

Background

Colorimeters are desirable, occasionally essential, pieces of equipment for use in secondary chemistry and biology laboratory work. A major factor which limits the availability of colorimeters is cost. Within SSERC we routinely use WPA CO7500 colorimeters (see www.wpald.co.uk) – the current price for each unit is £355 (excl. VAT and postage). It would be rare to find institutions which are able to provide class sets of such equipment. In this report we explore the design, reliability and scope of a new colorimeter marketed by Mystrica (see www.Mystrica.com) – the current (March 14 2009) listed price being £76 (excl. VAT and postage).



Figures 1 & 2
The Mystrica colorimeter.

Operation

The Mystrica colorimeter (MC) uses light emitting diodes as light sources. Three diodes are available – blue (470 nm), green (535 nm) and red (630 nm). The beam from the diode passes through a conventional cuvette of 1 cm pathlength (10 such cuvettes are supplied with the colorimeter). Operation is simple – a calibration reading is taken followed by a reading of the sample. The colorimeter comes supplied with 4 batteries (AA, 1.5 V) or alternatively can be operated without batteries by connecting it to the USB port of a PC (Mac versions of the software are under development at the time of writing this report). When operating on battery power the unit automatically shuts down after a period of 6 s in order to prolong battery life. Whilst such a time interval is relatively short, the calibration data is retained when the machine is switched off and so a new calibration is not required between individual sample readings. Readout of data in both absorbance and percentage transmittance is possible. The estimated battery lifetime is 100 hr of continuous use or 60 000 readings.

The USB link allows data to be collected either as individual events or at intervals of 1 s. Data can be stored and analysed utilising free software which can be downloaded at www.Mystrica.com. Alternatively data can be imported into a

range of different spreadsheet/data analysis packages (e.g. Microsoft Excel).

Test Samples

Neutral density filters

Utilising the green diode as the light source an empty cuvette was placed in the sample chamber and calibrated. Neutral density filters (available from Lee Filters - see www.leefilters.com) were used to test the response of the MC. Filters (each with an absorbance of 0.3 at the excitation wavelength) were placed in the cuvette and absorbance recorded.

The results from such experiments are shown in Figure 1. The slope of the plot shown in this figure is 0.29 (against a predicted value of 0.30).

Comparative experiments using the WPA CO7500 colorimeter using a 520 nm filter yielded a linear plot with a slope of 0.30 (data not shown).

Similarly impressive linear plots were obtained with the MC using blue (470 nm) and red (630 nm) diodes as the excitation source and neutral density filters as the absorbing sample.

Absorbance of Coloured Solutions (535 nm excitation)

Potassium manganate(VII)

The absorption spectrum of potassium manganate(VII) in water displays a

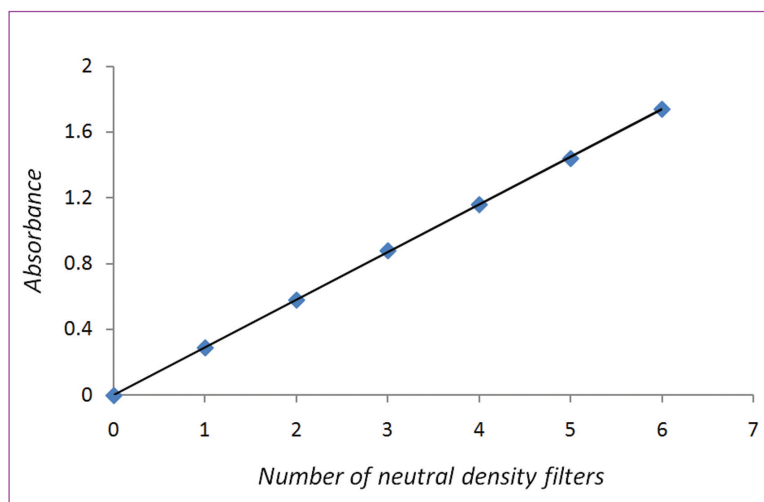


Figure 3 - Absorbance, as measured using the Mystrica Colorimeter, as a function of the number of neutral density filters present. Each neutral density filter is rated by the manufacturer as having an absorbance of 0.3. Excitation wavelength was 535 nm.

number of maxima in the visible region. At 535 nm the molar absorption coefficient of potassium manganate(VII) is *ca.* $2.0 \times 10^2 \text{ m}^2 \text{ mol}^{-1}$ [Perkampus and Schmiele, 1967]. A stock solution ($2 \times 10^{-3} \text{ mol dm}^{-3}$) of potassium manganate(VII) was prepared in water. Dilutions of the stock were prepared and the absorbance of each measured using the green diode as the excitation source. The resulting data are shown in Figure 4. An entirely adequate standard curve could readily be generated from such data.

Rhodamine 6G

The absorption spectrum of rhodamine 6G in water shows a maximum in the green portion of the visible spectrum with a molar absorption coefficient at 535 nm of *ca.* $7.0 \times 10^3 \text{ m}^2 \text{ mol}^{-1}$ [Johnson, 1995]. A range of rhodamine 6G solutions in water, in the range $0 - 2 \times 10^{-5} \text{ mol dm}^{-3}$, were prepared and the absorbance of these solutions was measured using both the WPA colorimeter with a 520 nm filter and the MC. The results are shown in Figure 5.

It should be recognised that the absolute values of absorbance in the two data sets in Figure 5 are not directly comparable since different wavelengths of observation are being used. However non-linearity is clearly observed in the case of data obtained using the MC.

On the basis of potassium manganate(VII) results (see Figure 4) one might have expected that the Mystrica colorimeter would also show a reasonably linear response when using rhodamine 6G solutions. The non-linearity shown is interesting and, at first glance one might argue, worrying.

The most logical explanation for the observed effect is that fluorescence from the sample (rhodamine dyes, in general, display fluorescence yields in excess of 50%) is being detected. In 'conventional' colorimeters and spectrophotometers, the detector collects light which has either passed through a filter or a monochromator and only light of those wavelengths being absorbed is detected (fluorescence being at longer wavelengths is minimised or excluded). A simple view of the design of the Mystrica colorimeter is shown in Figure 6. In the case of rhodamine 6G an alternative explanation may hold (Figure 7).

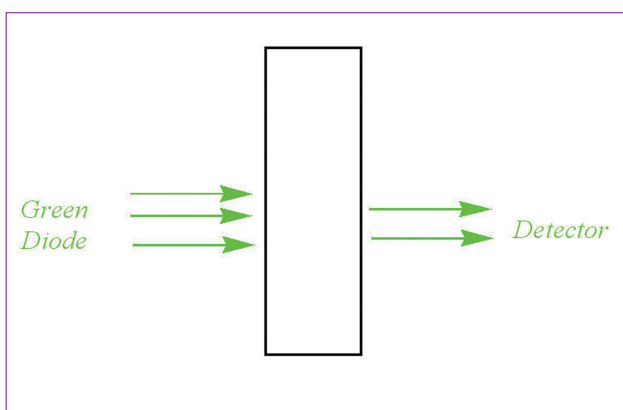


Figure 6 - The detector collects all light. Light which is absorbed in (say) a potassium manganate(VII) solution reduces the amount falling on the detector and the change in intensity is converted into an absorbance reading.

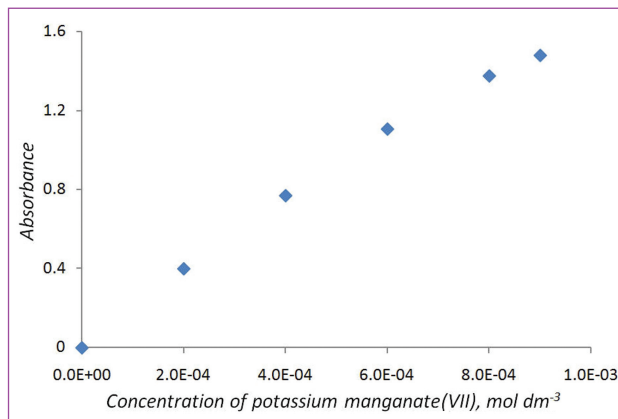


Figure 4 - Absorbance, as measured using the Mystrica Colorimeter, as a function of the concentration of aqueous solutions of potassium manganate(VII). Excitation wavelength was 535 nm and pathlength was 1 cm.

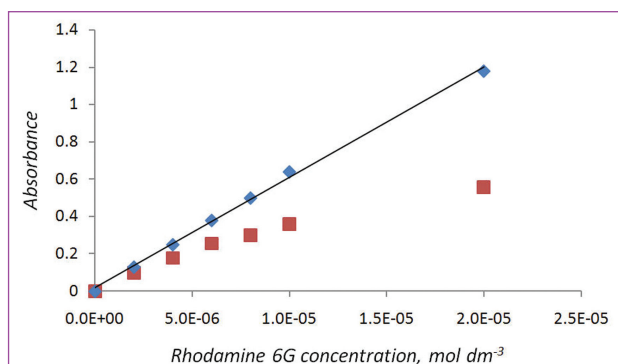


Figure 5 - Absorbance as a function of the concentration of aqueous solutions of rhodamine 6G. Excitation was 535 nm and pathlength was 1 cm. The colorimeter used was either a CO750 colorimeter using a 520 nm filter (♦) or the Mystrica Colorimeter (■).

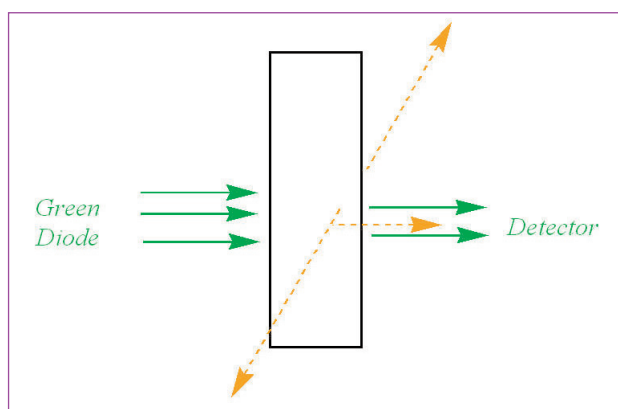


Figure 7 - Here fluorescence is emitted in all directions and, since there are no filters in place, some of this may fall on the detector. Even though light has been absorbed in the solution the detector does not 'see' this by way of the full reduction in intensity which might be expected since some of the green light has been converted into orange light which the detector also collects. So the apparent absorbance in the cuvette is lower than the actual/true value.

- Therefore, should the sample under investigation fluoresce with reasonable efficiency (no attempt has been made to determine the threshold above which fluorescence detection becomes a problem) then there is a chance that the MC will collect such fluorescence and give false readings. For most materials likely to be used in the school/college laboratory this will not be a major problem.

Absorbance of Coloured Solutions (630 nm excitation)

Methylene Blue

At 630nm the molar absorption coefficient of methylene blue is *ca.* $4.2 \times 10^3 \text{ m}^2 \text{ mol}^{-1}$ [Prah, 2008]. A stock solution of methylene blue was prepared at a concentration of $1 \times 10^{-4} \text{ mol dm}^{-3}$ in water. Dilutions of the stock were prepared and the absorbance measured using the MC; results are shown in Figure 8.

Absorbance of Coloured Solutions (470 nm excitation)

Potassium manganate (VII)

At 470 nm the molar absorption coefficient of potassium manganate (VII) is *ca.* $50 \text{ m}^2 \text{ mol}^{-1}$ [Perkampus and Schmiele, 1967]. A stock solution of potassium manganate (VII) was prepared at a concentration of $2 \times 10^{-3} \text{ mol dm}^{-3}$ in water. Dilutions of the stock were prepared and the absorbance of each measured using the green diode as the excitation source and the data are shown in Fig. 9.

Summary and Conclusions

The Mystrica Colorimeter represents excellent value for money as a simple, robust and reliable colorimeter for use in schools/colleges.

In a subsequent issue of the *SSERC Bulletin* we will explore how the Mystrica Colorimeter can be used in kinetic studies in biology and chemistry.

References

Johnson, D.G. (1995) An investigation of excited state properties of some rhodamine dyes, PhD Thesis, University of Salford, p 134.

Perkampus, H-H. and Schmiele, C. (1967) in '*UV atlas of organic compounds*', figure K1/12, Plenum Press, New York.

Prah, S. (2008) Optical absorption of methylene blue, <http://omlc.ogi.edu/spectra/mb/index.html> (accessed March 14th 2009).

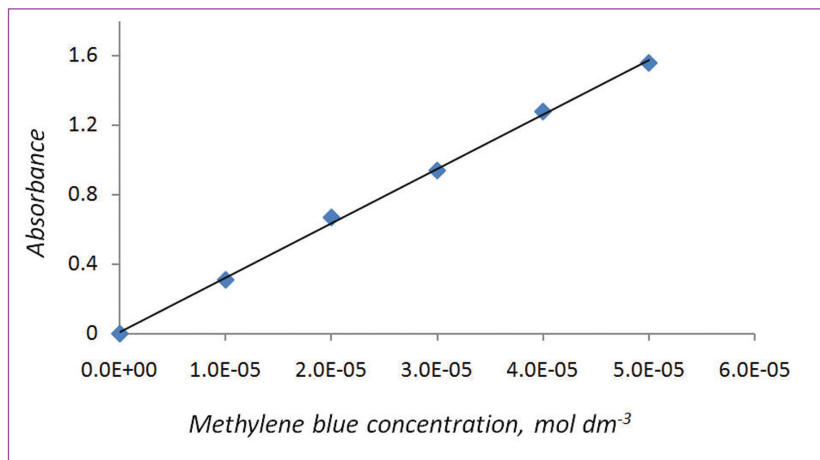


Figure 8 - Absorbance, as measured using the Mystrica Colorimeter, as a function of the concentration of aqueous solutions of methylene blue. Pathlength was 1 cm.

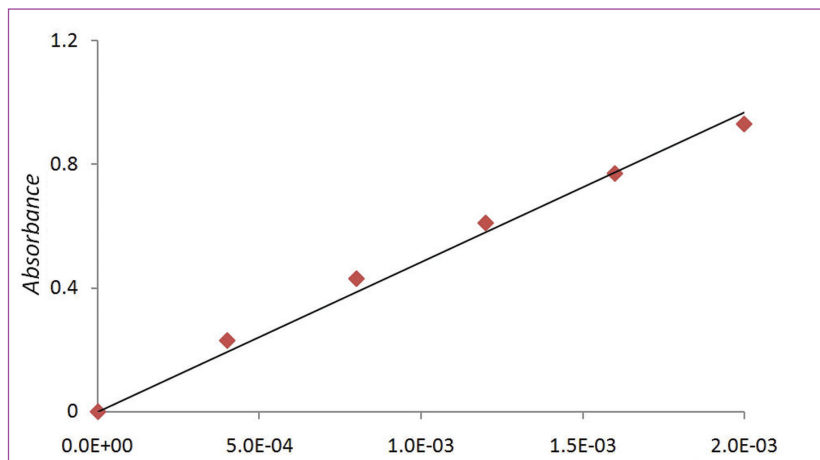


Figure 9 - Absorbance, as measured using the Mystrica Colorimeter, as a function of the concentration of aqueous solutions of methylene blue. Pathlength was 1 cm.