A Combined Spectrophotometer and Fluorometer to Demonstrate the Principles of Absorption Spectroscopy

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ABSTRACT

A dual-function student-crafted instrument is described as part of a laboratory activity aimed to teach both the principles and practical aspects of absorption spectroscopy to secondary and introductory undergraduate students. Using minimal changes in an arrangement that is based on interlocking bricks and low-cost components, both a fluorometer and photometer have been constructed. The former demonstrates the principles of the Beer-Lambert law visually and quantitatively by acquiring the spatial light attenuation through a fluorescent sample. The latter then demonstrates its practical application in a visible-light spectrometer by measuring the absorption spectrum of an aqueous permanganate solution.

GRAPHICAL ABSTRACT

INTRODUCTION

Absorption spectroscopy is an essential technique taught to undergraduate students for the analysis and characterization of chemical species. It gives access to information about physical properties of molecules related to their geometric and electronic structure and is often used to determine the concentration of molecules in, for example, kinetics experiments[1] or as a sensitive probe of the influence of an environment[2]. Consequently, UV-visible absorption spectroscopy is a staple of undergraduate chemistry courses and is also commonly taught in physics, biology, engineering, and medicine courses. The theory of absorption spectroscopy is generally introduced through the derivation of the Beer-Lambert law, which then leads to concepts of absorbance and the molar extinction coefficient[3]. While a diagram is often used to explain the practical implementation, in the laboratories UV-vis spectrometers are generally used as ‘black-box’ instruments: students insert a sample in an instrument and a spectrum is recorded, but no insight offered on how the spectrum was obtained, nor how it relates to concepts of the Beer-Lambert law.[4] In order to convey both these underpinning ideas in an accessible manner to undergraduate (and secondary school aged) students, we present a simple, low-cost instrument using interlocking bricks and few optical components that can (with minimal changes) function as both a spectrophotometer and fluorometer. Together, these provide students with the basic principles of absorption spectroscopy.

Qualitative and quantitative low-cost scientific instruments promote availability to students and are ideal practical tools to teach fundamental principles of spectroscopy.[5-11] Interlocking bricks, in particular, have been exploited for teaching various aspects of chemistry.[8,12-15] Several low-cost spectrophotometers have been developed using interlocking bricks as optical mounts, and/or as building blocks/supportive structures.[16-18] The construction of such visible spectrometers develops students’ scientific enquiry skills as they build the spectrometer, develops optical and practical skills, and brings a level of enthusiasm by using a “toy” to do science[19-20]. Previous studies using interlocking bricks have focused on the measurement of absorbance or to demonstrate the optical design features.[8,16-18] Instead, our instrument has been designed specifically as a practical tool to convey the *principles* of absorption spectroscopy. We have exploited the versatility of interlocking blocks to craft both a spectrophotometer as well as a fluorometer, which can be interconverted with minimal change in layout. The latter images the absorption of light along a path-length, thus providing a visual (as well as quantitative) validation of the Beer-Lambert law. This then lays the basis for the spectrophotometer configuration, which allows the measurement of an absorption spectrum. The spectrophotometer configuration is similar in spirit to some previous reports.[16-18] However, in these a photodiode with home-built electronics was used, whereas we have opted to simply capture the dispersed light (*i.e.* the entire spectrum) on a screen using a webcam, because it offers a direct measure of what the student can see. A key novelty of our spectrometer is that it teaches both the fundamentals *and* application of absorption spectroscopy in one activity. Finally, we aimed to ensure that the spectrometer allowed the collection of semi-quantitative data, requiring both data analysis and calibration, as these are essential components of practical spectroscopy. Our simple spectrometer, costing <\$100 (excluding personal computer), performed comparably to a commercial instrument when measuring the absorption spectrum of the permanganate anion.

SPECTROMETER CONSTRUCTION AND CONFIGURATIONS

With the design aim being a reconfigurable visible absorption spectrometer that is easy to understand and implement, at minimal cost, interlocking bricks offer an ideal solution. We developed two configurations as shown in Figure 1. The first configuration (Figure 1a) demonstrates the Beer-Lambert law using the fluorescence imaging of light passing through a fluorescent sample, while the second configuration (Figure 1b) enables visible absorption spectroscopy.

The critical components of a spectrometer are a light source, a slit, a lens, a dispersing element, a monitoring device and a sample. The construction uses an array of interlocking bricks to build a housing and mount for various components (see Figure 1a and b and a detailed description in the Supporting Information). For the light source, we used a bright white-light LED. The slit is constructed by the gap between two interlocking bricks. The lens is a plano-convex lens that is glued that can be translated on an “optical rail”. The dispersing optic is a low-cost polymer transmission grating, which diffracts the light at an angle that is wavelength dependent, thus dispersing the colors when viewed on a screen. As a sensor, a simple webcam was used to capture an image (either fluorescence or the dispersed light projected on a viewing screen). Finally, the liquid samples were held in a standard 1 × 1 cm plastic cuvette and placed in the light path as described below. To capture images of sufficient quality, a 'light-tight' box is constructed from interlocking bricks around the detection area with an open area made for the webcam to offer a view inside the box and a lid on top of the construction. The instrument can then be used in ambient light conditions in a typical laboratory. With experience, the construction of the spectrometer takes 10 to 15 min. Within an undergraduate laboratory setting, students typically take 45 – 60 min to complete the construction with clear instructions.

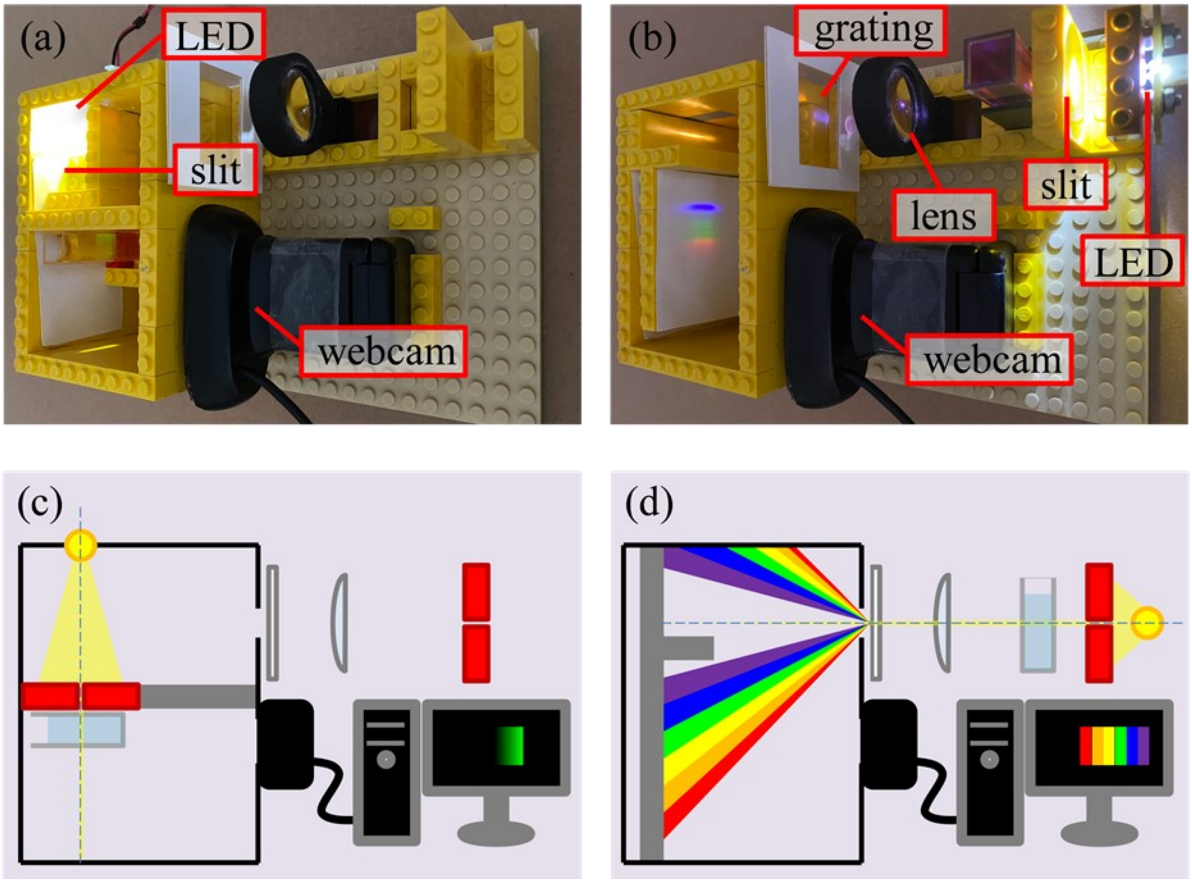

Figure 1. Fully assembled visible-range spectrometer. A first configuration, (a), allows the demonstration of the Beer-Lambert law by using fluorescence imaging; and a second configuration, (b), performs absorption spectroscopy; (c) and (d) are, respectively, the schematics of each configuration. The spectrometer is composed of a white-light LED source (and its power supply), a slit, a lens, a dispersing optic (transmission grating), and a webcam plugged to a computer.

In the fluorometer configuration, the light source passes through the slit and then through the sample placed in a plastic cuvette, where it is attenuated by the absorbance of the solution. The light intensity along the light propagation axis is monitored through the fluorescence of the sample by using the webcam placed perpendicularly to the propagation axis, as shown in the scheme (Figure 1c). The observed fluorescence is proportional to the amount of light present (at low fluorophore concentration) and so serves as a convenient measure of the light intensity. The webcam is plugged into a computer (by USB plug and play) and the software used to take still images is freely available[21]. The still images are subsequently used in the data analysis (see below). As a fluorophore, we used an aqueous solution (5×10^{-5} M) of fluorescein disodium salt (Sigma Aldrich). This has a very high quantum yield ($\Phi = 0.9$).[22-23] Stock solutions were made for the students to administer to the cuvette using a pipette.

In the absorption spectrometer configuration (Figure 1d), the light passes through a slit and then the sample. The transmitted light is focused using the lens, dispersed by the transmission grating, projected onto a screen (white paper), and monitored by the webcam plugged into a computer. The slit and lens together are critical in determining the resolution of the experiment. The lens position can be optimised by imaging the light emitted from the slit using the 0th order diffraction of the grating (*i.e.* its transmission). Specifically, the 0th order can be seen in Figure 2b and optimising its transverse focus will lead to acceptable results (see below). The focus can be further optimised using the 1st order diffraction spectrum of a high contrast sample (e.g. a color glass filter, see below), but we generally did not find that this made a large difference. Once the ideal position of the lens is determined, it should not be moved and all necessary reading should be done in a quick succession. In the laboratory course, the absorption spectrum of aqueous $KMnO_4$ (Sigma Aldrich) was measured. A stock solution of $KMnO_{4(aq)}$ made up so that the concentration of the permanganate anion, $[MnO_4^-]$, is 0.2 mM.

HAZARDS

Both fluorescein and potassium permanganate at these low concentrations pose minimal hazard using good laboratory practice. If in contact with skin or eye, it should be rinsed with plenty of water and in case of ingestion, clean mouth with water and drink plenty of water afterwards. To dispose, flush down fume cupboard sink with plenty of water.

RESULTS AND ANALYSIS

Experimental Demonstration of the Beer-Lambert Law

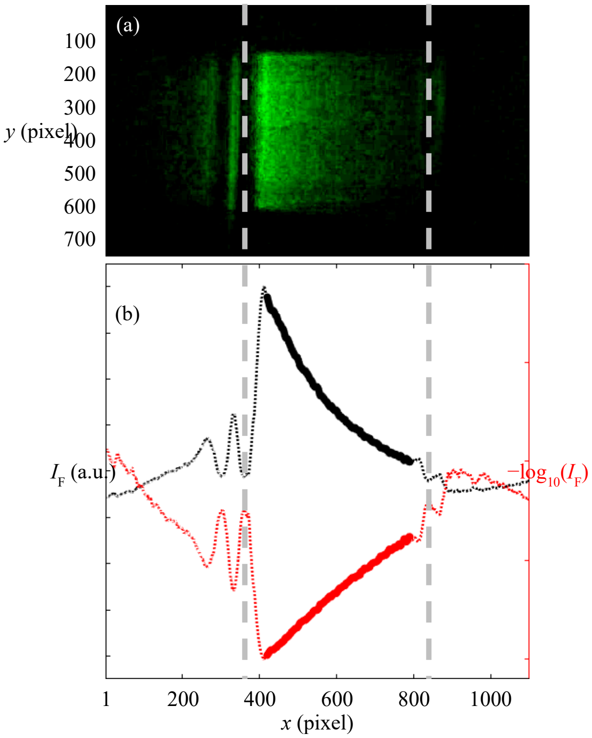

Figure 2. Observation of the Beer-Lambert law: (a) picture of a 5 × 10^{-5} mol L^{-1} fluorescein solution in a 1 × 1 cm plastic cuvette recorded using the spectrometer; (b) averaged fluorescence intensity (I_F) (in black) and $-\log(I_F)$ (in red). The light propagates from the left side of the image to the right side. The vertical dashed line represent the edges of the solution cell.

Using the first configuration (Figure 1a), the fluorescence produced by light passing through the fluorescein solution was monitored using the webcam. A region of the captured image is displayed in Figure 2a. The light propagates from left to right and the fluorescence emitted from the solution appears as green. In Figure 2a, the edges of the cuvette can be identified as breaks in the fluorescence signal; these are indicated by two vertical grey dashed lines. As the light propagates through the sample, the fluorescence intensity, I_F, can be seen to decay. At the left (input) side of the cuvette edge, an additional fluorescence signal can be seen that arises from a reflection of the solution's fluorescence on interlocking bricks wall.

To analyse the data in the image, I_F as a function of position along the propagation axis of the light, x, was calculated by summing the columns of the image over a chosen vertical range. This was achieved using a simple script (in Python, using either an online compiler or as an executable see Supporting Information) that converts the RGB image into a grey scale one and averages about 150 pixels along the y-axis of a slice centred on the cuvette[24]. The results are shown as a black dashed line in Figure 2b. In the region where the light passes through the sample, $I_F(x)$ shows an exponential decay. According to the Beer-Lambert law, the light transmitted after a sample is:

$$I = I_0\ 10^{-\varepsilon c \ell}, \tag{1}$$

where I_0 is the incoming light intensity. In the present case, I is proportional to I_F and is measured not just after the sample, but at all point along the sample. Hence, we can write:

$$I_F(x) = \kappa\ I_0\ 10^{-\varepsilon c x}, \tag{2}$$

where κ is a proportionality constant. I_F is an exponentially decaying function because the loss of light along the propagation axis, $-dI_F/dx$, is proportional to be much light there is, I_F.[25] That is to say, the more light there is, the more light will be absorbed. Note that edge effects mean that only the central region of the cuvette offers a usable exponential decay as highlighted with a thicker line in Figure 2.

To linearize the plot and demonstrate that the decay is exponential, Eq. (2) is recast in the familiar form of the absorbance:

$$A(x) = -\log_{10}[I_F(x)/I_0] = \varepsilon c x - \log_{10}(\kappa). \tag{3}$$

The function $A(x)$ is shown as a red line in Figure 2b (dashed is over all x and solid over exponential region of $I_F(x)$ as shown by solid black line) and confirms that the decay is exponential. The gradient of the line is εc. To be quantitative, the x-axis can be converted from pixels to cm. This can be done from Figure 2b by recognising that 1 cm is the distance between the two edges of the cuvette, which suggest that there are approximately 480 pixels cm^{-1}. From Figure 2b, we obtain that the gradient $\varepsilon c = 1.6 \times 10^{-3}$ pixel^{-1}, which yields 7.7×10^{-1} cm^{-1}. Given the concentration of fluorescein $c = 5 \times 10^{-5}$ M, we obtain a molar extinction coefficient $\varepsilon = 1.5 \times 10^4$ $\text{M}^{-1}\ \text{cm}^{-1}$ in water. This value is in reasonable agreement with the literature value (9.23×10^4 $\text{M}^{-1}\ \text{cm}^{-1}$ at 500 nm).[20] As we use a white-light LED to produce the fluorescence, we did not measure it at a specific wavelength and, hence, there is a discrepancy between the two values.

Absorption spectroscopy

The absorption spectrum of $KMnO_{4(aq)}$ was measured using the absorption spectrometer configuration (Figure 1b). The absorbance of a sample at a range of wavelengths, λ, can be expressed as:

$$A(\lambda) = -\log_{10}[I(\lambda)/I_0(\lambda)] = \varepsilon(\lambda)c\ell. \qquad (4)$$

Hence, to determine $A(\lambda)$, both $I(\lambda)$ and $I_0(\lambda)$ must be measured independently. Figure 3 shows a series of images of the dispersed light as measured on the screen by the webcam after passing through a sample of: pure water, the $KMnO_{4(aq)}$ solution, air (*i.e.* no sample), and a filter with a well-known absorption spectrum (FGB67, Thorlabs).

The water and air spectra are very similar and simply represent the emission profile of the LED as measured by the webcam. The limited range of the spectrum naturally limits the spectrometer to those molecules that absorb in this spectral range. Using different LED sources could enable absorption spectroscopy in various spectral ranges (including in the UV as most bleached paper fluoresces UV light). From the images in Figure 3, the respective spectra $I_{\mathrm{w}}(x)$, $I_{\mathrm{pm}}(x)$, $I_{\mathrm{air}}(x)$ and $I_{\mathrm{filt}}(x)$ can be obtained by integrating each over a given range along the vertical axis, y-axis, and ensuring the same range is used in all cases. These transmission spectra are included as white lines on the images. The image of the $KMnO_{4(aq)}$ sample, shows that light in the green spectral region appears diminished (*i.e.* absorbed). Additionally, on top of the reduced transmission in this range, some darker stripes can be discerned. The corresponding spectrum, $I_{\mathrm{pm}}(x)$, confirms the depletion with respect to the spectrum of the solvent, $I_{\mathrm{w}}(x)$. The image and spectrum for air are similar to those of water and effectively represent the spectral profile of the white-light LED. Finally, the reference filter image shows transmission over several narrow ranges as seen in the spectrum $I_{\mathrm{filt}}(x)$. The filter is used to calibrate the x-axis of the spectra, as explained below.

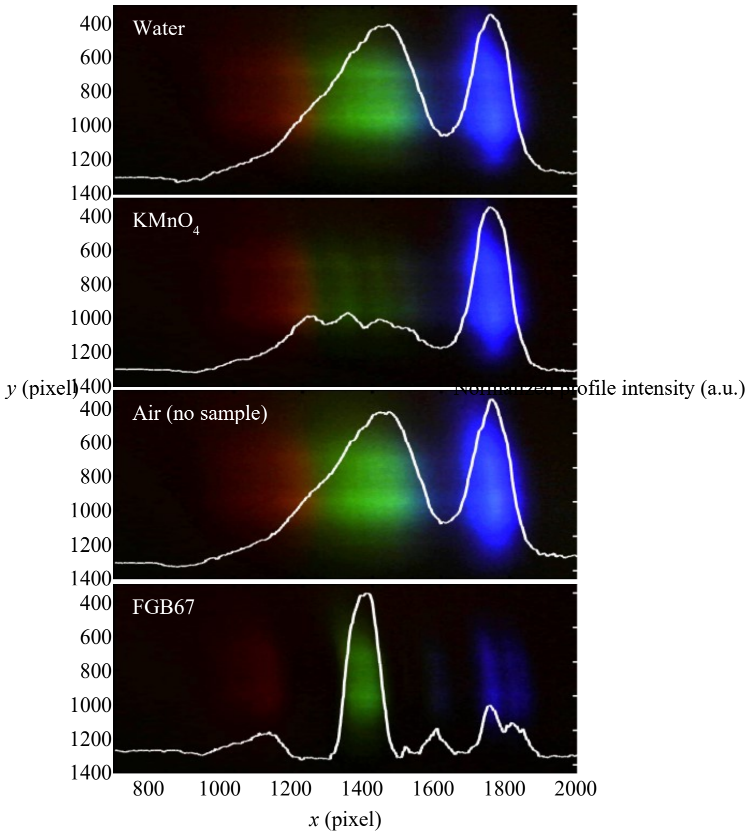

Figure 3. Images of diffracted light measured on the screen by the webcam of a sample of pure water, the KMnO$_{4(aq)}$ solution, no sample (i.e. air) and a 2 mm thick bandpass color filter with a well-known transmission-spectrum (FGB67, Thorlabs).

Spectral calibration

Once the data are acquired, the four functions $I_{\mathrm{w}}(x)$, $I_{\mathrm{pm}}(x)$, $I_{\mathrm{air}}(x)$ and $I_{\mathrm{filt}}(x)$ can be tabulated. This is done in Microsoft Excel in the laboratory class and all the analysis is done on these data (see examples in Supporting Information). The first step is to convert the variable x from units of pixels to the appropriate unit of wavelength, nm. For this, the characteristic bands of $I_{\mathrm{filt}}(x)$ are ideal. A reference absorption spectrum for the filter, $A_{\mathrm{ref}}(\lambda)$, is available from the vendor and is shown in Figure 4a (black line, taken from Thorlabs[26]). The reference spectrum can be compared to the absorption spectrum of the filter measured, which can be calculated as $A_{\mathrm{filt}}(x) = -\log_{10}[I_{\mathrm{filt}}(x)/I_{\mathrm{air}}(x)]$. To convert $A_{\mathrm{filt}}(x)$ to $A_{\mathrm{filt}}(\lambda)$, a conversion function was determined by aligning characteristic points (maxima and minima) of $A_{\mathrm{ref}}(\lambda)$ (shown as black crosses in Figure 4a) to the same points in $A_{\mathrm{filt}}(x)$. Hence, tabulation a series of x values that are associated with the corresponding λ from $A_{\mathrm{ref}}(\lambda)$ offers a graph as shown in Figure 4b (see Excel example in Supporting Information). A fit to this graph produces the function $x(\lambda)$ that can be used to convert x (in pixel) to λ (in nm). In Figure 4b, both a quadratic and linear fits are shown, generated using Microsoft Excel's in-build solver by minimising the root-means-square of the differences. The quadratic fit shown in Figure 4b has a $R^2 = 0.9991$, while the linear fit offers $R^2 = 0.9984$; either would be acceptable for the undergraduate lab course. Using the function $x(\lambda)$, the spectrum, $A_{\mathrm{filt}}(x)$ can be converted to $A_{\mathrm{filt}}(\lambda)$ as shown in Figure 4a, and this aligns very well with the reference spectrum $A_{\mathrm{ref}}(\lambda)$.

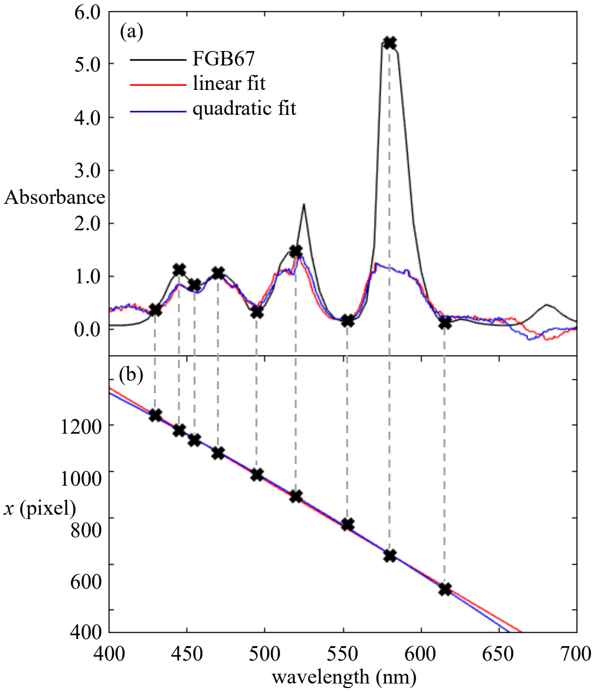

Figure 4. Absorption spectrum of a FGB67 filter (THORLABS data) in black compared to absorption spectra obtained with the spectrometer using a linear (in red) and a quadratic (in blue) fit to turn pixel into wavelength.

Absorption spectrum of aqueous potassium permanganate $KMnO_{4(aq)}$

The absorbance of $KMnO_{4(aq)}$ was calculated using $A_{\mathrm{pm}}(x) = -\log_{10}[I_{\mathrm{pm}}(x) / I_{\mathrm{w}}(x)]$ and subsequently converted to $A_{\mathrm{pm}}(\lambda)$ using the calibration function determined above. The absorption spectrum is shown in Figure 5 between 450 and 625 nm along with a spectrum recorded with a commercial UV-Vis spectrometer (Perkin Elmer Lambda 40). The overall agreement is surprising: even the vibrational structure is resolved. The spectrum is also in reasonable quantitative agreement, especially in terms of wavelength. For the solution with [MnO_4^-] = 0.2 mM, a peak absorbance of $A_{pm} = 0.51$ is seen from Figure 5, which yields $\varepsilon = 2.6 \times 10^3$ M^{-1} cm^{-1}. This is in good agreement with the extinction coefficient of 2.4×10^3 M^{-1} cm^{-1} from the literature.[27]

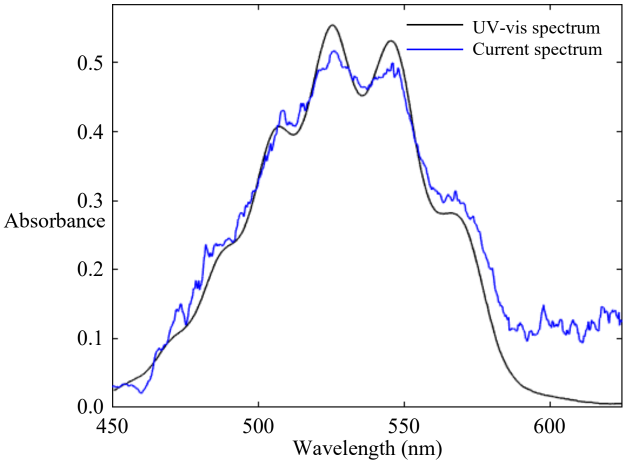

Figure 5. Absorption spectrum of $KMnO_4$ obtained using the spectrometer in blue and using a commercial UV-Vis spectrometer in black.

DISCUSSION AND OUTLOOK

We set out to develop a stand-alone experiment that would offer students hands-on experience of the principles and practices of absorption spectroscopy. With regards to the latter, the use of interlocking bricks provides and develops practical skills in terms of experimental design concepts (*e.g.* data acquisition and calibration), as well as optical alignment principles, that are often not taught in undergraduate physical chemistry courses. Moreover, it uncovers the “black-box” of an absorption spectrometer that is commonly used in many undergraduate and postgraduate laboratories. With regards to the principles of absorption spectroscopy, the current experiment aligns directly with the taught material in most undergraduate courses in which the Beer-Lambert law is derived by considering that the loss of light traversing an absorbing material is proportional to how much light there is initially, $-dI/dx \propto I$. This is shown directly using the first configuration of the experiment and also offers an opportunity to explain why exponential functions are so common in physical sciences. With regards to absorption spectroscopy, the absorption spectrum of $MnO_4^-{}_{(aq)}$ (Figure 5) not only demonstrates that the extinction coefficient depends on λ, but also shows vibrational structure from which structural changes can be derived upon excitation.[28] This can be conveniently used to show concepts of the Franck-Condon principle and can be linked to inorganic transition metal spectroscopy. The transition is a ligand-to-metal charge-transfer (LMCT) transition that arises from promoting an electron from the $1t_1$ molecular orbital (an oxygen lone-pair) to the 2e molecular orbital (the Mn-centred d_{x2-y2} and d_{z2}). The vibrational mode that is predominantly excited as the totally symmetric a_1 Mn−O stretch.[29]

One important driver was to ensure that the cost of the spectrometer remained low. The current instrument costs <\$100 (excluding the computer required to interface the webcam), with the interlocking bricks making up a large fraction of the cost. The cost can be dramatically reduced by: 3D printing a basic layout with suitable compartment for all components, replacing the lens with a much cheaper one, and potentially using students' mobile phones to capture images[30]. This could reduce the overall cost to <\$25 per instrument. At this cost, one could envisage that the students can simply buy (or to be provided with) a spectrometer. Ownership would allow students to explore absorption spectra in their own time or in other laboratory classes that require the measurement of absorption spectra and to potentially use in later years of their degree.

A key difference between the current work and previous student-crafted spectrometers[16-18] is that it serves two functions in a single practical, for which the interlocking bricks are very convenient. Specifically, it teaches the principles underlying absorption spectroscopy as well as the practise. Moreover, it offers both a visual learning tool (through the fluorescence and using screens to project transmitted light) as well as analytical (through calibration and absolute measurement of molar extinction coefficients). Finally, by exploiting interlocking blocks, students can extrapolate uses of the instrument to explore the variables underpinning absorption spectroscopy such as cell path-length and sample concentration.

CONCLUSION

A low-cost reconfigurable visible spectrometer using interlocking bricks is described. The flexibility of our spectrometer allows (i) the practical demonstration of the Beer-Lambert law by monitoring the fluorescence emitted from the sample as the light passes through; (ii) the recording of an absorption spectrum of a sample that is comparable to a commercial UV-vis spectrometer. The experiments provide an appreciation of optical design processes, calibration requirements, and the conversion from transmittance to absorbance. The spectrometer provides a practical basis to discuss and understand the fundamental scientific principles of absorption spectroscopy while developing basic analysis skills. Moreover, the low cost will potentially enable many instruments to be present in a laboratory offering the students a sense of ownership that can be transferred to other experiments in both physical chemistry and general chemistry laboratories.

ASSOCIATED CONTENT

Supporting information

- Detailed laboratory instruction for students (DOCX)
- Detailed builder's manual to construct instrument (DOCX)
- Example experimental data and workbook layout (XLS)
- The Supporting Information is available at https://pubs.acs.org/doi/XXXX.

Additional example images from measurements (JPG) and the Image to spectrum converter source code (PY) are available at https://doi.org/10.5281/zenodo.5552379

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