The Beer-Lambert Law

Learning Objectives

Students should be able to:

Content

- Identify each term in the Beer-Lambert law and explain its effect on absorbance.
- Explain the relationship between transmittance and absorbance.
- Use Beer's Law in quantitative measurements.

Process

- Prepare and interpret graphs (information processing).
- Develop mathematical expressions to describe data (problem solving).

Prior knowledge

- Calibration curves and linear equations.

Further Reading

- D.C. Harris, *Quantitative Chemical Analysis,* 7th Edition, 2007 W.H. Freeman: USA, Section 18-2, pp. 380.

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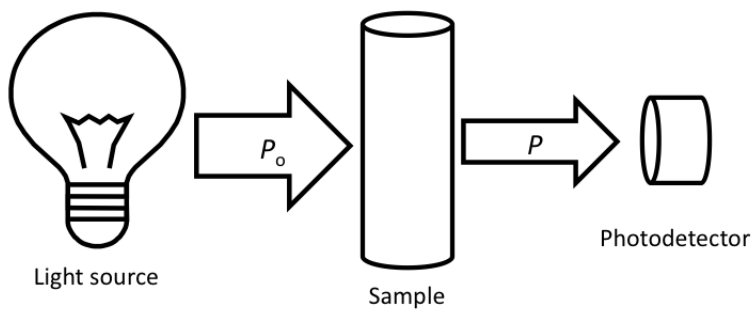
Consider this…

Suppose that we have a solution that absorbs light.

Suppose further, that we propose to analyze the solution using an instrument organized as shown below:

Figure 1 ***Simple spectrometer***



The light source might be an ordinary light bulb, a flashlight, or the sun. The photodetector could be a simple solar cell like one might buy at Radio Shack. The light shining on the sample has power P_0 and the light that manages to get through the sample has power P. P_0 and P may be expressed in Lamberts or lumens or ergs/sec/cm^2 or any other units, as long as the same units are used for both.

Transmittance is defined as $T = P/P_0$.

Percent transmittance, or $\%T$, is simply 100 T.

Key Questions

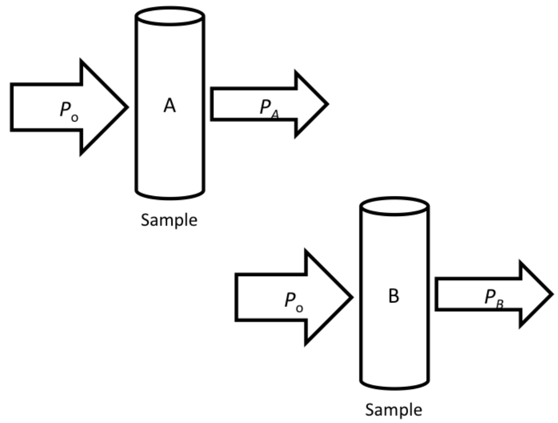
1. If some of the light is absorbed by the sample….

Is P greater than, less than, or equal to P_0? Justify your answer to your group members.

Is T greater than, less than, or equal to 1? Justify your answer to your group members.

2. Absorbance indicates how much light is absorbed by a sample. If sample A (below) absorbs more light than sample B, which would have the higher transmittance? Which would have the higher absorbance? Explain your reasoning.

Figure 2 ***Two absorbing samples***

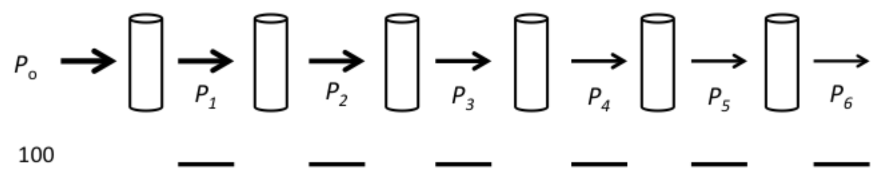


3. Discuss with your group members what happens to transmittance if absorbance increases or decreases. In 2-3 complete sentences explain why transmittance and absorption are related in this way.

Consider this…

Suppose that we have multiple identical samples of a light-absorbing solution (in containers that do not absorb light) and we set them up in a line, with a light source with power P_0 shining down the line from one end as shown in Figure 3.

Figure 3 ***Consecutive absorbing solutions***



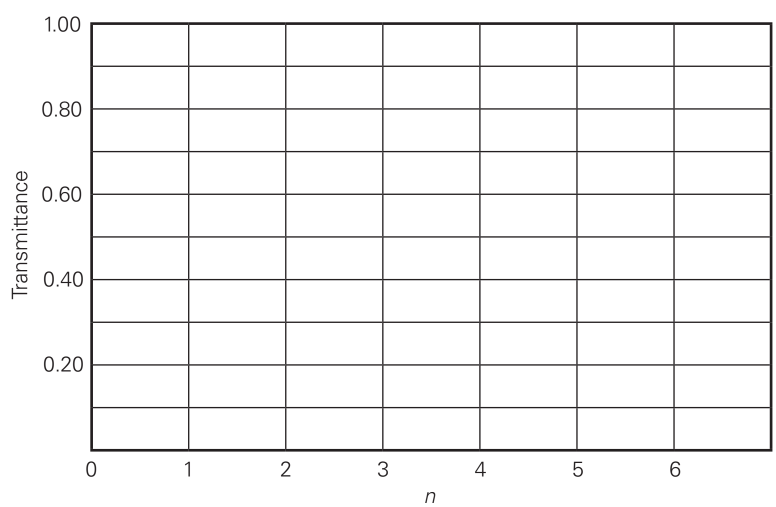
Key Questions

4. If 50% of the light is transmitted through each sample and the initial power, P_0, is 100 arbitrary units, calculate values for P_1 through P_6 and fill in the blanks in the figure with these values. Compare your values with your group members.

5. Using the equation for transmittance, $T = P/P_0$, each group member should calculate the total transmittance value for two different n values where n is the number of solutions through which light passes. Share your answers and graph the T values *vs. n*:

Figure 4 ***Transmittance vs. n***

n	T
0	1.00
1	
2	
3	
4	
5	
6	

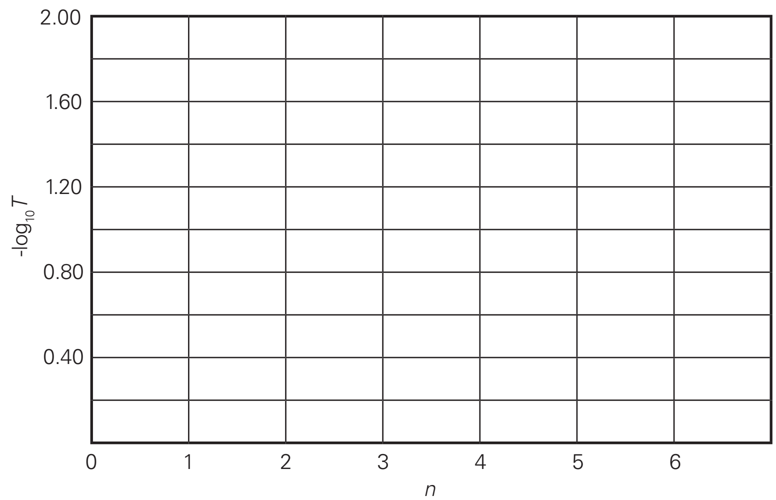


6. Does the transmittance increase or decrease as *n* increases? Is there a linear relationship between the transmittance and the number of samples through which the light passes?

7. Repeat the exercise above but this time plot ($-\log_{10}T$) *vs.* *n* (number of solutions through which light passes).

Figure 5 ($-\log_{10}T$) vs. n

n	$-\log_{10}T$
0	
1	
2	
3	
4	
5	
6	



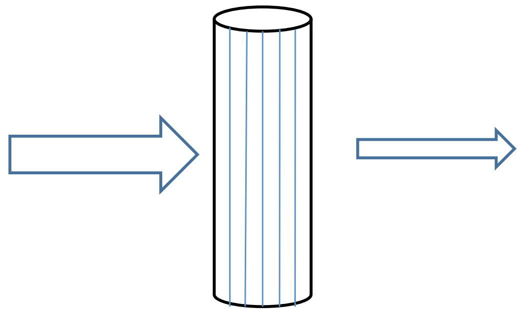
Does ($-\log_{10}T$) appear to vary linearly with *n*?

8. Using the standard form for a linear equation ($y = mx + b$, where m is the slope and b is the intercept), write an equation for the relationship shown on the graph in Figure 5.

What value should be expected for the intercept?

Now it is convenient to describe $-\log_{10} T$ as the absorbance *(A)* such that $A = -\log_{10} T$.

Figure 6 ***A sample can be considered a series of identical thin slices***



9. Suppose that each individual sample in the line of samples in Figure 3 is a slice of a mega-sample. In other words, instead of six successive solutions there is one large container with 6 slices of solution put together (Figure 6). Look back at your data in Figure 5, but now think of n as the number of slices in the sample. In words, describe the relationship between distance the light travels through the sample and $-\log_{10} T$ (or A).

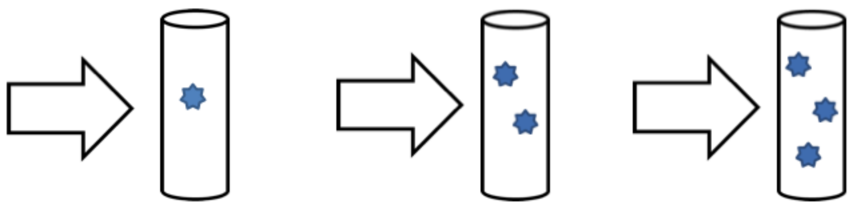
10. Using b to represent the total distance the light travels—the pathlength—modify your mathematical expression in Q8 to express the relationship between absorbance *(A)* and pathlength *(b).*

Consider this…

Below are three samples with different amounts of absorbing compound which are represented by  .

The distance the light travels through each sample (pathlength) and the power of the incoming light *(P_0)* are the same for each sample.

Figure 7 ***Comparing absorbance by different amounts of an absorbing compound***



11. Compare the concentrations of the absorbing species in the three samples. What assumption are you making about the volume of the samples?

12. Discuss with your group the relationship between concentration (c) and absorbance. Draw arrows in Figure 7 to represent the transmitted light through each sample. Modify your mathematical expression in question 10 to include both pathlength and concentration.

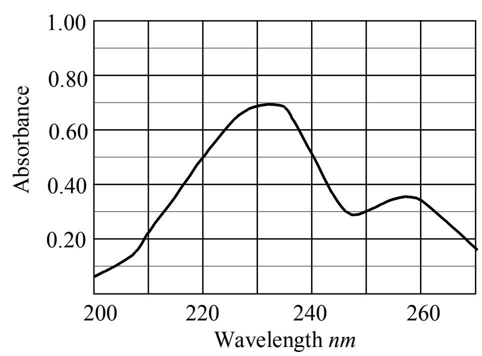
The absorbance, A, of a solution is related to the distance through which light travels in a sample and the concentration of the sample by means of the Beer-Lambert Law or simply Beer's Law:

A = abc
***a =* absorptivity, a characteristic of a substance that determines extent of absorption**
***b =* pathlength, distance light travels through the sample.**
***c =* concentration of absorbing species in the sample**

Consider this…

A spectrophotometer is an instrument that can measure the absorbance of a sample at different wavelengths of light. A graph of the absorbance of a sample as a function of wavelength obtained from such an instrument is called a spectrum and is shown in Figure 8. The spectrum shown below is for a 100 ppm solution of Compound X using a 1.00 cm wide sample vial.

Figure 8 ***UV spectrum of Compound X at 100 ppm.***



Key Questions

13. From the description above this spectrum, what terms in Beer's Law have been held constant to obtain the spectrum in Figure 8?

14. What is the absorbance of Compound X under these conditions at 233 nm? At 257 nm?

15. Discuss with your group how the data collected in Figure 8 illustrates Beer's Law. What term in Beer's Law must be wavelength-dependent?

16. Beer's Law assumes monochromatic light, but monochromatic light is not very practical to use for most analyses. Choosing a wavelength with little variation in absorbance over a short range of wavelengths can approximate monochromatic light and therefore Beer's Law will hold. What wavelengths from Figure 8 would best meet this criterion?

17. Determine with your group which wavelength in Figure 8 would allow you to measure the lowest concentration of Compound X and explain why this wavelength was chosen.

18. Sketch the absorbance vs. concentration plot for Compound X at both 233 nm and 257 nm. How are they similar and how are they different?

19. What are two things you did not know before, but learned about Beer's Law during this activity?

20. In what ways did this activity enhance your ability to write mathematical expressions to describe relationships?

Applications

21. What is the value of absorbance that corresponds to 35.0%T? Spacing seemed off here.

22. At very low values of T (less than 5%), describe the absorbance values. What problems could arise in detecting the light passing through this sample?

23. Molar absorptivity (ε) is the absorptivity when concentration is in units of molarity and the pathlength is in centimeters. If a 1.53×10^{-5} M solution has an absorbance of 0.426 in a 1.00 cm cell when measured at a wavelength of 254 nm, what is the molar absorptivity of the compound?

24. Iron (II) is measured in abandoned mine drainage using a phenanthroline method (Standard Methods for the examination of water and wastewater, method 3500-Fe). In this method 1.00 ml of duplicate water samples are acidified, added to phenanthroline solution and diluted to 100.0 ml. The solution turns deep red and is measured at 510 nm. A series of standards from 0.50-2.00 ug/ml Iron(II) are prepared and their absorbance measured. The data for these solutions is given below. Using this data calculate the concentration of iron(II) in the original mine drainage samples.

Fe Conc (ug/ml)	Absorbance
0.00	0.002
0.50	0.045
1.00	0.096
1.50	0.134
2.00	0.196
Mine drainage	0.107 0.103

25. Fresh meat slowly changes from bright red to a brown color due to the oxidation of Oxy-myoglobin to met-myoglobin upon aging. The concentrations of both of these proteins can be measured in ground meat (after extraction, purification, and separation) using a spectrophotometer. The oxy-myoglobin is measured at 417 nm with a molar absorbtivity of 12800 $M^{-1}cm^{-1}$. The met-myoglobin is measured at 409 nm with a molar absorbtivity of 17900 $M^{-1}cm^{-1}$. In an extract of 10.0 g of ground meat (in 3.0 mL total solution) the absorbance of the oxy-myoglobin sample was found to be 0.769 using a 1 cm cell and the met-myoglobin was 0.346. Determine the moles of oxy-myoglobin and met-myoglobin per gram of ground meat. (Bylkas, S.A.; and Andersson, L.A. J. *Chem. Ed.* 1997, 74, 426-430.)

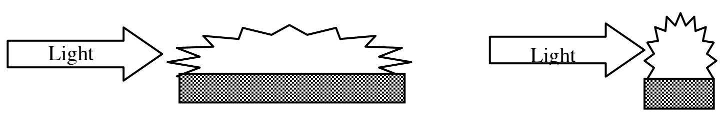
26. A solution suspected of containing copper is analyzed. To determine the standard curve, the absorbance of five standards ranging from 0.125 mg/L Cu to 1.25 mg/L Cu was measured and graphed against the concentration. The slope of the linear best-fit line was determined to be 0.6136 and the y-intercept was 0.0142. If three samples of the unknown solution have absorbance values of 0.438, 0.434, and 0.439 what is the concentration of the unknown?

27. A solution of Compound X is to be measured using Beer's Law. Based on Figure 6, what are two advantages of choosing a wavelength of 233 nm instead of 240 nm?

28. A spectrophotometer will often produce values of both Absorbance and % Transmittance. Which would be the easiest for you to use to determine the concentration of a compound in solution? Explain.

29. Suppose that in measuring a sample of iron using an absorption spectrophotometer, you find that the absorbance is too high for the instrument to measure accurately. Based on Beer's Law, what are two options you might employ to lower the absorbance value?

30. In an Atomic Absorption (AA) instrument a flame is the sample holder. In the configurations below would the short, wide flame or the tall, narrow flame give the highest absorbance values for the same concentration of sample? Explain why.



31. Beer's Law has some limitations. It works well for monochromatic radiation, dilute solutions, and when the absorbing compounds are not participating in a concentration-dependent equilibrium. Plot the following data using Excel and determine the highest concentration in which Beer's Law applies.

Conc (mg/L)	Absorbance
1.00	0.0334
5.00	0.1635
10.00	0.3284
15.00	0.4965
20.00	0.6026
30.00	0.7781
50.00	0.8652