Beers Law Instructor's Guide

David T. Harvey

Introduction

This learning module provides an introduction to Beer's law that is designed for an introductory course in analytical chemistry. The module consists of seven investigations:

- Investigation 1: Recalling Beer's Law
- Investigation 2: Absorbance Spectra
- Investigation 3: Calibration Curves
- Investigation 4: Fundamental Limitation to Beer's Law
- Investigation 5: Instrumental Limitation to Beer's Law: Polychromatic Radiation
- Investigation 6: Instrumental Limitation to Beer's Law: Stray Light
- Investigation 7: Chemical Limitation to Beer's Law

The learning module is programmed in R (www.r-project.org) using the Shiny package, which allows for interactive features. Each investigation includes a brief introduction, an explanation of the controls—sliders, radio buttons, and check boxes— available to the user and the type of plots, tables, and statistical summaries produced by the underlying code. Each investigation also includes one or more questions to answer.

The purpose of this document is to provide instructors with additional background on the program's features and data sets, to provide representative examples of the results students might generate, and to provide suggestions of possible ways to make use of the module's investigations.

Note: The dimensions for the figures in this document are not the same as their respective dimensions when running the application in a browser; as a result, the legends included with the figures in this document partially overlap the data, although not in a way that obscures meaning.

Some Background Details on the Learning Module's Data

The data included with this learning module consists of two objects: a data frame with 351 rows and 20 columns, and a numerical vector with 14 values. As shown here, each row of the data frame provides information for one wavelength in the range 400 nm to 750 nm, and each of the 20 columns provides values for a different variable:

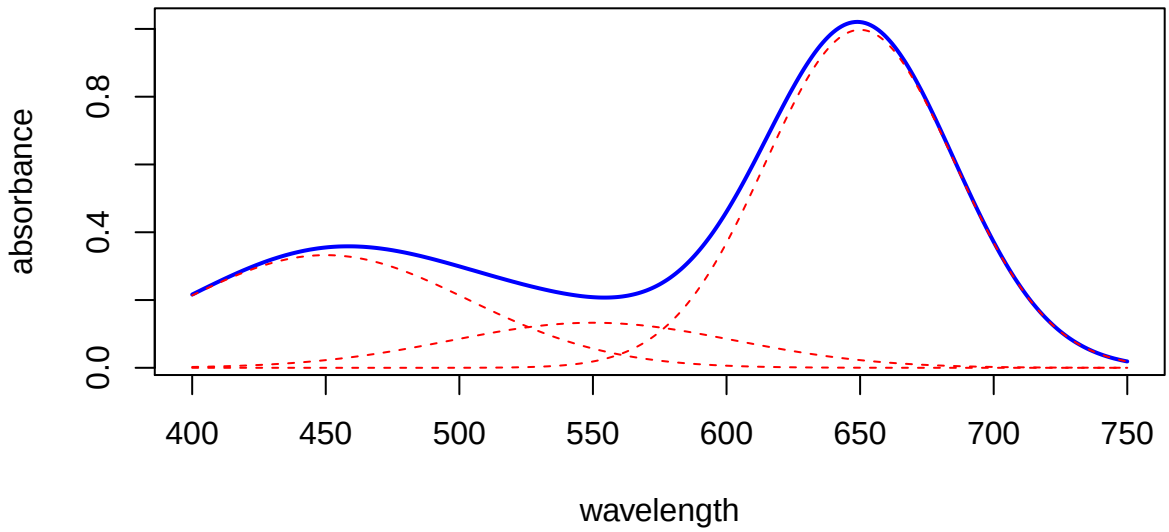
- Column 1: wavelengths
- Column 2: absorbance values for a 5.000 mM standard solution of the analyte (noise free)
- Column 3: transmittance values for a 5.000 mM standard solution of the analyte (noise free)
- Column 4: power spectrum for the reference (noise free)
- Column 5: power spectrum for the sample (noise free)
- Column 6: ϵb values for the analyte (noise free)
- Column 7: absorbance values for a blank (with 0.2%T noise added)
- Columns 8-20: absorbance values for a series of standards with concentrations of 0.100 mM, 0.200 mM, 0.400 mM, 0.500 mM, 0.600 mM, 0.800 mM, 1.000 mM, 2.000 mM, 4.000 mM, 5.000 mM, 6.000 mM, 8.000 mM, and 10.000 mM (with 0.2%T noise added)

The numerical vector contains the concentrations of analyte in each of the standards, as noted above.

For those wishing to explore the data, files are available at the application's Github site. The file BL.RData contains both the dataframe and the vector and can be loaded directly in R. The file rawdata.csv contains the dataframe only and be opened in Excel or read into R.

A brief description of how this data was created is in order. The power spectrum for the reference is taken from the emission profile of a tungsten-halogen visible source; the original data is from Thor labs for their model SLS201 light source and gives power as a function of wavelength from 400 nm to 2600 nm in 10 nm intervals. This data was trimmed to the range 400 nm to 750 nm and additional values were added at 1 nm intervals using a linear interpolation over each 10 nm interval. The resulting power spectrum was assigned as the reference and is stored in the data frame's fourth column.

The absorbance values for the standard solution of 5.000 mM analyte was simulated in R using a function written for that purpose; the resulting spectrum is the sum of three Gaussian curves centered at 450 nm, 550 nm, and 650 nm, with respective standard deviations of 30 nm, 30 nm, and 20 nm, and respective peak heights of 25, 10, and 50. The resulting absorbance spectrum is shown here in blue (with values stored in the data frame's second column) and has a maximum absorbance at 649 nm; the dashed red lines show the three Gaussian functions that make up the spectrum.



This absorbance spectrum was used to generate the ϵb values in the data frame's sixth column by dividing by the standard's concentration of 0.00500 M, and the transmittance values in the data frame's third column were calculated as $T = 10^{-A}$. The sample's power spectrum was calculated as $P_{samp} = T \times P_{ref}$ and stored in the data frame's fifth column.

Finally, the absorbance spectrum for the blank and for each standard was created using the following equation

$$A = \epsilon b C \times (corr)$$

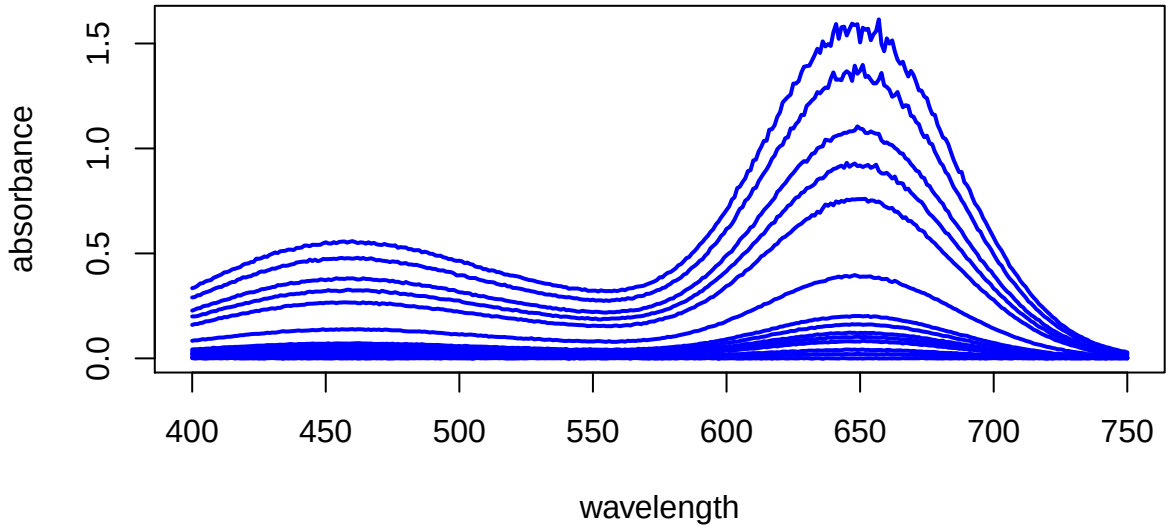
where the standard values for ϵb are in the data frame's sixth column and the concentrations of the standards are in their vector, and where the correction factor $(corr)$

```
corr = c(1.000, 0.9987, 0.9974, 0.9948, 0.9934, 0.9922, 0.9896,
         0.9870, 0.9624, 0.9271, 0.9040, 0.8835, 0.8316, 0.7733)
```

is designed to make obvious a fundamental deviation from Beer's law when the analyte's concentration is greater than 2.000 mM.

Noise was added to each absorbance value by first converting it to a transmittance, then adding a value drawn from a random normal distribution centered on zero with a standard deviation of 0.002 (0.2%T), and,

finally, converting the transmittance back to an absorbance value. The resulting spectra for the blank and for all 13 standards are shown here



Investigation 1

This investigation asks students to remember that Beer's law relates a sample's absorbance to the concentration of the absorbing species, the distance light travels through the sample, and the absorbing species' molar absorptivity. The intent here simply is to review how these four values relate to each other.

Question 1. For the first set of cuvettes, students should recognize that the absorbance values are identical when viewed from the side because ϵ, b, and C are constant, but that when viewed from the top, the absorbance values increase proportionally with the increase in the solution's height. For the second set of cuvettes, students should recognize that the product of b and C is the same for all four cuvettes when viewed from the top, a result that is consistent with a constant value for ϵ.

Question 2. Students should recall that molar absorptivity provides a measure of the relative probability that the analyte will absorb a photon of light of a particular wavelength, and that it has general units of cm^{-1} M^{-1}, or, more generally, $length^{-1}$ $concentration^{-1}$.

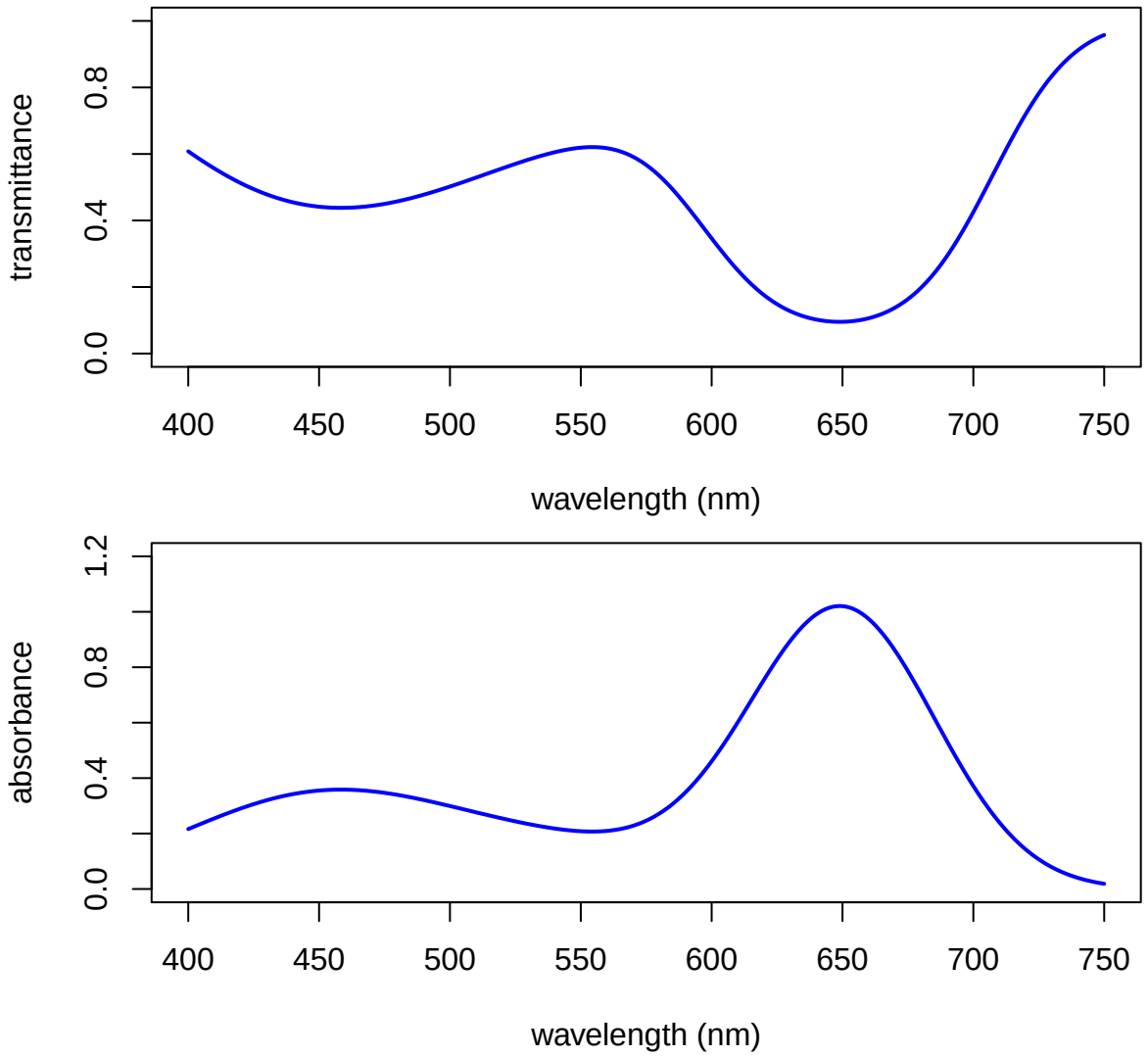
Question 3. The molar absorptivity is $(4X)^{-1}$ cm^{-1} M^{-1}.

Investigation 2

This investigation presents students with the power spectra for the reference and for a standard solution that is 5.000 mM in analyte, the data for which are available as a .csv file using the "Download" button.

Question 1. Students should recall that $A = -\log T$ and that $-\log T = \epsilon b C$.

Question 2. To create the transmittance spectrum and the absorbance spectrum, students should use the equations $T = P_{sam}/P_{ref}$ and $A = -\log T$, which yields the results shown here

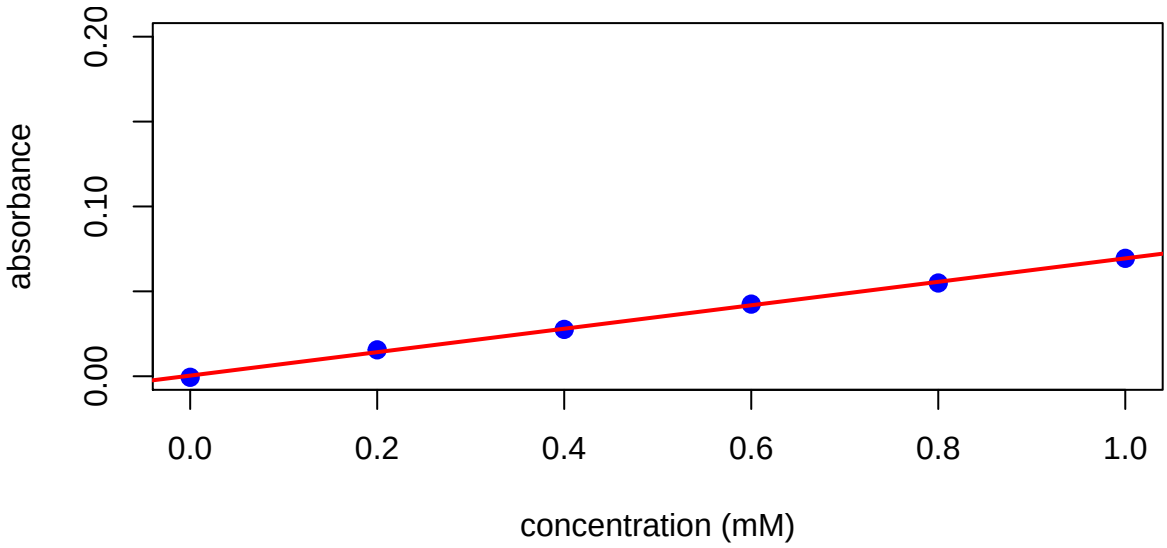


Students can check their work by using the radio buttons to display the transmittance or the absorbance spectrum and to view the first six values for each.

Investigation 3

In this investigation, students are presented with the absorbance spectra for the blank and for a set of standards with concentrations of 0.200 mM, 0.400 mM, 0.600 mM, 0.800 mM, and 1.000 mM. The slider, which increments in steps of 1 nm, allows students to adjust the analytical wavelength and to retrieve the absorbance data for the standards at this wavelength. After plotting the calibration data and completing a regression analysis, students can evaluate their results by checking the box for "Show Model?" and examining the resulting regression model.

Question 1. The calibration curve and the regression model when using the default wavelength of 450 nm are shown here




```
##
## Call:
## lm(formula = abs ~ conc)
##
## Residuals:
##          1          2          3          4          5          6
## -9.842e-04  1.363e-03 -4.008e-04  6.801e-04 -6.901e-04  3.164e-05
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.0003692  0.0007172   0.515    0.634
## conc        0.0690523  0.0011844  58.302 5.18e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.0009909 on 4 degrees of freedom
## Multiple R-squared:  0.9988, Adjusted R-squared:  0.9985
## F-statistic:  3399 on 1 and 4 DF,  p-value: 5.183e-07
```

Question 2. Students should note that the intercept's value essentially is constant for all wavelengths with all values clustering near zero; they should note, as well, that the slope varies with wavelength and is steepest where the analyte has the strongest absorbance. Both observations, of course, are consistent with Beer's law, which predicts that the slope of the calibration curve is ϵb, and thus increases as the molar absorptivity increases, and that there is no absorbance in the absence of analyte.

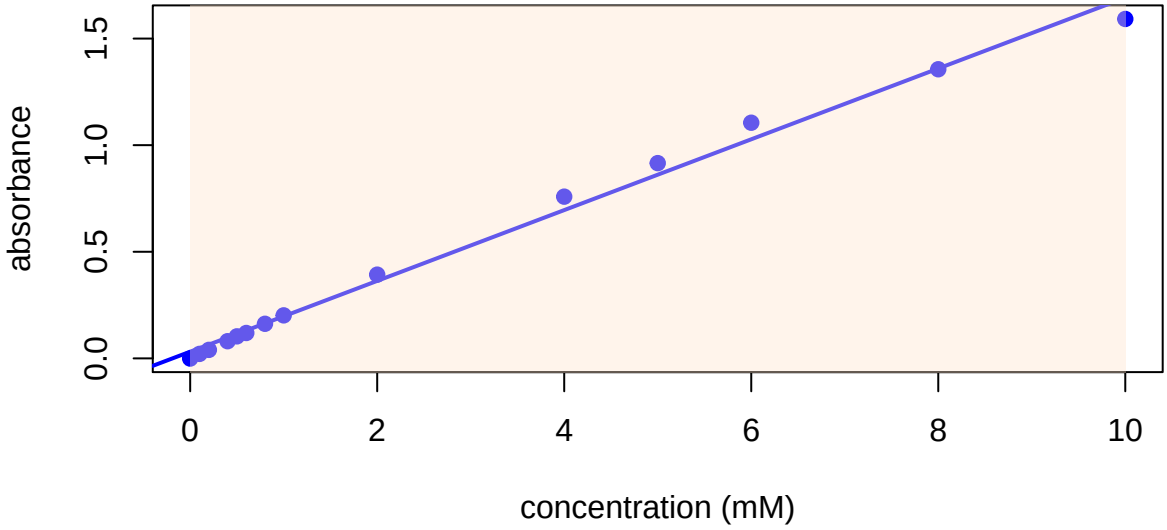
Question 3. The optimum analytical wavelength is 649 nm as this is where the analyte has its maximum absorbance; any value within ± 2 nm or ± 3 nm is reasonable.

Investigation 4

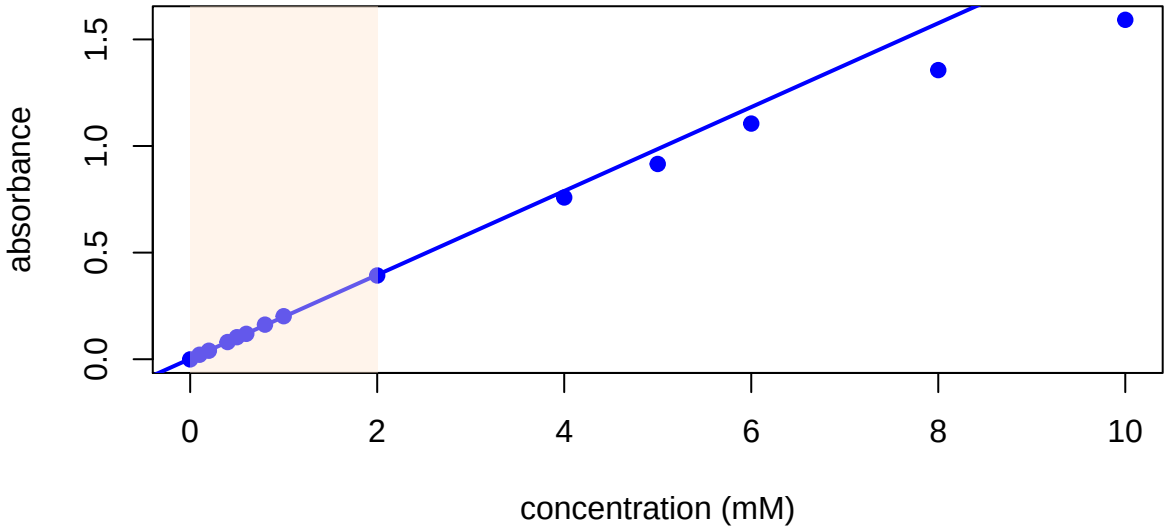
This investigation introduces students to the fundamental limitation of Beer's law. Using the two available sliders, students select the analytical wavelength and adjust the range of concentrations to include in the calibration curve. Highlighting in the calibration plot shows which standards are included in the calibration model.

Question 1. Students will need to consider what it means for a particle to be an independent absorber. A reasonable assumption is that a particle of analyte is independent if its behavior is not affected by the presence of other particles, and that this is more likely for lower concentrations of analyte.

Question 2. When using 649 nm as the analytical wavelength, and when including all 14 standards, the calibration curve



is a poor fit to the data, showing evidence, in particular, of distinct curvature. When limiting the standards to those with concentrations less than or equal to 2.000 mM analyte, the calibration curve provides a good fit to the data.



Students may choose to limit the calibration curve to a maximum concentration of 1.000 mM or extend it to a maximum concentration of 4.000 mM; although these are not unreasonable choices, for standards not included in the calibration curve, consistent negative deviations from the calibration equation are evident only for concentrations of analyte greater than 2.000 mM.

Question 3. Students should recall that to interpolate is to predict an analyte's concentration given an absorbance that falls within the absorbance values used to construct the calibration curve, and that to extrapolate is to predict an analyte's concentration given an absorbance that is greater than that for the most concentrated standard. The calibration curve from Question 1 should convince students that interpolation is not possible if the mathematical model—a straight-line in this case—does not correctly match the data; they should recognize from the calibration curve from Question 2 that extrapolating beyond the calibration data

will lead to a determinate error and that this error becomes larger for greater extrapolations.

Investigation 5

In this investigation, students examine the effect on Beer's law of a non-monochromatic source, using sliders to select the analytical wavelength and the offset for the interfering wavelength.

Question 1. Students should recall from Investigation 2 that transmittance is the ratio of P_{sam} to P_{ref}, where each term is a sum of contributions at each wavelength. Students should recognize that

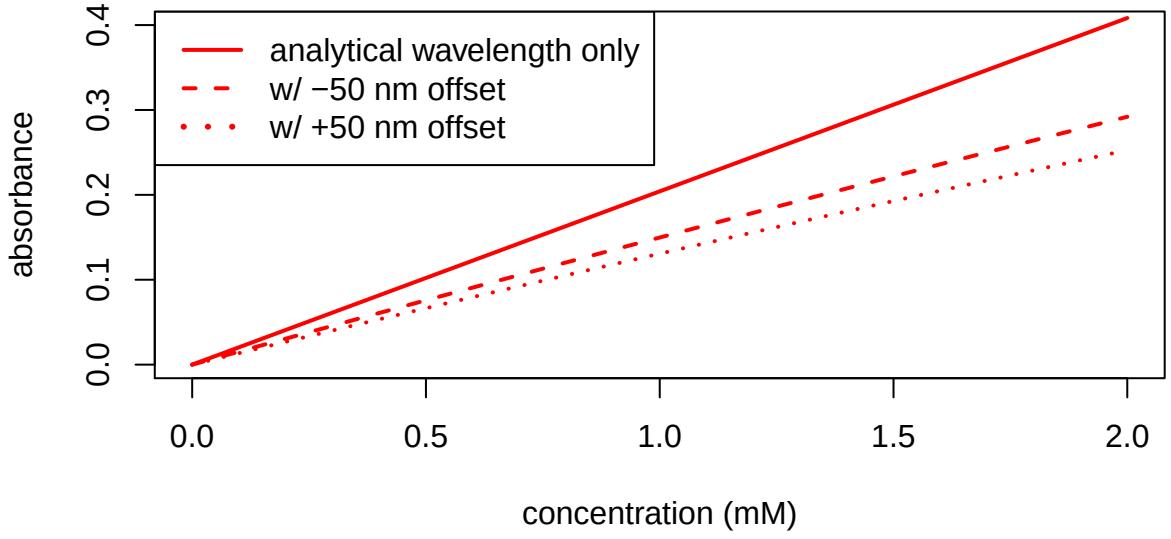
$$T = \frac{P_{sam\lambda 1} + P_{sam\lambda 2}}{P_{ref\lambda 1} + P_{ref\lambda 2}}$$

and, perhaps with additional guidance, they should be able to derive the following equation that relates the combined transmittance of light at both wavelengths to the source's power at each wavelength and the analyte's absorbance at each wavelength; note: it may help to nudge students in this direction.

$$T = \frac{P_{sam\lambda 1} + P_{sam\lambda 2}}{P_{ref\lambda 1} + P_{ref\lambda 2}} = \frac{T_{\lambda 1} P_{ref\lambda 1} + T_{\lambda 2} P_{ref\lambda 2}}{P_{ref\lambda 1} + P_{ref\lambda 2}} = \frac{\left(A^{-\epsilon bC}\right)_{\lambda 1} P_{ref\lambda 1} + \left(A^{-\epsilon bC}\right)_{\lambda 2} P_{ref\lambda 2}}{P_{ref\lambda 1} + P_{ref\lambda 2}}$$

With this equation, it is easy to see that a negative deviation from Beer's law occurs when the the analyte absorbs less strongly at the interfering wavelength than at the analytical wavelength.

Question 2. For an analytical wavelength of 649 nm, increasing the offset from ±5 nm to ±50 nm results in an increasingly more negative deviation from Beer's law. Students should note that the deviation from Beer's law is negligible for an offset of ±5 nm because there is little difference in absorbance at these two wavelengths. They also should note that the deviation is greater for an offset of +50 nm than an offset of -50 nm, as shown here



because the absorbance peak is not symmetrical around the wavelength of maximum absorbance such that the absorbance of 0.371 at 699 nm is much smaller than the absorbance of 0.448 at 599 nm.

Question 3. Students should discover two things as they explore this question. First, as the offset increases from its default value of ±0 nm, the negative offset yields a negative deviation from Beer's law and the positive offset yields a positive deviation from Beer's law; students should be able to explain this using the same logic as in Question 2. Second, between a positive offset of +45 nm and a positive offset of +50 nm, there is no apparent deviation from Beer's law; students should recognize that there is no deviation from

Beer's law if the absorbance at the analytical wavelength is identical to the absorbance at the interfering wavelength.

Question 4. Students should recall that a scanning monochromator's slit width determines the range of wavelengths that pass through the sample, and that a narrower slit width will make the source radiation appear less polychromatic, resulting in a smaller deviation from Beer's law; they should recognize, as well, that a narrower slit width will decrease the total power of source radiation that reaches the detector, which increases the impact of noise.

Investigation 6

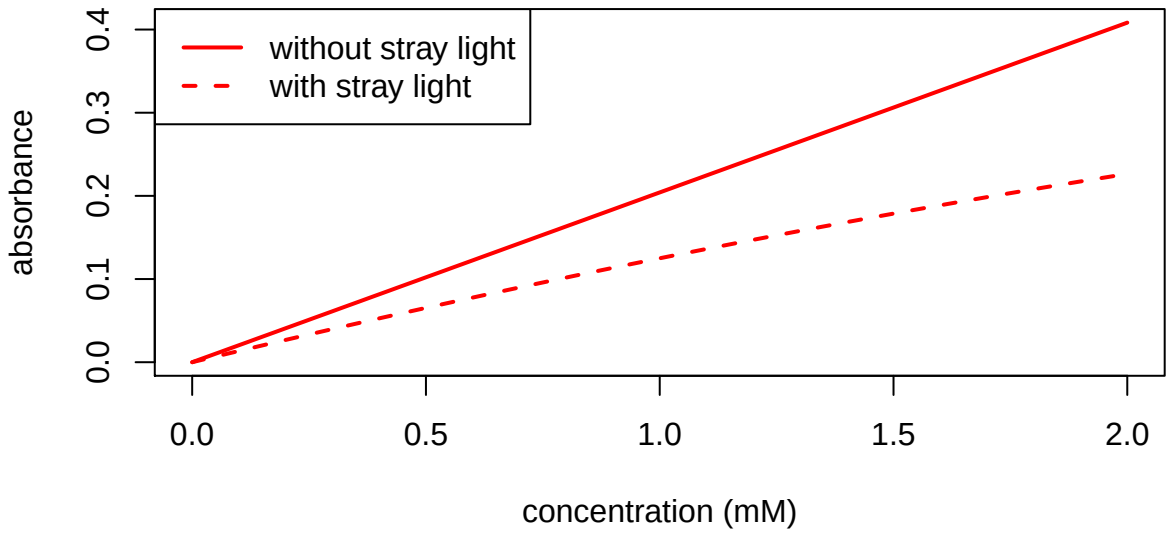
This investigation allows students to explore how stray light affects the linearity of a Beer's law calibration curve using sliders to select the analytical wavelength and the amount of stray light, which is given as a percentage of P_{ref} for the analytical wavelength.

Question 1. Using the same general approach as in Investigation 5, but replacing $P_{ref\lambda 2}$ and $P_{sam\lambda 2}$ with P_{stray}

$$T = \frac{P_{sam} + P_{stray}}{P_{ref} + P_{stray}}$$

students should recognize that the effect of stray light is to increase $P_{sam} + P_{stray}$ relatively more than $P_{ref} + P_{stray}$; thus, T increases and A decreases, resulting in a negative deviation to Beer's law.

Question 2. For all wavelengths and for all amounts of stray light, students should see that the effect of stray light on absorbance is greater for higher concentrations of analyte and greater when using an analytical wavelength where the analyte has a stronger absorbance. A typical result when using an analytical wavelength of 649 nm and 50% stray light is shown here



Using the values from the tables, in the presence of 50% stray light the 2.000 mM standard's absorbance is 55.4% of its absorbance in the absence of stray light; for the 0.200 mM standard, the absorbance in the presence of 50% stray light is 65.7% of its absorbance in the absence of stray light.

Question 3. Students should have little problem identifying as a source of stray light external radiation from the room that finds its way into the spectrometer's detector compartment; students may need some nudges to recognize that scattering of source radiation within the spectrometer might allow some of the scattered radiation to reach the detector without passing through the sample. The first source of stray light is minimized by better designing the spectrophotometer so that external radiation cannot enter the spectrophotometer; the second source of stray light is minimized by improving the spectrophotometer's optics.

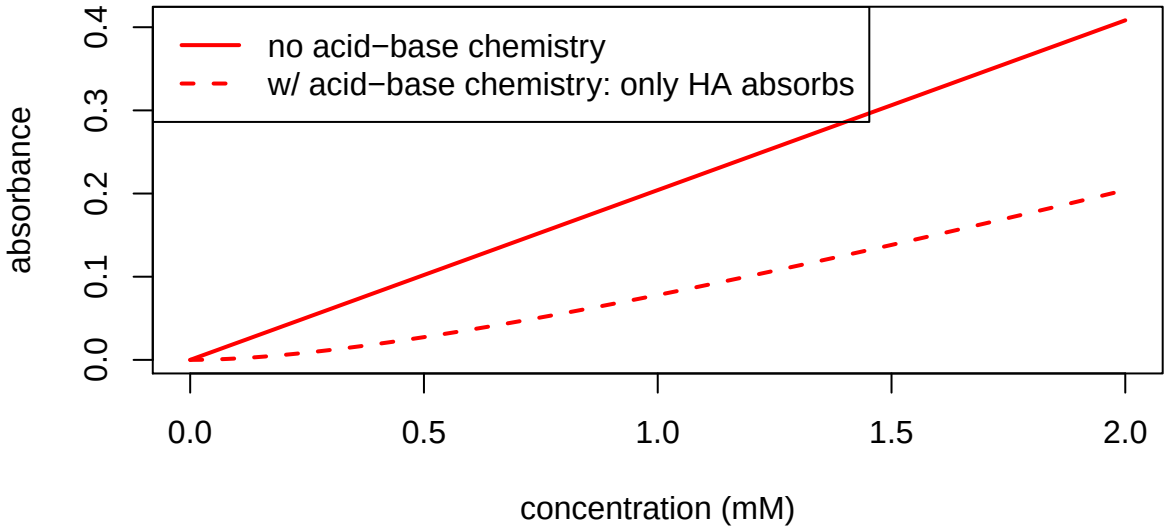
Investigation 7

The final investigation allows students to explore the effect of an analyte's acid-base chemistry on its Beer's law calibration curve. Controls are provided to select the analyte's pK_a value, to select the absorbing species, to allow for buffering, and, when using buffering, to set the buffer's pH.

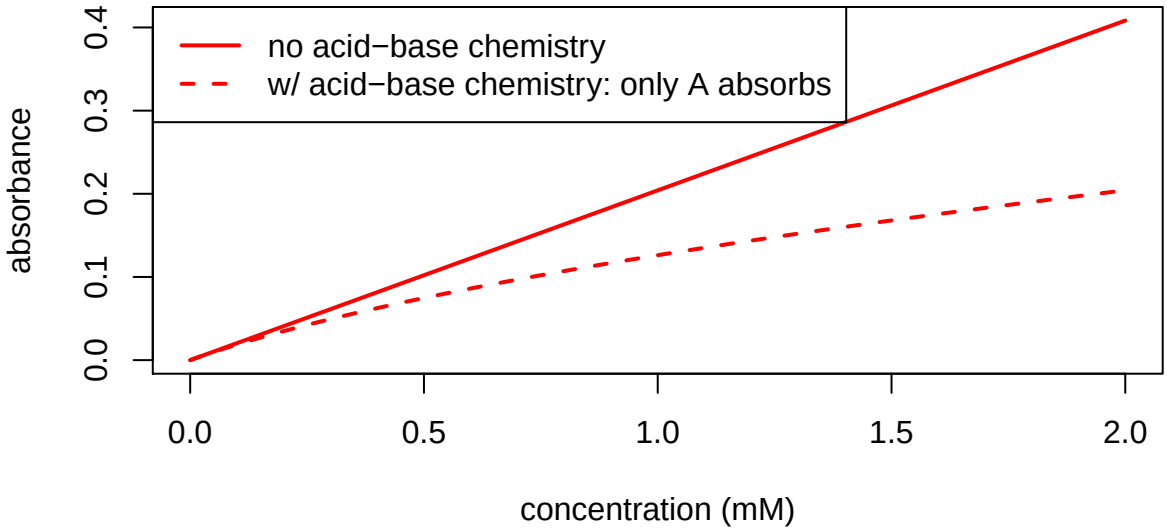
Question 1. Students should recognize that the negative deviation when HA is the sole absorbing species indicates that the concentration of HA is not the same as the analyte's reported total concentration. With additional thought—and some hints, as needed—students should recognize that because HA is a weak acid, some of it has dissociated into its weak base form, A^-.

Question 2. Students should predict that the stronger HA is an acid, the greater the deviation from Beer's law when HA is the absorbing species; thus, they should predict that the deviation is greater when HA has a pK_a of 3 and smaller when HA has a pK_a of 7.

Question 3. Although both sets of conditions result in a negative deviation from Beer's law, the deviation is concave when HA is the absorbing species



and convex when A^- is the absorbing species



Students can explain this behavior by writing the K_a expression

$$K_a = \frac{\left[\mathrm{H}^+\right]\left[\mathrm{A}^-\right]}{[\mathrm{HA}]}$$

and noting that as the concentration of HA increases, the pH decreases, the $\left[\mathrm{H}^+\right]$ increases, and the ratio

$$\frac{\left[\mathrm{A}^-\right]}{[\mathrm{HA}]}$$

becomes smaller. As a result, the percentage of analyte present as HA increases as the total concentration of analyte increases.

Question 4. For an analyte with a pK_a of 5 and HA as the absorbing species, students should recognize that they need to buffer the pH such that HA is the only significant species; a pH of 3 or less will work.

Question 5. For an analyte with a pK_a of 7 and A^- as the absorbing species, students should recognize that they need to buffer the pH such that A^- is the only significant species; a pH of 9 or more will work.

Question 6. To prepare the calibration standards, students should recognize that they need to dilute the stock standard using a solution of buffer with the appropriate pH. The buffer, of course, will need sufficient buffer capacity to hold the pH constant for all concentrations of analyte. To prepare samples for analysis, a suitable amount of sample is brought into solution using the same buffer such that the analyte's concentration falls within the calibration curve; again, the buffer capacity must be sufficient to ensure that the pH of the sample matches that of the standards.