity which follows let us assume that the 'true/correct absorbance' of a 0.2 mol dm^{-3} solution of $CuSO_4.5H_2O$ at 680 nm is indeed 0.66. What we did next was to take an empty standard cuvette (referenced against an 'identical' cuvette containing distilled water) and added 0.2 cm^3 aliquots of the $CuSO_4.5H_2O$ solution measuring absorbance after each addition. The results are shown in Table 1 and plotted in Figure 3.

Reading	Volume (cm^3) of 0.2 mol dm^{-3} $CuSO_4.5H_2O$ present	Absorbance (680 nm)
1	0.0	0.00
2	0.2	0.00
3	0.4	0.00
4	0.6	0.01
5	0.8	0.07
6	1.0	0.48
7	1.2	0.66
8	1.4	0.66
9	1.6	0.66
10	1.8	0.66
11	2.0	0.66

Table 1

The explanation for the data shown in Figure 3 is that at low volumes (< 0.6 cm^3) the light beam passes through the cuvette but there is insufficient volume of $CuSO_4$ present to absorb any of the light. At volumes between 0.8 and 1.2 cm^3 the light beam is probably passing partly through solution and partly through air; this causes scattering and so the absorbance recorded is not accurate. At volumes above 1.2 cm^3 the readings represent 'true/correct absorbance'. So, what is the impact of this on measurements using a colorimeter? Well, we would recommend that:

- when using a WPA CO7500 colorimeter, you use a minimum volume of 1.4 cm^3 in your cuvette to ensure accurate readings;
- when using a WPA CO7500 colorimeter, there is no need to have more than 2.0 cm^3 of solution in your cuvette - keeping the volume at about this level not only reduces the amount of solution needed but also reduces the risks of spillages;
- if you use a different colorimeter you cannot assume that the light beam will travel at the same height through the cuvette and so you will need to do similar experiments as outlined above to determine the minimum volume required.

The same experiment was run using a semi-micro cuvette using aliquots of 0.05 cm^3 of the $CuSO_4.5H_2O$ solution (Figure 4). In this case the scattering is more pronounced but from the plot one can see that a minimum volume of ~ 0.55 cm^3 is required to ensure that measurements of the 'true/correct absorbance' are obtained.

References

[1] Course/Unit Support Notes for Advanced Higher Chemistry and Course/Unit Support Notes for Advanced Higher Biology are available via the SQA website www.sqa.org.uk/ (accessed August 20th 2014).

[2] The Mystrica Colorimeter, SSERC Bulletin (2009), **228**, 10-12.

[3] Available at www.sserc.org.uk/index.php/biology-2/biology-resources/advanced-higher/cells-proteins/3438-laboratory-techniques-for-biologists (accessed August 22nd 2014).

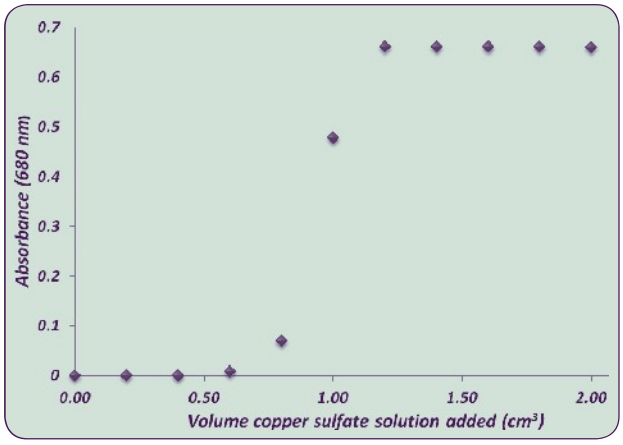

Figure 3 *- Plot of measured absorbance of 680 nm as a function of volume of 0.2 mol dm^{-3} $CuSO_4.5H_2O$ contained in a 'standard' 1 cm pathlength cuvette.*

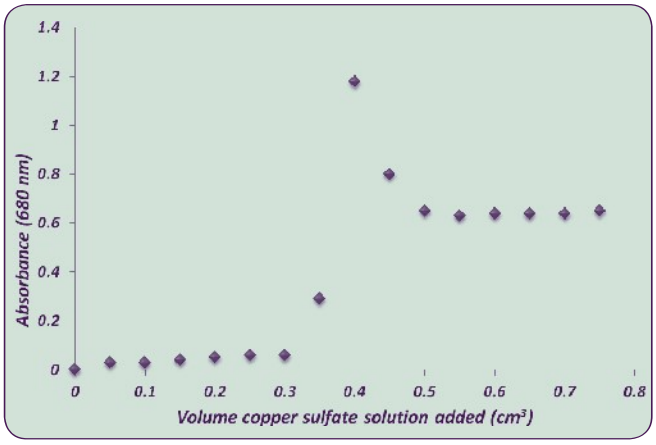

Figure 4 *- Plot of measured absorbance at 680 nm as a function of volume of 0.2 mol dm^{-3} $CuSO_4.5H_2O$ contained in a 'semi-micro' 1 cm pathlength cuvette.*